Published in final edited form as:

Thromb Res. 2017 March ; 151: 57–62. doi:10.1016/j.thromres.2017.01.010.

Platelet function as a risk factor for venous thromboembolism in the Framingham Heart Study

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Abstract

Introduction—The relationship of venous thromboembolism (VTE) with platelet reactivity is unclear. Platelet function plays a key role in arterial thrombosis. Evidence suggests antiplatelet agents also effects in reducing VTE. Our aim is to describe the role of baseline platelet function in development of VTE in the community-based Framingham Heart Study (FHS) cohort.

Materials and Methods—Participants in the Framingham Offspring cohort fifth examination and Omni cohort first examination were eligible. We used light transmission aggregometry to measure platelet aggregation in response to collagen and a range of ADP and epinephrine doses. The study population consisted of 2831 participants [average age 54.3 y; 57% female].

Results and Conclusions—During a median follow-up of 20.4 years, we observed 138 incident VTE events. In age-, sex- and cohort-adjusted analysis an increase in collagen lag time was associated with increased risk for incident VTE (HR 1.01 [1.00–1.02]; $p=0.049$). Increased maximal aggregation to low dose epinephrine (1.0uM) was associated with lower VTE risk (HR 0.84 [0.71–0.99]; $p=0.042$). However, additional multivariable analyses attenuated the collagen-VTE and epinephrine-VTE associations to trends, primarily due to adjustment for baseline body mass index (BMI), a VTE risk factor and potential modifier of platelet function. Secondary analyses considering varying follow-up periods, cancer incidence and interim aspirin use did not dramatically affect the collagen and epinephrine trends observed. Baseline platelet aggregability was only weakly associated with incident VTE, and in a paradoxical direction, in a community-based population. Other markers of platelet function and hemostasis could prove to be more useful predictors.

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Keywords

Platelets; Platelet aggregation; Platelet Function Tests; Venous Thromboembolism; Pulmonary Embolism; Deep Vein Thrombosis

Introduction

The central role of platelets in forming an arterial thrombosis is well established, and this function is the target of drug treatment in preventing arterial thrombosis. [1] The relationship of venous thromboembolism (VTE), consisting of pulmonary embolism (PE) and deep venous thrombosis (DVT), with platelet reactivity is largely unknown. Traditionally, pharmacological intervention in VTE has been targeted to coagulation proteins (anticoagulants). However, recent evidence shows that antiplatelet agents, primarily aspirin, have an effect in reducing VTE, too. [2–5]

Mean platelet volume (an indirect measure of platelet function), and circulating soluble P-selectin (a marker of platelet activation), have been previously shown to associate with VTE. [6,7] Several small studies have variously reported an exaggerated platelet aggregation response to epinephrine, collagen or thrombin in patients with thrombosis but have not been completely consistent. [8,9] However, to our knowledge, no studies on platelet aggregation measures as prospective predictors of VTE risk have been published to date. Our aim is to describe the role of platelet function at baseline in the development of VTE in the community-based Framingham Heart Study (FHS) cohort.

Materials and Methods

Study sample

The Framingham Heart Study (FHS) is a longitudinal community-based cohort. The design, selection criteria, and enrollment of the FHS participants have been detailed previously. [10,11] The Offspring cohort consists of almost exclusively white participants of European ancestry. The Omni cohort consists of participants who self-identified as non-white. [12] Participants in the Framingham Offspring cohort attending the fifth examination cycle (1991–1995) and Omni cohort attending the first examination cycle (1994–1998) during which platelet function was assayed were eligible for the present investigation.

A total of 3799 persons attended the fifth examination cycle of the Offspring cohort, and 505 persons attended the first examination cycle of the Omni cohort. Of these, 1473 were excluded for the following reasons: use of aspirin or other antiplatelet agents at the time of platelet function analyses (N=859); missing platelet function measures (N=614) since these were started later in the examination. We also excluded 12 participants due to lack of information on BMI or cigarette smoking, including 1 VTE case. The Institutional Review Board of Boston University Medical Center approved the study protocol, and all participants provided written informed consent.

Determination of platelet aggregability

We tested platelet aggregation in response to three agonists – adenosine diphosphate (ADP), epinephrine, and collagen. Blood samples were collected from the antecubital vein in the morning, with subjects in the supine position after an overnight fast. Blood was drawn into an evacuated blood collection tube that contained a 3.8 % solution of sodium citrate as an anticoagulant and centrifuged at $160 \times g$ for 5 min at room temperature to separate out platelet-rich plasma (PRP). We used a four-channel light transmission aggregometer (BioData, Horsham, PA) to measure platelet aggregation. We tested aggregation using a final 0.5 mg/mL concentration of arachidonic acid and increasing concentrations of ADP (from 0.05 to 15 μM) and epinephrine (from 0.01 to 15 μM) [13]. Because doses tested for ADP and epinephrine were titrated up or down depending on responsiveness on a per individual basis after entry doses, not all individuals were tested at all doses. Collagen lag time was measured in response to a single 190 $\mu\text{g/mL}$ dose of Type I calf skin-derived collagen. We excluded participants who reported any aspirin use within 2 weeks of examination or who had no response to arachidonic acid stimulation, a sensitive measure of recent aspirin exposure.

We characterized platelet aggregation by generating dichotomous and continuous aggregation traits. Participants who responded ($\geq 50\%$ maximal aggregation) at least at one low dose of ADP (0.05, 0.1, 0.5, and/or 1.0 μM) were considered *hyper-responders* for ADP (hyper_ADP). Similarly, hyper-reactivity to epinephrine (hyper_epi) was defined as $\geq 50\%$ maximal aggregation with at least one low dose of epinephrine (0.01, 0.03, 0.05, 0.1, 0.5, 1.0 μM). In contrast, those participants who failed to aggregate ($< 50\%$ maximal aggregation) at a higher dose (5.0, 10.0, 15.0 μM) of ADP or epinephrine were considered *hypo-responders* (hypo_ADP, hypo_epi). This scheme was chosen to share information across doses since not all participants were tested at all doses. The threshold of 50% was chosen based on response distributions and prior studies in healthy donor samples that indicated this a reasonable inflection point to distinguish partial versus full responses. [14]

As mentioned above, different participant populations were available for aggregation responses at various agonist concentrations due to the dose titration scheme. In all association studies presented here a sample size > 500 at each dose/condition was analyzed to ensure sufficient numbers of events. Due to skewed distributions of aggregation measures, levels were analyzed using quartiles.

Definition of covariates

We defined current smoking as use of at least one cigarette per day during the preceding year. We defined hypertension as a systolic blood pressure ≥ 140 mmHg, or a diastolic blood pressure ≥ 90 mmHg, or the use of any antihypertensive medication. Blood pressures represent the average of two blood pressures obtained by the physician on seated participants at the time of the Framingham clinic examination using a standardized measurement protocol. Criteria for diabetes mellitus were a fasting glucose level of ≥ 126 mg/dL (7.0 mmol/L) or greater, or use of medications to treat hyperglycemia. Heavy alcohol drinking was defined as weekly alcohol amount greater than 14 drinks for men or 7 drinks for women. Levels of total, high density lipoprotein (HDL)-cholesterol, and triglycerides were

analyzed in fasting blood samples collected at the clinic exam following standard measurement protocols. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of the height in meters.

Definition of outcomes

The primary outcome for this study was the development of first symptomatic VTE. The data collection protocol has been previously reported. [15] Briefly, medical records were obtained for all hospitalizations and physician visits related to VTE during follow-up and were reviewed by a committee of 3 investigators; events were adjudicated following established criteria. The diagnostic criteria for VTE included clinical symptoms and signs of VTE and objective evidence of venous thrombosis on imaging studies or findings at autopsy. PE was diagnosed using either ventilation perfusion (V/Q) scans, computed tomography (CT) angiography, or at autopsy. DVT was diagnosed using venograms, Doppler ultrasound studies of culprit veins, impedance plethysmography (IPG), 125I fibrinogen leg scan, or a combination of the IPG and leg radionuclide scans. DVT included thrombosis in the lower and upper extremities; thrombosis in the superficial veins, restricted to the calf, in untypical sites, or intracranial veins were not included. While some of these measures are not contemporary they were included in event adjudication due to the time period of baseline consideration. 125I fibrinogen leg scan only identified 1 case of DVT and IPG identified no cases.

Statistical methods

Distributions of demographic characteristics and cardiovascular disease (CVD)-related risk factors were illustrated as mean $\pm$ standard deviation for continuous variables and number $\pm$ frequency for dichotomous or categorical variables. We applied Cox's Proportional hazard models to test for significant associations between platelet function and development of VTE.

Time of follow-up was calculated as the year between date of baseline examination and date of VTE diagnosis using a deadline of December 31, 2013. Time of follow-up for participants free of VTE was either years between baseline examination and date of death or study end whichever came first. Every covariate was tested for validation of semi-parametric assumptions. In addition to age at the baseline and sex, we adjusted for study cohort (Offspring or Omni), BMI, and cigarette smoking in multivariable models. A two-tailed P value <0.05 was considered statistically significant.

Since aspirin use may potentially reduce the risk of VTE events [2–5], we considered it may be possible that individuals with differential platelet reactivity at baseline might be more likely to later receive aspirin and thus have reduced VTE risks due to treatment biases. Likewise, cancer-provoked VTE likely has different etiology and has worse prognosis than non-cancer provoked VTE as observed in several studies [15]. Due to the potential effects of cancer and aspirin on outcomes, and the possibility these varied among platelet reactivity extremes, for the two significant results (collagen and epinephrine 1.0 μ M) we ran final multivariable models at all lengths of follow-up, additionally adjusting for cancer and aspirin

use reported in the interim at those follow-up times (5 years, 10 years, 15 years, maximum). Statistical analyses were performed with SAS (version 9.3, SAS Institute Inc., Cary, NC).

Results

Study population

The study population consisted of 2819 FHS participants [average age 54.3 y; 57% female] mainly from the Offspring cohort (89%). Only ~7.8% of VTE cases and ~11.0% of controls were of self-identified Asian, African or Hispanic ancestry. Platelet aggregation data using different agonists was available as follows: N=2368 for epinephrine, N=2771 for ADP, and N=2721 for collagen. Baseline characteristics are shown in Table 1. During a median follow-up of 20.1 years (mean±std, 18.9±4.5), we observed a total of 137 incident VTE events (79 DVT and 58 PE with or without DVT). Cancer prevalence was not significantly different among cases (n=8 of 137) and controls (n=72 of 2,682), but cancer incidence was significantly higher among VTE cases (31%) than controls (14%). Most VTE cases (77%) were classified as provoked (e.g., fracture, infection, trauma, heart failure, myocardial infarction, stroke).

Platelet aggregation and incident VTE

Mean values and sample sizes in each group are shown in Table 2. In regression models adjusted for age, sex, and study cohort, platelet aggregation as measured by % maximal aggregation induced by 1.0 or 3.0 μ M epinephrine was statistically significantly (1.0 μ M), or had a trend toward association (3.0 μ M), with a decreased risk for VTE at maximal time of follow-up surveillance [HR 0.84 (95% CI 0.71–0.99), $p=0.04$, and HR 0.82 (95% CI 0.66–1.00), $p=0.053$, respectively]. However, after further adjustment for baseline BMI and smoking the association did not remain statistically significant [HR 0.85 (95% CI 0.72–1.01), $p=0.06$, and HR 0.86 (95% CI 0.70–1.06), $p=0.16$, respectively].

We observed a trend toward increase in collagen lag time associated with increased risk for VTE in the age and sex adjusted model [HR 1.01 (95% CI 1.01–1.02); $p=0.049$]. However, this association was not seen in the model with additional adjustment for BMI and cigarette smoking [HR 1.01 (95% CI 1.00–1.02); $p=0.07$]. Hyper- or hypo-reactivity to either ADP or epinephrine were not associated with VTE risk, nor was platelet aggregation measured using any of the tested concentrations of ADP (Table 3).

Since the strongest findings in models adjusted only for age, sex and study cohort were observed with epinephrine 1.0 μ M and collagen lag time, we conducted secondary analysis for these measures to see if these findings varied or were consistent across different periods of follow-up (5 years, 10 years, 15 years). The trend of the results during those periods is consistent with those at the maximal follow-up period, with slightly more significant results observed at 10 years follow-up (Table 4). The number of events in time periods of months to several years after platelet function testing are too low to test statistically. Since cancer incidence varied across VTE cases and controls, cancer-provoked cases may have different etiology, and differences in interim aspirin treatments could affect VTE outcomes, we analyzed multivariable models at all lengths of follow-up additionally adjusting for cancer

incidence and aspirin use. These results were very similar to the multivariable model adjusting for age, sex, BMI and smoking (Table 4). To further elucidate the effects of baseline BMI, or obesity (defined as BMI ≥ 30), versus smoking on the results we ran additional models of different combinations for collagen lag time and epinephrine 1.0 μM at maximal follow-up. These results show that adjustment for baseline BMI or obesity is the main factor that attenuates the platelet VTE incidence associations (Table 5). In analysis of provoked VTEs alone ($n=102$ cases for collagen lag time; $n=88$ cases for epinephrine), similar trends were observed (collagen: 1.01 [1.00–1.02] $p=0.12$; epinephrine 1.0 μM : 0.84 [0.70–1.02] $p=0.08$) but were not significant likely due to the smaller sample size.

Discussion

Our study is the first to report on the association of baseline platelet aggregability and VTE. Despite the plausible hypothesis of platelet function being associated with thrombotic events, we did not detect strong statistically significant associations between platelet aggregation measures and VTE in the community-based Framingham Heart Study cohort, after accounting for other likely competing risk factors. There was a trend for a paradoxical association with epinephrine induced aggregation and decreased risk of VTE which did not remain significant after adjusting for BMI and smoking. Likewise, the trend of longer collagen lag time being associated with increased risk of VTE seems paradoxical to mechanistic expectations. Further analyses for epinephrine and collagen indicated that adjustment for BMI at baseline attenuated these associations. BMI has been previously shown to be an important predictor of VTE. [16] Varying the follow-up time period from 5 years to the maximum, or additionally adjusting for cancer incidence or aspirin usage after baseline, did not appreciably change the interpretation of the results. Since few of the events in the study were classified as unprovoked VTEs (23%) we combined unprovoked and provoked VTE in our analyses. Analyses in provoked VTE cases alone showed similar trends to the combined analyses.

To our knowledge no longitudinal population-based studies on the topic of platelet function and VTE previously exist. The association of platelet aggregability with VTE was studied cross-sectionally in 63 patients with a history of VTE, with a control sample of 14 healthy volunteers. [8] In the study patients with arterial events ($n=31$) were further combined in analysis with VTE patients for a total of 94 samples. Light transmission aggregometry with normal and low-dose epinephrine or ADP as well as spontaneous aggregation were recorded within a few weeks after venous or arterial thrombosis. An exaggerated response to low-dose epinephrine (1.0 μM), defined by Hayes et al. as $>18\%$ aggregation, was observed in 69% of thrombosis patients ($n=64/94$), but also in 36% of healthy volunteers ($n=5/14$) [$p=0.034$]. Our study examined a much larger case and control sample and observed no such finding with epinephrine at 1.0 μM and disease incidence after ~ 20 years of follow-up. In fact, the direction of effect of any trend in our study was opposite (increasing % maximal aggregation at Epi 1.0 μM , multivariable model: HR 0.85 [0.72–1.01], $p=0.06$). No association with ADP or arachidonic acid was reported by Hayes et al., and responses to collagen were not measured. [8]

Additionally, there have been a number of studies of platelet aggregation abnormalities referred to as sticky platelet syndrome (SPS), generally performed on patients with unexplained thrombotic events, both venous and arterial, sometimes in families and generally presenting at younger ages [17]. Features of sticky platelet syndrome include definitions based on threshold hyperaggregability to ADP and/or epinephrine, usually at lower dose ranges in LTA. However, the magnitude of these responses do overlap with normal ranges observed in population studies [13, 14] and thus the phenotype of being hyperaggregable at these doses of ADP and/or epinephrine may not be rare in the general population. While SPS studies often had small or no control samples, they have generally concluded that there may be enrichment of low dose aggregability to ADP and/or epinephrine particularly among individuals with unexplained thrombosis. They have also concluded that aggregation to collagen, ristocetin and thrombin appears normal in these samples [17]. Notably, Weber et al. studied patients with unexplained VTE (n=34) for 3 doses each of ADP, epinephrine, collagen and TRAP-6 (a thrombin receptor activating peptide) versus healthy individuals (n=53). [9] They found that ADP (0.58, 1.17, or 2.34 μ M) or epinephrine (0.55, 1.1, 11.0 μ M) aggregation were not associated with VTE at any of the doses examined. Aggregation to moderate or higher doses of collagen (0.1, 0.25 μ M) and TRAP-6 (5.0, 7.5 μ M) were not associated with VTE. However, higher percent aggregation to low dose collagen (0.05 μ M) was associated VTE (P=0.00076), as was higher aggregation to low dose TRAP-6 (2.0 μ M) (P=0.006). In our current study we did not have data available to evaluate thrombin or low dose collagen effects in order to compare to the results of Weber et al. [9], and these pathways of activation were not evaluated by Hayes et al. [8]

The inconsistency in results across these studies may be a result of the small number of control samples in some prior studies, or differences in methodology and study context. Other studies were conducted in the context of presenting clinical cases. Inclusion criteria adopted in many of these studies focusing on unexplained thrombosis or cases without family history of thromboembolic events, or bleeding or coagulation disorders, differs significantly from our population-based study. Hayes et al. [8] and many of the SPS [17] studies conducted dichotomized threshold tests for ADP and epinephrine whereas most of our analyses focused on continuous trait data. Some studies have combined arterial and venous events in their analysis, making it possible that their findings were driven in part by associations with arterial events. Furthermore, some studies tested samples post-thrombosis in a clinical context with a high degree of anti-platelet treatment and potential comorbidities whereas we tested a population sample at baseline with low rates of anti-platelet treatment due to our exclusions. To our knowledge, body mass index and other potential covariates of importance were not adjusted or accounted for in many of the prior studies on SPS, platelet function and thrombosis. Weber et al. did have well matched age- and sex- cases and controls and found no association of age, sex or smoking on their platelet measurements. [9]

Our observations on platelet aggregation, although still statistically underpowered due to limited case numbers, are unexpected and somewhat counter-intuitive. Increased reactivity to epinephrine appeared to be associated with decreased risk for VTE. Similarly, increased collagen lag-time was associated with increased risk for VTE; i.e., our observations suggest people with less active platelets (longer collagen lag-time) being more prone to thrombosis, and those with more active platelets (response to epinephrine) being protected from VTE.

Another study found an unexplained paradoxical association between ADP-induced aggregation and arterial thrombosis (stroke) in the Caerphilly cohort. [18,19] Whether these paradoxical associations have validity remains to be determined in larger future studies. Overall, based on the available evidence in the literature and the modest HRs observed here, we conclude that platelet function, as measured here by a single timepoint LTA test with ADP, epinephrine or collagen, is unlikely to be a useful biomarker for VTE prediction, at least in the general population. A lack of evidence for strong ADP or epinephrine association with VTE is also supported by Weber et al. [9], although their study raises the prospect that hyperaggregability to lose dose thrombin or collagen receptor activators could associate with VTE, hypotheses we were unable to test.

Even though our study sample is among the largest available, we still had a limited number of VTE events (maximum of n=137). Lack of association between VTE and platelet function could thus be partly explained by lack of statistical power, or by limitations in the methods and extensiveness (e.g., dose ranges available, PRP and not whole blood tests) of assessing platelet function. A single baseline biomarker measurement may also be less reliable than repeated measures, but repeated or longitudinal measures were not available in FHS for these phenotypes. Our study has general implications for potential future incidence-based studies of VTE. Despite ~2,400 individuals at baseline we had limited number of events precluding analysis at shorter time periods (e.g., 5-year follow-up). Future studies might need very large samples and long follow-up, or they may need to consider alternate designs such as prevalence-based designs, or sampling older and higher risk populations.

Another alternate approach may be to rely on mean platelet volume (MPV) as a surrogate for platelet function instead of measuring directly platelet aggregability [20–22]. Higher MPV indicates larger platelets on average which may be more reactive, and which can be measured more routinely in labs as part of complete blood cell counts. Baseline MPV in healthy individuals was assessed in the Tromsø study and shown to be predictive of VTE in this longitudinal population. [6] Other recent studies have suggested that lower [23–27] or higher [27] MPV values are associated with increased VTE risk in either cancer or general clinical settings. The measure of MPV does exhibit significant heterogeneity across testing platforms and centers which makes standardization or meta-analysis across studies potentially challenging in clinical settings [28]. Our study could not address the hypothesis of MPV and VTE, as MPV was only measured at baseline in the youngest FHS cohort (Gen3) and recently in the FHS Offspring/Gen2 Exam 9, in both cases allowing insufficient follow-up to accrue VTE events for analysis [29]. Larger, deeply platelet phenotyped population-based or case-control cohorts may be needed to examine and solidify hypotheses regarding platelet reactivity, MPV and VTE in the future. It is possible that MPV as a general pan-marker of larger and potentially more reactive platelets may be superior in risk prediction to platelet markers of specific pathways of activation such as studied here.

In conclusion, platelet reactivity as measured by lower doses of ADP, epinephrine or standard collagen dose are not strong biomarkers of incident VTE, a finding that is somewhat inconsistent with some small clinical studies [8,17], while being partly consistent in null findings with another study [9]. Future studies may do well to focus on alternate

venous biomarkers, or other approaches to platelet testing such as flow-cytometry based assays, whole blood assays or indices like MPV.

Acknowledgments

M. K. Puurunen, S-J. Hwang, C. J. O'Donnell, G. Tofler and A. D. Johnson all contributed to the concept and design of the study and measures, and approved the manuscript content. S-J. Hwang conducted analyses, and M. K. Puurunen, S-J. Hwang and A. D. Johnson provided interpretation of analyses. M. K. Puurunen, S-J. Hwang and A. D. Johnson wrote and revised the manuscript materials. Funding Sources: This work was supported by the National Heart, Lung, and Blood Institute (NHLBI) contracts NO1-HL 25195 and HHSN268201500001I, NHLBI Intramural Funds (ADJ) and Paulo Foundation (MP). The funding source played no role in study design, data analysis, or reporting. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

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Highlights

- A large study of platelet reactivity with long surveillance for VTE incidence
- Paradoxical association of lower collagen reactivity with higher VTE risk
- Paradoxical association of higher epinephrine reactivity with lower VTE risk
- Results in this study and among the clinical literature are inconsistent
- Further studies (e.g., for alternate platelet biomarkers) may be needed

Table 1

Baseline characteristics of the study population (n=2819).

Baseline characteristic	
Age (year) (mean, SD)	54 (10)
Female sex (n, %)	1605 (56.9%)
European ancestry (n, VTE cases/controls) *	117/2357
Hispanic-American (n, VTE cases/controls) *	3/138
African-American (n, VTE cases/controls) *	6/90
Asian-American (n, VTE cases/controls) *	1/64
BMI (kg/m ²) (mean, SD)	27.3 (5.1)
Systolic blood pressure (SBP) (mmHg) (mean, SD)	125 (19)
Diastolic blood pressure (DBP) (mmHg) (mean, SD)	74 (10)
Alcohol per week (drinks) (mean, SD)	5 (8)
Fasting blood glucose (mg/dL) (mean, SD)	100 (28)
Total cholesterol (mg/dL) (mean, SD)	205 (37)
HDL-cholesterol (mg/dL) (mean, SD)	51 (16)
Triglycerides (mg/dL) (mean, SD)	144 (106)
Hypertension (n, %)	860 (30.5%)
Diabetes (n, %)	165 (5.8%)
Prevalent cardiovascular disease (n, %)	101 (3.6%)
Current smoker (n, %)	547 (19.4%)
Heavy alcohol drinking (n, %)	533 (18.9%)
Anti-hypertensive treatment (n, %)	445 (15.8%)
Medication for hyperlipidemia (n, %)	122 (4.4%)
Anti-depression medication (n, %)	265 (9.4%)
Non-steroidal anti-inflammatory drugs (n, %)	96 (3.5%)

* Ancestry group is based on baseline self-report. Case/control numbers by ancestry are shown for those participants with ADP 3.0 μ M data acquired, the platelet trait that had the largest available sample size for analyses

Table 2

Mean platelet aggregation response to various agonists.

Platelet function trait	N	N VTE cases, controls	Mean (units)	SD
ADP 1.0 μ M	2659	125 cases, 2534 controls	19.48 (%)	22.56
ADP 3.0 μ M	2772	136 cases, 2636 controls	72.11 (%)	23.31
ADP 5.0 μ M	1667	82 cases, 1585 controls	80.98 (%)	16.01
ADP hyper-response	2782	16 cases, 292 controls		
ADP hypo-response	2782	5 cases, 79 controls		
Epinephrine 0.1 μ M	1575	70 cases, 1505 control	24.44 (%)	25.88
Epinephrine 0.5 μ M	2224	100 cases, 2124 controls	52.27 (%)	30.76
Epinephrine 1.0 μ M	2368	114 cases, 2254 controls	59.45 (%)	30.30
Epinephrine 3.0 μ M	1338	76 cases, 1262 controls	67.86 (%)	27.09
Epinephrine hyper-response	2347	14 cases, 318 controls		
Epinephrine hypo-response	2347	10 cases, 166 controls		
Collagen lag time	2721	133 cases, 2588 controls	79.22 (s)	22.03

Table 3

Hazard ratio of incident venous thromboembolism by platelet function in Framingham Heart Study participants.

Platelet function trait	Adjusted for age, sex, study cohort				Multivariable adjusted			
	HR	CI	P	HR	CI	P	HR	P
Hyper_ADP (yes/no)	1.13	0.66	1.92	0.65	1.15	0.67	1.95	0.62
Hyper_Epinephrine (yes/no)	0.87	0.50	1.53	0.63	0.88	0.50	1.54	0.66
Hypo_ADP (yes/no)	1.52	0.62	3.74	0.36	1.52	0.62	3.75	0.36
Hypo_Epinephrine (yes/no)	1.27	0.66	2.44	0.48	1.18	0.61	2.28	0.62
ADP 1.0 μ M (N=2659)	1.00	0.85	1.18	0.96	1.01	0.86	1.18	0.94
ADP 3.0 μ M (N=2772)	0.88	0.75	1.02	0.10	0.88	0.76	1.03	0.11
ADP 5.0 μ M (N=1667)	0.90	0.74	1.09	0.28	0.90	0.74	1.10	0.29
Epinephrine 0.1 μ M (N=1575)	0.98	0.78	1.22	0.82	0.97	0.77	1.21	0.76
Epinephrine 0.5 μ M (N=2224)	0.94	0.78	1.12	0.48	0.95	0.79	1.13	0.54
Epinephrine 1.0 μ M (N=2368)	0.84	0.71	0.99	0.042	0.85	0.72	1.01	0.06
Epinephrine 3.0 μ M (N=1338)	0.82	0.66	1.00	0.053	0.86	0.70	1.06	0.16
Collagen lag time (N=2721)	1.01	1.00	1.02	0.049	1.01	1.00	1.02	0.07

Table 4

Hazard ratios for incident venous thromboembolism for collagen lag time and epinephrine 1.0 μ M at varying follow-up periods from baseline in Framingham Heart Study participants.

Platelet function trait	Follow-up period	VTE cases n	Adjusted for age, sex, study cohort			Multivariable adjusted		
			HR	CI	p	HR	CI	p
Collagen lag time (N=2721)	5 years	17	1.00	0.98	1.03	0.65	0.98	0.73
	10 years	37	1.02	1.00	1.03	0.002	1.00	0.004
	15 years	71	1.00	1.00	1.02	0.02	1.00	0.03
	maximum	133	1.01	1.00	1.02	0.049	1.00	0.07
Epinephrine 1.0 μ M (N=2368)	5 years	13	0.76	0.46	1.26	0.28	0.48	0.35
	10 years	29	0.69	0.49	0.97	0.03	0.50	0.05
	15 years	61	0.81	0.64	1.01	0.06	0.65	0.08
	maximum	114	0.84	0.71	0.99	0.042	0.72	0.03

p* indicates the p-value in the multivariable model adjusted for age, sex, smoking, BMI, cancer incidence and interim reported aspirin use after baseline at each follow-up period

Table 5

Hazard ratios for incident venous thromboembolism at maximal follow-up for collagen lag time and epinephrine 1.0 μ M with varying baseline covariate adjustments in Framingham Heart Study participants.

Platelet function trait	Covariate(s) in addition to age, sex and study cohort	HR	CI	p
Collagen lag time (N=2721)	---	1.01	1.00 1.02	0.049
	Baseline BMI	1.01	0.99 1.02	0.076
	Baseline obesity (BMI $\geq$ 30)	1.01	0.99 1.02	0.070
	Smoking	1.01	1.00 1.02	0.047
	Smoking, baseline BMI	1.01	0.99 1.02	0.072
Epinephrine 1.0 μ M (N=2368)	Smoking, baseline obesity	1.01	0.99 1.02	0.067
	---	0.84	0.71 0.99	0.042
	Baseline BMI	0.85	0.72 1.01	0.058
	Baseline obesity (BMI $\geq$ 30)	0.85	0.72 1.00	0.050
	Smoking	0.84	0.71 0.99	0.042
	Smoking, baseline BMI	0.85	0.72 1.01	0.062
	Smoking, baseline obesity	0.85	0.72 1.00	0.052