Emerging risk factors for venous thromboembolism: The role of commonly prescribed drugs for cardiovascular disease and inflammatory disorders

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Chapter 3

Rosuvastatin use reduces thrombin generation potential in patients with venous thromboembolism: a randomized controlled trial

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Summary

Background: Statin therapy could form an alternative prophylactic treatment for venous thromboembolism (VTE) if statins are proven to downregulate hemostasis and prevent recurrent VTE, without increasing bleeding risk. **Objectives**: The STAtins Reduce Thrombophilia (START) trial investigated whether statin affects coagulation in patients with prior VTE. **Patients/Methods:** After anticoagulation withdrawal, patients were randomized to rosuvastatin 20mg/day for 4 weeks or no intervention. Plasma samples taken at baseline and at the end of the study were analyzed employing thrombin generation assay. **Results and Conclusions:** The study comprised 126 rosuvastatin users and 119 non-users. Mean age was 58 years, 61% were men, 49% had unprovoked VTE and 75% had cardiovascular (CV) risk factors. Endogenous thrombin potential (ETP) increased from baseline to end of study in non-statin users (mean 97.22nM*min; 95%CI 40.92 to 153.53) and decreased in rosuvastatin users (mean -24.94nM*min; 95%CI -71.81 to 21.93). The mean difference in ETP change between treatments was -120.24nM*min (95%CI -192.97 to -47.51), yielding a 10.4% ETP reduction by rosuvastatin. Thrombin peak increased in both non-statin (mean 20.69nM; 95%CI 9.80 to 31.58) and rosuvastatin users (mean 8.41nM; 95%CI -0.86 to 17.69). The mean difference in peak change between treatments was -11.88nM (95%CI -26.11 to 2.35), yielding a 5% peak reduction by rosuvastatin. Other thrombin generation parameters did not change substantially. The reduction in ETP and peak by rosuvastatin was more pronounced in the subgroups of participants with CV risk factors and with unprovoked VTE. We conclude that rosuvastatin reduces thrombin generation potential in patients who had VTE.

INTRODUCTION

Venous thromboembolism (VTE) contributes significantly to the global disease burden, and therefore, preventive measures and adequate treatment are warranted [1]. While anticoagulation is the treatment of choice for preventing VTE episodes [2], bleeding complications are a major concern and may lead to treatment avoidance in many cases [3]. The latter underscores the need for alternative treatment options for VTE prophylaxis. Statins may provide a promising alternative treatment for thromboprophylaxis because these drugs are alleged to have pleiotropic effects on hemostasis and may reduce VTE risk, although strong clinical evidence supporting these effects is still scarce [4].

Previous studies have reported that statins reduce the risk of first VTE by 14 to 54% [5-9] and the risk of recurrent VTE by 27% [10]. However, healthy user effects, survivor bias and adherence bias could have influenced these results [11]. Moreover, the strongest evidence on the effect of rosuvastatin on first VTE still comes from one randomized clinical trial [8], while no randomized trials have investigated the impact of statin therapy on the risk of recurrent VTE. Despite the need for additional randomized trials, the lack of knowledge on the mechanisms that are at the basis of the supposed causal association between statin use and a reduced risk of VTE may discourage the conduction of such interventional studies.

Recently, we have shown in the STAtins Reduce Thrombophilia (START) trial that one month of treatment with rosuvastatin at 20 mg/day led to an improved coagulation profile as compared with non-statin users in patients with prior VTE, most notably by reducing factor VIII plasma levels [12]. These observations from the START trial were the first randomized evidence indicating that rosuvastatin reduces coagulation factor levels in patients with prior VTE and confirmed similar findings previously observed for other statins [13-15]. To better understand the effect of rosuvastatin on individual prothrombotic profiles, we evaluated here whether rosuvastatin could interfere with thrombin generation, a global coagulation test that reflects not only the coagulation potential [16-18] of an individual but also predicts the risk of a first and recurrent VTE [19-21].

METHODS

Trial design

The START trial is a randomized, open label, controlled, clinical trial conducted in the Netherlands that investigated whether the coagulation profile in persons with a history of VTE

and not taking anticoagulants is improved when using rosuvastatin. Details of the study design are described elsewhere [12]. The study was undertaken in accordance with the Declaration of Helsinki and International Conference on Harmonization guidelines for Good Clinical Practice. All participants gave written informed consent prior to participation. START was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands, and is registered at www.clinicaltrials.gov as NCT01613794.

Participants

Participants were recruited at three anticoagulation clinics in the Netherlands (Leiden, Hoofddorp and Rotterdam) between June 2012 and January 2017. Individuals aged 18 years or older, with confirmed symptomatic proximal deep vein thrombosis or pulmonary embolism, were eligible for inclusion in the study if their physicians approved the cessation of oral anticoagulant treatment. Exclusion criteria were: current use of statins or lipid lowering drugs, or any contraindications for rosuvastatin at 20 mg/day use as provided by the instruction leaflet of the manufacturer.

Intervention

Informed consent was obtained at the study baseline visit. The study baseline visit was defined at the time of the last regular visit of the patient to the anticoagulation clinic. After informed consent, participants were screened on acquired risk factors for thrombosis through a questionnaire and tested for liver and kidney functions. At randomization, participants were allocated to receive either rosuvastatin at 20 mg/day or no study medication. The random allocation sequence was implemented by central telephone and the sequence was concealed until interventions were assigned.

The duration of the study was 28 days, based on the consideration that some small, non-randomized, studies showed beneficial effects of statins on the coagulation system as early as after a three-day statin administration [12].

Measurements

Patients stopped using their vitamin K antagonist for one month (to allow the anticoagulant drugs to wear off) after which a blood sample was drawn at randomization visit and at the end

of the study period (*i.e.* 28 days later). All blood drawings were performed between 08:00 and 15:00. Blood was collected in tubes containing sodium citrate (3.2%) and centrifuged within 3 hours of venepuncture at 2500 g for 15 minutes at 18°C, after which plasma was immediately stored at -80°C. Laboratory technicians, who were unaware of which participants were rosuvastatin-users, performed the assays after all participants had completed the study.

The thrombin generation potential was assessed by means of the thrombin generation assay (TGA), which is a global coagulation test that reproduces the kinetics of thrombin formation [22, 23], using the Calibrated Automated Thrombogram® (Diagnostica Stago, France) according to the manufacturer's specifications [24]. Briefly, plasma samples were mixed with the assay reagents (tissue factor and phospholipids) and tested in duplicate. As internal control, normal pooled plasma, derived from citrated plasma from 64 healthy men and women not taking oral contraceptives, was tested in each assay and a thrombin calibrator was used for each plasma duplicate. The fluorescent signal representing generated thrombin was monitored in a Fluoroskan Ascent fluorometer (Thermo Scientific, USA) and the parameters were calculated with the Thrombinoscope software (Thrombinoscope BV). The TGA parameters determined were: endogenous thrombin potential (ETP), thrombin peak, time to peak, lag time and velocity index. ETP, or area under curve, represents the total amount of thrombin generated over time. The thrombin peak represents the maximum amount of thrombin that can be generated. Time to peak indicates the time required to reach the maximum amount of thrombin formed. The lag time measures the length of time between the start of the assay (addition of triggers) and the initiation of thrombin generation. Velocity index is defined as [peak height/(time to peak – lag time)] and represents the rate of thrombin generation [20].

Outcomes

Because the ETP and thrombin peak have been consistently associated with VTE risk [25-31], the primary endpoints were defined as the difference in change in ETP and thrombin peak from baseline to the end of the study between rosuvastain users and non-users. The differences in the change in lag time, time to peak or velocity index were considered secondary endpoints. The study was originally powered on factor VIII [12]. Nevertheless, we observed in the non-statin users that the mean ETP was 1245 mM*min (SD 322) at randomization. Therefore, we a-priori expected to find a powered mean difference of at least 76 nM*min or 6% decrease between participants at end of study with a 2-sided alpha of 0.05 and 80% power.

Statistical analysis

Final analyses were done by modified intention-to-treat since there were post-randomization exclusions. The mean levels and 95% confidence intervals (95% CI) of all pre-specified thrombin generation assay parameters were calculated at the time of randomization (baseline), at the end of the study period and for the change between these two time periods within each treatment group. We also calculated the percentage of change within groups by subtracting the baseline value from the end of the study value, dividing it by the baseline value, and multiplying the result by 100%.

To determine the between-groups difference in thrombin generation parameters, the mean difference in change and 95% CI between treatment groups (rosuvastatin users vs. non-users) was calculated by means of linear regression methods. We performed both unadjusted and age and sex adjusted analysis, because more men were randomized to non-rosuvastatin use and non-rosuvastatin users were slightly older than those who were randomized to rosuvastatin. In a predefined sensitivity analysis, we excluded all participants who reported signs or symptoms of an infection during the study, as infections may affect thrombin generation [32, 33].

Next, we plotted the end of study-expected and the end of study-observed thrombin generation among rosuvastatin users. To do so, we assumed that if patients assigned to rosuvastatin had not received the drug, they would have had the same change in thrombin generation as those assigned to non-statin treatment. Thus, the expected end of study-thrombin generation among rosuvastatin users was estimated by adding the mean change in thrombin values (at each time point of the thrombin generation curve) within non-statin users to the corresponding baseline-thrombin value in rosuvastatin users.

Additionally, we performed a subgroup analysis according to the following potential or established prognostic determinants of recurrent venous thrombosis: male/female sex, unprovoked/provoked first event, deep vein thrombosis or pulmonary embolism, and presence or absence of self-reported cardiovascular risk factors.

A post-hoc analysis was performed to investigate whether the coagulation factors VIII, VII, XI and D-dimer was associated with the effect of rosuvastatin on ETP. For this purpose, we performed linear regression with those coagulation factors entering as independent

variables, along with the randomization groups and sex and age, and ETP entering as dependent variables. All analyses were performed with SPSS version 23.0 (SPSS Inc, Chicago, Ill).

RESULTS

Study population

A total of 255 patients were randomized between December 2012 and December 2016, 131 were assigned to receive rosuvastatin and 124 were allocated to non-statin treatment. Figure 1 shows the trial profile. Two participants allocated to rosuvastatin treatment did not start treatment and another six randomized, three in each study arm, did not complete the study. The thrombin generation assay could not be performed in two patients because of technical issues, who both had been assigned to non-statin treatment. Table 1 presents baseline characteristics in the 245 participants who completed the study; 126 assigned to rosuvastatin and 119 assigned to non-rosuvastatin treatment.

Non-rosuvastatin users were slightly older than rosuvastatin users, mean ages were 58.4 years (range 21 to 80) and 56.8 years (range 19 to 82), respectively. More men were assigned to non-statin treatment, the proportion of men was 54% among rosuvastatin users and 69% among non-users. Other reported exposures, such as body mass index (BMI), type and classification of venous thromboembolism, and presence of cardiovascular risk factors, were balanced at baseline. (Table 1)

Outcomes

Results of all measured thrombin generation parameters are shown in Table 2. ETP increased 7.8% from baseline to end of study in non-statin users (mean change, or intraindividual variability, within non-users 97.22 nM*min; 95%CI 40.92 to 153.53) and decreased 1.9% from baseline to end of study in rosuvastatin users (mean change in rosuvastatin users: -24.94 nM*min; 95%CI -71.81 to 21.93). The mean difference between treatments, after adjustment for age and sex, was -120.24 nM*min (95%CI -192.97 to -47.51). After the exclusion of patients who reported an infection at the end of study, as pre-specified by the study protocol, the age and sex- adjusted mean difference in ETP between treatments was -129.39 nM*min (95%CI -202.29 to -56.49). The mean difference between treatments yielded a treatment effect

of 10.4% (95%CI 4.5 to 16.2%) reduction in ETP by rosuvastatin, when compared with non-statin treatment (Figure 2).

While the thrombin peak increased in both rosuvastatin and non-statin users from baseline to the end of the study, the percentage change was higher for non-users (7.6%) relative to the rosuvastatin users (2.9%). The mean change in thrombin peak was 20.69 nM (95%CI -9.80 to 31.58) for the non-users and 8.41 nM (95%CI -0.86 to 17.69) for the rosuvastatin users, which resulted in a mean difference in change between both treatments, adjusted for age and sex, of -11.88 nM (95%CI -26.11 to 2.35). The mean difference between the treatments yielded a treatment effect of 5.0% (95%CI -0.2 to 10.2%) reduction in thrombin peak by rosuvastatin, when compared with non-statin treatment (Figure 2).

The time to peak decreased 6.4% from baseline to the end of the study in rosuvastatin users (mean change -0.28 min; 95%CI -0.35 to -0.21), and 1.5% in non-statin users (mean change -0.07 min; 95%CI -0.23 to 0.09). The mean difference in these changes between treatments was -0.21 min (95%CI -0.38 to -0.03), which was equivalent to a treatment effect of 4.8% (95%CI 0.9 to 8.5) reduction in time to peak by rosuvastatin, when compared with non-statin treatment (Figure 2). The results were not materially affected by excluding the 8 participants who reported an infection. Changes in lag time and velocity index were not different between treatments (Figure 2).

Figure 3 illustrates the difference between expected and observed thrombin generation in rosuvastatin users by the end of the study.

Supplementary tables 1 to 5 show all measures of thrombin generation parameters in the subgroups of sex, unprovoked or provoked first VTE, deep vein thrombosis or pulmonary embolism, and presence or absence of self-reported cardiovascular risk factors. These subgroup analyses revealed that the decrease in ETP and thrombin peak by rosuvastatin was more pronounced in patients with unprovoked venous thrombosis, pulmonary embolism or cardiovascular risk factors, than in those with provoked venous thrombosis, deep vein thrombosis or without cardiovascular risk factors, respectively (Figure 4). A relative decrease in ETP following rosuvastatin use was also more pronounced in men than in women, while the effects of rosuvastatin on thrombin peak were similar between sexes. Subgroup analysis of the effect of rosuvastatin on other thrombin generation parameters revealed similar results as in the main analysis.

As we have recently reported that treatment with rosuvastatin led to a decrease in the levels of D-dimer and coagulation factors VIII, VII and XI as compared with non-statin in START, we performed a post-hoc analysis to evaluate whether the observed effect of rosuvastatin on thrombin generation could be explained by the levels of these factors at the end of the study. As shown in table 3, the effect of rosuvastatin on thrombin generation was reduced by 33% with factor VII, but not by the other coagulation factors/ D-dimer.

DISCUSSION

In this randomized clinical trial (START), we have shown that treatment with rosuvastatin leads to a relative reduction in thrombin generation potential, decreasing the ETP by 10.4% (adjusted mean difference between treatments -129.39 nM*min) and decreasing the thrombin peak by 5% (adjusted mean difference between treatments -13.69 nM), in comparison with non-statin treatment. Our results confirm previous clinical studies that also demonstrated that statin therapy, either with rosuvastatin [30], simvastatin [34], atorvastatin [35, 36] or cerivastatin [37], affects coagulation factors and thrombin generation.

Additionally, these findings are consistent with previous results from the START trial, in which rosuvastatin treatment was shown to decrease the plasma factor VIII levels by 6% (adjusted mean difference in change between treatments -8.2 IU/dL; 95%CI -13.6 to -2.9), those of FXI by 4% (adjusted mean difference in change between treatments -4.9 IU/dL; 95%CI 9.9 to -0.1), coinciding with a decrease in D-dimer by 3% and factor VII levels by 4% [12]. The results from the START trial point to the same direction of an effect of rosuvastatin on the individual coagulation profile, but the observed decrease in thrombin generation potential was only partially mediated by factor VII and by D-dimer, factor VIII or XI. Since thrombin generation is a product of a synergic combination of multiple coagulation factors [38], [18], it is possible that the mechanism behind the effect of rosuvastatin on decreasing thrombin generation potential relies on the reduction of several coagulation factors, some of them not measured in the START trial. Whether this effect of rosuvastatin on changing the coagulation profile has clinical significance in terms of reducing VTE risk deserves to be addressed in clinical trials aimed to evaluate this question. However, it is possible to speculate on a potential clinical impact of statins on VTE risk if the current findings are evaluated in the light of previous studies. Studies on thrombin generation and VTE risk have demonstrated that both the ETP and thrombin peak are associated with a first VTE [16, 28, 29, 31] and can predict the risk of recurrent VTE [25-27, 30]. A cohort study of 188 patients with VTE [28] described that the

risk of recurrent VTE increased by 25% per 100 nM*min increase in ETP (hazard ratio 1.25 per 100 nM*min increase, 95% CI 1.01 to 1.55). The Austrian Study on Recurrent Venous Thromboembolism (AUREC), which is a cohort study with patients with an unprovoked first episode of VTE, showed that the risk of recurrent VTE increased by 1.4% for each 1% increase in ETP (hazard ratio 1.014 per 1% increase in ETP, 95% CI 1.0 to 1.03; P 0.06) [25]. Another study derived from the AUREC cohort showed that the relative risk of recurrent VTE increased by 4% (relative risk [RR] 1.04; 95% CI, 1.02 to 1.06) for each 10nM increase in thrombin peak [27]. The Vienna Cancer and Thrombosis Study (CATS), a prospective cohort study of patients with cancer, demonstrated that patients who developed VTE had 10% higher thrombin peak at baseline than those without VTE events (peak values 556nM; 95% CI 432 to 677 and 499nM; 95%CI 360 to 603, respectively) [39]. Considering ETP and thrombin peak as surrogate markers of recurrent VTE risk, as described in the aforementioned trials, our results suggest that rosuvastatin has the potential to decrease the risk of recurrent VTE by 14 to 25%. Interestingly, a meta-analysis of observational studies reported that statins reduced the overall risk of recurrent VTE by 27% (RR 0.73; 95% CI, 0.68 to 0.79) [10]. Therefore, our finding that statins are capable of modulating the pro-thrombotic profile in patients after a first VTE episode could be interpreted as statins having the potential to decrease the risk of recurrence.

We also observed that the relative treatment effect of rosuvastatin on ETP was mainly driven by the absence of an increase in this parameter among rosuvastatin users, in contrast to a significant increase in ETP in patients not using statins. This is consistent with a previous observation from this trial demonstrating that the difference in D-dimer levels between both treatment groups was driven by the absence of an increase in D-dimer following rosuvastatin use [12]. As both thrombin generation and D-dimer are markers of hypercoagulability [25, 26], the current results provide further evidence that rosuvastatin may prevent a rebound phenomenon; *i.e.,* a shift to a more procoagulant profile along with increased risk of a recurrence of VTE after the sudden withdrawal of anticoagulant treatment [40, 41]. Preventing such a rebound hypercoagulability may be further beneficial to patients with previous VTE in whom anticoagulation is withdrawn.

It is worth noting that the decrease in ETP and thrombin peak appeared strongest in participants with unprovoked VTE and in those with cardiovascular risk factors. This potential benefit for patients who had unprovoked VTE is interesting because these patients are at high risk of recurrent VTE [2], and anticoagulants may not be prescribed if a patient is considered to be at high risk of anticoagulation-related bleeding [42]. Secondary prevention with statin

therapy may be a convenient alternative treatment, as statins do not increase the risk of bleeding complications [43]. In addition, a benefit among patients with cardiovascular risk factors is noteworthy because most of these patients are already likely to receive statins [44]. Therefore, the possibility of using one single drug to prevent both cardiovascular diseases and VTE could diminish the medication burden associated with the use of several classes of drugs and decrease the risk of adverse effects, thus increasing the changes of treatment efficacy [45].

Although our results point to a decrease in thrombin generation potential by rosuvastatin, not all thrombin generation parameters were modified after the treatment. The lag time and velocity index did not change substantially, while the time to peak decreased in rosuvastatin users, in comparison with non-statin users. Despite the fact that a reduced time to peak may indicate a hypercoagulable state [20], the real significance of this parameter is not known, since it is not associated with the risk of VTE. Conversely, as time to peak is calculated based on the thrombin values, a shortened time to peak may be explained by a relative reduction in ETP and thrombin peak [46]; a similar phenomenon was reported in a previous study, wherein a protraction of the thrombin generation curve lengthened the time to peak [47].

There are some aspects in this study that need to be highlighted. First, the trial was not blind to participants and physicians involved; however, it was considered unlikely that knowledge on the treatment could affect a laboratory surrogate outcome. Second, we previously noticed that the distribution of sex and age after randomization was different between the groups, for which we *a-priori* decided to adjust the analysis for these potential confounding factors [12]. These adjustments did not influence our results. Third, we decided *a-priori* to perform a sensitivity analysis excluding participants who developed an infection during the follow-up due to the possibility of an acute phase reaction affecting the thrombin generation potential, which did not materially change the results. Fourth, although the results from our subgroup analyses suggest that statins may have the strongest potential to decrease thrombin potential in individuals with CV risk factors or unprovoked VTE, these subgroup analyses must be handled with caution as the study was not designed or powered to analyze differences in subgroups [48]. Finally, the assessment of thrombin generation potential is dependent on the assay conditions, which vary according to different laboratory protocols and may affect the clinical interpretation of the results [49]. Besides the potential limitations, the START trial evaluated the effect of rosuvastatin on six coagulation parameters related with the risk of VTE: VWF, factors VIII, VII, XI, D-dimer, ETP and thrombin peak. The values of all parameters were consistently pointing towards a decreased level with rosuvastatin treatment,

as compared with no statin. Altogether, these results confirm that rosuvastatin is capable of affecting several components of coagulation and modifying the coagulation profile of patients with a prior VTE.

We conclude that rosuvastatin use of 20mg/day improves the coagulation profile in patients with VTE by reducing the thrombin generation potential after anticoagulation withdrawal. These results of the START trial suggest that statin therapy might be beneficial in patients at risk of recurrent VTE and provide a clinical rationale for the conduction of a randomized controlled trial to evaluate the effectiveness of rosuvastatin in decreasing the risk of recurrent VTE.

Addendum

F.A.Orsi performed the statistical analyses and drafted the manuscript; J.S. Biedermann performed the statistical analyses and revised the manuscript; M.J.H.A. Kruip, F.J. van der Meer, F.R. Rosendaal, A. van Hylckama Vlieg and F.W.G. Leebeek revised the manuscript; M.H.A. Bos was responsible for the laboratory analyses and revised the manuscript; S.C. Cannegieter designed the analyses and revised the manuscript and W.M. Lijfering was responsible for the START study concept, designed the analyses and revised the manuscript.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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Legends to Figures and Tables

Figure 1. Trial profile. study enrolment, randomization, follow-up and reasons for withdrawal (*one participant admitted to hospital with a diagnosis of acute asthma exacerbation)

Table 1. Baseline characteristics of participants

Table 2. Effects of rosuvastatin on measures of thrombin generation parameters

Table 3. Mean difference in change in thrombin generation parameters between roasuvastatin users and non-users (reference) adjusted for coagulation factors

Figure 2. Relative effect of rosuvastatin treatment on thrombin generation. This figure illustrates the changes in endogenous thrombin potential, from baseline to the end of treatment, compared between rosuvastatin users versus non-statin users.

Figure 3. Thrombin generation curves (A) mean values of thrombin generation over time in non-statin users at baseline and at the end of study. (B) mean values of thrombin generation over time in rosuvastatin users at baseline and at the end of study. (C) expected mean thrombin generation values (if rosuvastatin would not have a treatment effect on thrombin generation) and observed mean thrombin generation values by the end of study in patients receiving rosuvastatin.

Figure 4. Relative effect of rosuvastatin treatment on thrombin generation potential by subgroups. The relative effect of rosuvastatin treatment on endogenous thrombin potential (A) and on thrombin peak (B) in pre-specified subgroups: sex (female/male), type of VTE (DVT/PE), VTE classification (provoked/unprovoked) and presence of cardiovascular (CV) risk factors (no CV risk/CV risk) compared with non-statin treatment.

Reference List

1. ISTH Steering Committee for World Thrombosis Day. Thrombosis: a major contributor to the global disease burden. *J Thromb Haemost*. 2014; 12: 1580-90.

2. Kearon C, Akl EA, Ornelas J, Blaivas A, Jimenez D, Bounameaux H, Huisman M, King CS, Morris TA, Sood N, Stevens SM, Vintch JRE, Wells P, Woller SC, Moores L. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. *Chest*. 2016; 149: 315-52.

3. Kearon C, Akl EA. Duration of anticoagulant therapy for deep vein thrombosis and pulmonary embolism. *Blood*. 2014; 123: 1794-801.

4. Lijfering WM, Biedermann JS, Kruip MJ, Leebeek FW, Rosendaal FR, Cannegieter SC. Can we prevent venous thrombosis with statins: an epidemiologic review into mechanism and clinical utility. *Expert Rev Hematol*. 2016; 9: 1023-30.

5. Kunutsor SK, Seidu S, Khunti K. Statins and primary prevention of venous thromboembolism: a systematic review and meta-analysis. *Lancet Haematol*. 2017; 4: e83-e93.

6. Pai M, Evans NS, Shah SJ, Green D, Cook D, Crowther MA. Statins in the prevention of venous thromboembolism: a meta-analysis of observational studies. *Thromb Res*. 2011; 128: 422-30.

7. Hippisley-Cox J, Coupland C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. *BMJ*. 2010; 340: c2197.

8. Glynn RJ, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Ridker PM. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. *N Engl J Med*. 2009; 360: 1851-61.

9. Rahimi K, Bhala N, Kamphuisen P, Emberson J, Biere-Rafi S, Krane V, Robertson M, Wikstrand J, McMurray J. Effect of statins on venous thromboembolic events: a meta-analysis of published and unpublished evidence from randomised controlled trials. *PLoS Med*. 2012; 9: e1001310.

10. Kunutsor SK, Seidu S, Khunti K. Statins and secondary prevention of venous thromboembolism: pooled analysis of published observational cohort studies. *Eur Heart J*. 2017; 38: 1608-12.

11. Rosendaal FR. Statins and venous thrombosis: a story too good to be true? *PLoS Med*. 2012; 9: e1001311.

12. Biedermann JS, Kruip M, van der Meer FJ, Rosendaal FR, Leebeek FWG, Cannegieter SC, Lijfering WM. Rosuvastatin use improves measures of coagulation in patients with venous thrombosis. *Eur Heart J*. 2018; 39: 1740-7.

13. Adams NB, Lutsey PL, Folsom AR, Herrington DH, Sibley CT, Zakai NA, Ades S, Burke GL, Cushman M. Statin therapy and levels of hemostatic factors in a healthy population: the Multi-Ethnic Study of Atherosclerosis. *J Thromb Haemost*. 2013; 11: 1078-84.

14. Sahebkar A, Serban C, Mikhailidis DP, Undas A, Lip GY, Muntner P, Bittner V, Ray KK, Watts GF, Hovingh GK, Rysz J, Kastelein JJ, Banach M, Lipid, Blood Pressure Meta-analysis Collaboration G. Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials. *Thromb Haemost*. 2015; 114: 546-57.

15. Sahebkar A, Serban C, Ursoniu S, Mikhailidis DP, Undas A, Lip GY, Bittner V, Ray K, Watts GF, Hovingh GK, Rysz J, Kastelein JJ, Banach M, Lipid, Blood Pressure Meta-analysis Collaboration G. The impact of statin therapy on plasma levels of von Willebrand factor antigen. Systematic review and meta-analysis of randomised placebo-controlled trials. *Thromb Haemost*. 2016; 115: 520-32.

16. Segers O, van Oerle R, ten Cate H, Rosing J, Castoldi E. Thrombin generation as an intermediate phenotype for venous thrombosis. *Thromb Haemost*. 2010; 103: 114-22.

17. ten Cate-Hoek AJ, Dielis AW, Spronk HM, van Oerle R, Hamulyak K, Prins MH, ten Cate H. Thrombin generation in patients after acute deep-vein thrombosis. *Thromb Haemost*. 2008; 100: 240-5.

18. Dielis AW, Castoldi E, Spronk HM, van Oerle R, Hamulyak K, Ten Cate H, Rosing J. Coagulation factors and the protein C system as determinants of thrombin generation in a normal population. *J Thromb Haemost*. 2008; 6: 125-31.

19. Ten Cate H. Thrombin generation in clinical conditions. *Thromb Res*. 2012; 129: 367-70.

20. Tripodi A. Thrombin Generation Assay and Its Application in the Clinical Laboratory. *Clin Chem*. 2016; 62: 699-707.

21. van Veen JJ, Gatt A, Makris M. Thrombin generation testing in routine clinical practice: are we there yet? *Br J Haematol*. 2008; 142: 889-903.

22. Brummel-Ziedins K, Vossen CY, Rosendaal FR, Umezaki K, Mann KG. The plasma hemostatic proteome: thrombin generation in healthy individuals. *J Thromb Haemost*. 2005; 3: 1472-81.

23. Morishima Y, Kamisato C. Laboratory measurements of the oral direct factor Xa inhibitor edoxaban: comparison of prothrombin time, activated partial thromboplastin time, and thrombin generation assay. *Am J Clin Pathol*. 2015; 143: 241-7.

24. Spronk HM, Dielis AW, De Smedt E, van Oerle R, Fens D, Prins MH, Hamulyak K, ten Cate H. Assessment of thrombin generation II: Validation of the Calibrated Automated Thrombogram in platelet-poor plasma in a clinical laboratory. *Thromb Haemost*. 2008; 100: 362-4.

25. Eichinger S, Hron G, Kollars M, Kyrle PA. Prediction of recurrent venous thromboembolism by endogenous thrombin potential and D-dimer. *Clin Chem*. 2008; 54: 2042-8.

26. van Hylckama Vlieg A, Baglin CA, Luddington R, MacDonald S, Rosendaal FR, Baglin TP. The risk of a first and a recurrent venous thrombosis associated with an elevated D-dimer level and an elevated thrombin potential: results of the THE-VTE study. *J Thromb Haemost*. 2015; 13: 1642-52.

27. Hron G, Kollars M, Binder BR, Eichinger S, Kyrle PA. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. *JAMA*. 2006; 296: 397-402.

28. Besser M, Baglin C, Luddington R, van Hylckama Vlieg A, Baglin T. High rate of unprovoked recurrent venous thrombosis is associated with high thrombin-generating potential in a prospective cohort study. *J Thromb Haemost*. 2008; 6: 1720-5.

29. Lutsey PL, Folsom AR, Heckbert SR, Cushman M. Peak thrombin generation and subsequent venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE) study. *J Thromb Haemost*. 2009; 7: 1639-48.

30. Tripodi A, Legnani C, Chantarangkul V, Cosmi B, Palareti G, Mannucci PM. High thrombin generation measured in the presence of thrombomodulin is associated with an increased risk of recurrent venous thromboembolism. *J Thromb Haemost*. 2008; 6: 1327-33.

31. van Hylckama Vlieg A, Christiansen SC, Luddington R, Cannegieter SC, Rosendaal FR, Baglin TP. Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence. *Br J Haematol*. 2007; 138: 769-74.

32. Visser MR, Tracy PB, Vercellotti GM, Goodman JL, White JG, Jacob HS. Enhanced thrombin generation and platelet binding on herpes simplex virus-infected endothelium. *Proc Natl Acad Sci U S A*. 1988; 85: 8227-30.

33. Regnault V, Boehlen F, Ozsahin H, Wahl D, de Groot PG, Lecompte T, de Moerloose P. Anti-protein S antibodies following a varicella infection: detection, characterization and influence on thrombin generation. *J Thromb Haemost*. 2005; 3: 1243-9.

34. Szczeklik A, Musial J, Undas A, Gajewski P, Gora P, Swadzba J, Jankowski M. Inhibition of thrombin generation by simvastatin and lack of additive effects of aspirin in patients with marked hypercholesterolemia. *J Am Coll Cardiol*. 1999; 33: 1286-93.

35. Cortellaro M, Cofrancesco E, Arbustini E, Rossi F, Negri A, Tremoli E, Gabrielli L, Camera M. Atorvastatin and thrombogenicity of the carotid atherosclerotic plaque: the ATROCAP study. *Thromb Haemost*. 2002; 88: 41-7.

36. Macchia A, Laffaye N, Comignani PD, Cornejo Pucci E, Igarzabal C, Scazziota AS, Herrera L, Mariani JA, Bragagnolo JC, Catalano H, Tognoni G, Nicolucci A. Statins but not aspirin reduce thrombotic risk assessed by thrombin generation in diabetic patients without cardiovascular events: the RATIONAL trial. *PLoS One*. 2012; 7: e32894.

37. Ural AU, Yilmaz MI, Avcu F, Yalcin A. Treatment with cerivastatin in primary mixed hyperlipidemia induces changes in platelet aggregation and coagulation system components. *Int J Hematol*. 2002; 76: 279-83.

38. Mann KG, Butenas S, Brummel K. The dynamics of thrombin formation. *Arterioscler Thromb Vasc Biol*. 2003; 23: 17-25.

39. Ay C, Pabinger I. Predictive potential of haemostatic biomarkers for venous thromboembolism in cancer patients. *Thromb Res*. 2012; 129 Suppl 1: S6-9.

40. Martinez C, Katholing A, Folkerts K, Cohen AT. Risk of recurrent venous thromboembolism after discontinuation of vitamin K antagonist treatment: a nested case-control study. *J Thromb Haemost*. 2016; 14: 1374-83.

41. Palareti G, Legnani C, Guazzaloca G, Frascaro M, Grauso F, De Rosa F, Fortunato G, Coccheri S. Activation of blood coagulation after abrupt or stepwise withdrawal of oral anticoagulants--a prospective study. *Thromb Haemost*. 1994; 72: 222-6.

42. Konstantinides SV, Torbicki A, Agnelli G, Danchin N, Fitzmaurice D, Galie N, Gibbs JS, Huisman MV, Humbert M, Kucher N, Lang I, Lankeit M, Lekakis J, Maack C, Mayer E, Meneveau N, Perrier A, Pruszczyk P, Rasmussen LH, Schindler TH, Svitil P, Vonk Noordegraaf A, Zamorano JL, Zompatori M, Task Force for the D, Management of Acute Pulmonary Embolism of the European Society of C. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J*. 2014; 35: 3033-69, 69a-69k.

43. Undas A, Brummel-Ziedins KE, Mann KG. Anticoagulant effects of statins and their clinical implications. *Thromb Haemost*. 2014; 111: 392-400.

44. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC, Jr., Watson K, Wilson PW, American College of Cardiology/American Heart Association Task Force on Practice G. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014; 63: 2889-934.

45. Chapman RH, Benner JS, Petrilla AA, Tierce JC, Collins SR, Battleman DS, Schwartz JS. Predictors of adherence with antihypertensive and lipid-lowering therapy. *Arch Intern Med*. 2005; 165: 1147-52.

46. Hemker HC, Kremers R. Data management in thrombin generation. *Thromb Res*. 2013; 131: 3-11.

47. Cohen H, Hunt BJ, Efthymiou M, Arachchillage DRJ, Mackie IJ, Clawson S, Sylvestre Y, Machin SJ, Bertolaccini ML, Ruiz-Castellano M, Muirhead N, Dore CJ, Khamashta M, Isenberg DA, Investigators RT. Rivaroxaban versus warfarin to treat patients with thrombotic antiphospholipid syndrome, with or without systemic lupus erythematosus (RAPS): a randomised, controlled, open-label, phase 2/3, non-inferiority trial. *Lancet Haematology*. 2016; 3: E426-E36.

48. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Obstet Gynecol*. 2010; 115: 1063-70.

49. Kintigh J, Monagle P, Ignjatovic V. A review of commercially available thrombin generation assays. *Res Pract Thromb Haemost*. 2018; 2: 42-8.

Table 1. Baseline characteristics of participants

	Rosuvastatin users (n=126)		Non-users (n=119*)	
General				
Age (years)	56.8	(19-82)	58.4	(21-80)
Male	68	(54)	82	(69)
Body mass index (kg/m2)	27.4	(19.2-43.5)	27.7	(17.2-43.3)
Aspirin use	5	(4)	5	(4)
Venous thrombosis characteristics				
Type of venous thromboembolism				
Deep vein thrombosis	72	(57)	64	(54)
Pulmonary embolism	54	(43)	55	(46)
Unprovoked	57	(45)	62	(52)
Provoked, by	69	(55)	57	(48)
Surgery/ Trauma/ Immobilization	32	(25)	30	(25)
Travel > 4 hrs	22	(18)	14	(12)
Estrogen use (% in women)	24	(41)	14	(38)
Pregnancy/ puerperium (% in women)	0	(0)	2	(5)
Malignancy	2	(2)	8	(7)
Recurrent venous thrombosis	10	(8)	8	(7)
Cardiovascular risk factors				
Cardiovascular risk	89	(71)	94	(78)
Current smoking	18	(14)	16	(13)
Hypertension	24	(19)	20	(17)
Diabetes	3	(2)	0	(0)
Overweight[#]	54	(43)	51	(43)
Obesity[##]	29	(23)	34	(28)

Continous variables denoted as mean (range), categorical variables as number (%)
*technical issued in 2 non-users
[#] Overweight was defined as body mass index (BMI) between 25 and 30kg/m2
[##] Obesity was defined as BMI above 30kg/m2

Table 2. Effects of rosuvastatin on measures of thrombin generation parameters

	Mean levels (SD)		Mean*	Mean difference‡	Mean^	Mean difference#
	Baseline	End of study	change (95% CI)	in change (95% CI)	change (95% CI)	in change (95% CI)
THROMBIN GENERATION						
ETP (nM*min)						
Non users	1245.01 (321.47)	1343.85 (290.17)	97.22 (40.92, 153.53)	Reference	94.62 (37.78, 151.46)	Reference
Rosuvastatin users	1284.04 (263.97)	1259.10 (205.37)	-24.94 (-71.81, 21.93)	-120.24 (-192.97, -47.51)	-38.49 (-85.19, 8.21)	-129.39 (-202.29, -56.49)
Thrombin Peak (nM)						
Non users	273.33 (62.09)	294.47 (52.32)	20.69 (9.80, 31.58)	Reference	20.39 (9.42, 31.37)	Reference
Rosuvastatin users	288.86 (62.68)	297.27 (52.25)	8.41 (-0.86, 17.69)	-11.88 (-26.11, 2.35)	5.99 (-3.31, 15.29)	-13.69 (-27.98, 0.60)
Lag Time (min)						
Non users	2.23 (0.49)	2.19 (0.72)	-0.04 (-0.16, 0.08)	Reference	-0.04 (-0.17, 0.08)	Reference
Rosuvastatin users	2.16 (0.43)	2.05 (0.38)	-0.11 (-0.15, -0.07)	-0.07 (-0.20, 0.05)	-0.12 (-0.16, -0.08)	-0.08 (-0.21, 0.05)
Time to peak (min)						
Non users	4.55 (0.89)	4.48 (1.06)	-0.07 (-0.23, 0.09)	Reference	-0.06 (-0.22, 0.10)	Reference
Rosuvastatin users	4.37 (0.77)	4.09 (0.71)	-0.28 (-0.35, -0.21)	-0.21 (-0.38, -0.03)	-0.28 (-0.35, -0.21)	-0.22 (-0.39, -0.04)
Velocity Index						
Non users	126.68 (47.07)	137.39 (43.90)	10.37 (3.64, 17.09)	Reference	9.96 (3.15, 16.77)	Reference
Rosuvastatin users	140.33 (57.35)	154.69 (50.87)	14.36 (7.38, 21.34)	4.41 (-5.35, 14.17)	12.52 (5.60, 19.44)	3.07 (-6.66, 12.80)

Abbreviations: ETP, endogenous thrombin potential. * Paired analysis. ‡ Between comparison analysis, adjusted for age and sex. ^ Paired analysis to eight participants who reported an infection at time of end of study excluded. # Between comparison analysis, adjusted for age and sex to 8 participants who reported an infection at time of end of study excluded.

Table 3. Mean difference in endogenous thrombin potential between rosuvastatin users and non-users (reference) at the end of the study, adjusted for coagulation factors

	Mean difference (95% CI)‡		
ETP (nM*min) at the end of the study			
No coagulation factor	-89.46	(-153.18,	-25.74)
+ factorVIII	-87.66	(-148.43,	-26.89)
+factorXI	-73.13	(-133.58,	-12.68)
+factorVII	-59.93	(-120.02,	0.17)
+DD	-87.20	(-151.41,	-22.99)
+factors VIII, XI, VII, DD	-54.98	(-111.99,	2.03)

‡ comparison between rosuvastatin treatment and no treatment at the end of the study, adjusted for age and sex. DD=D dimer CI= confidence interval

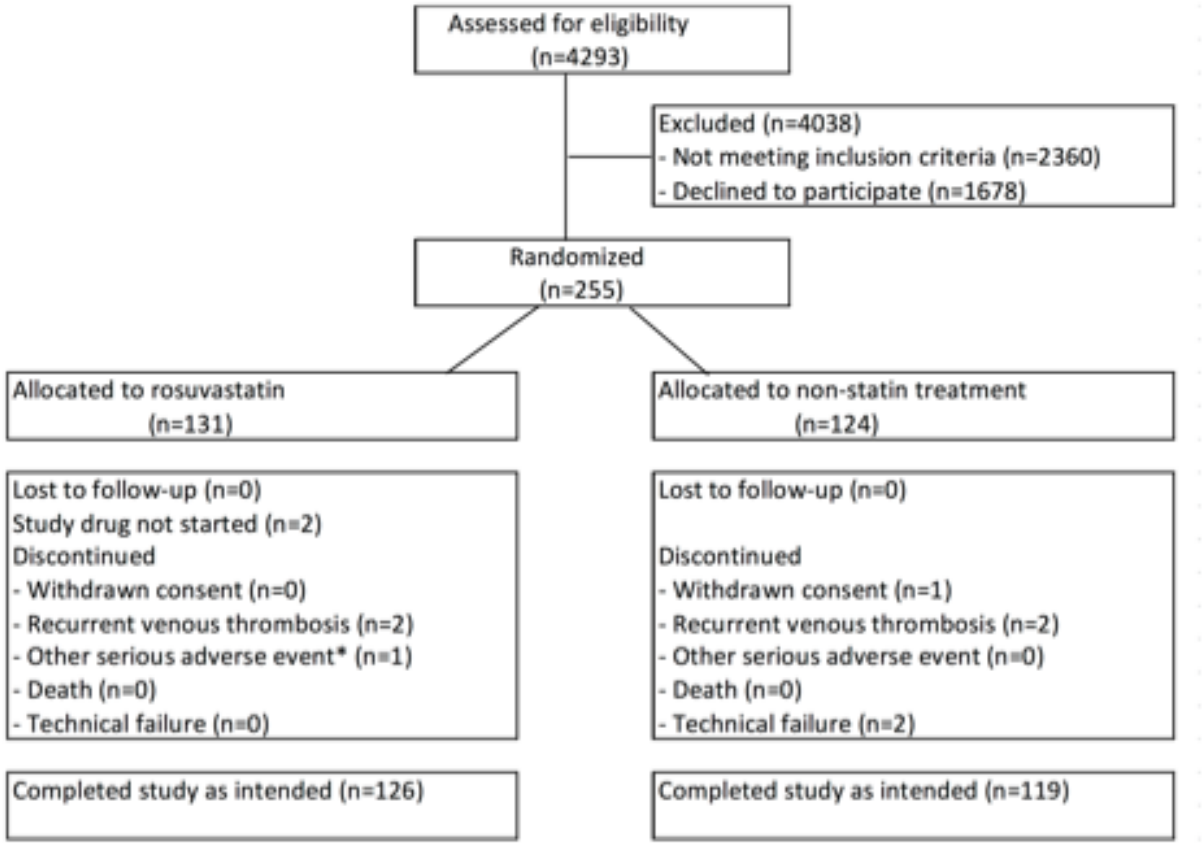


Figure 1. Trial profile. study enrolment, randomization, follow-up and reasons for withdrawal (*one participant admitted to hospital with a diagnosis of acute asthma exacerbation)

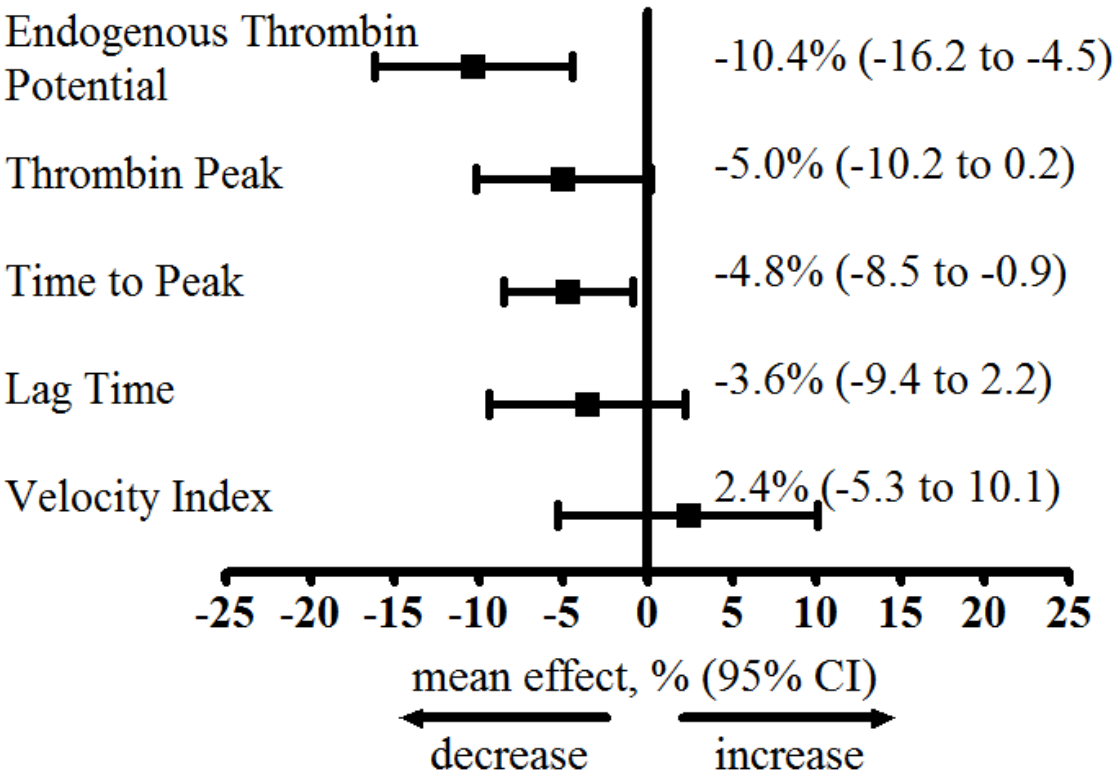


Figure 2. Relative effect of rosuvastatin treatment on thrombin generation. This figure illustrates the changes in endogenous thrombin potential, from baseline to the end of treatment, compared between rosuvastatin users versus non-statin users.

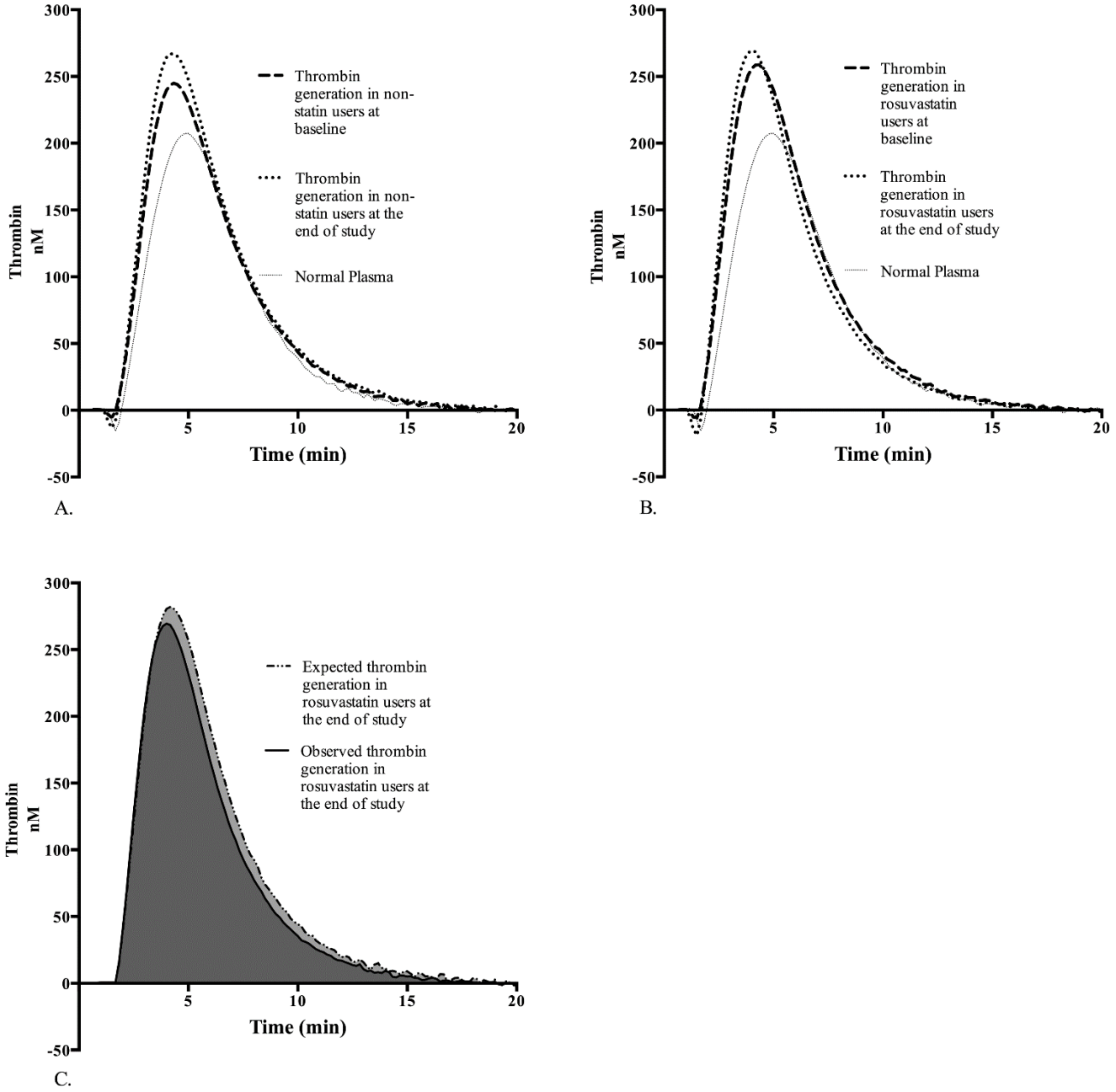
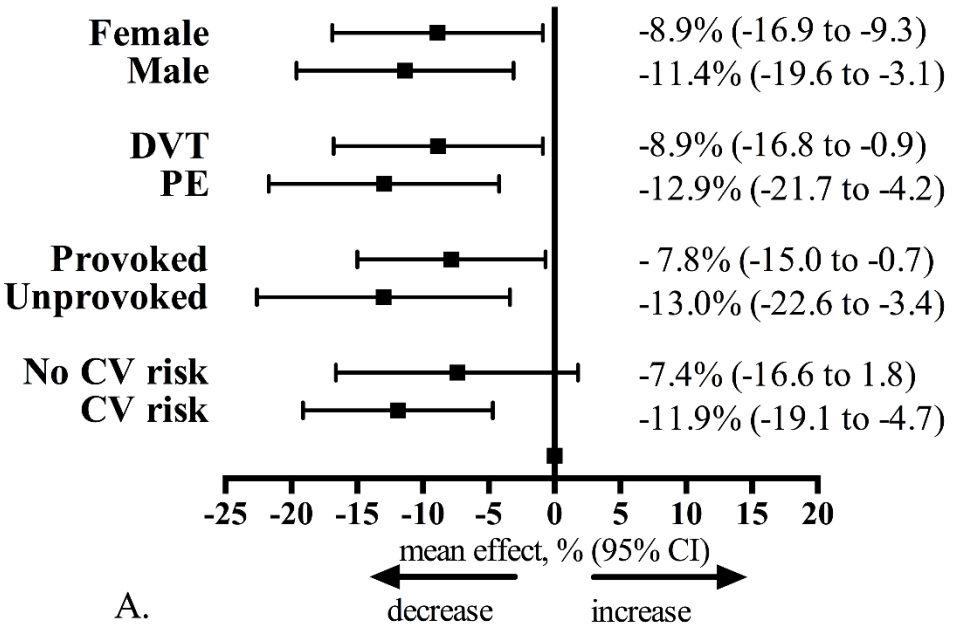


Figure 3. Thrombin generation curves (A) mean values of thrombin generation over time in non-statin users at baseline and at the end of study. (B) mean values of thrombin generation over time in rosuvastatin users at baseline and at the end of study. (C) expected mean thrombin generation values (if rosuvastatin would not have a treatment effect on thrombin generation) and observed mean thrombin generation values by the end of study in patients receiving rosuvastatin.



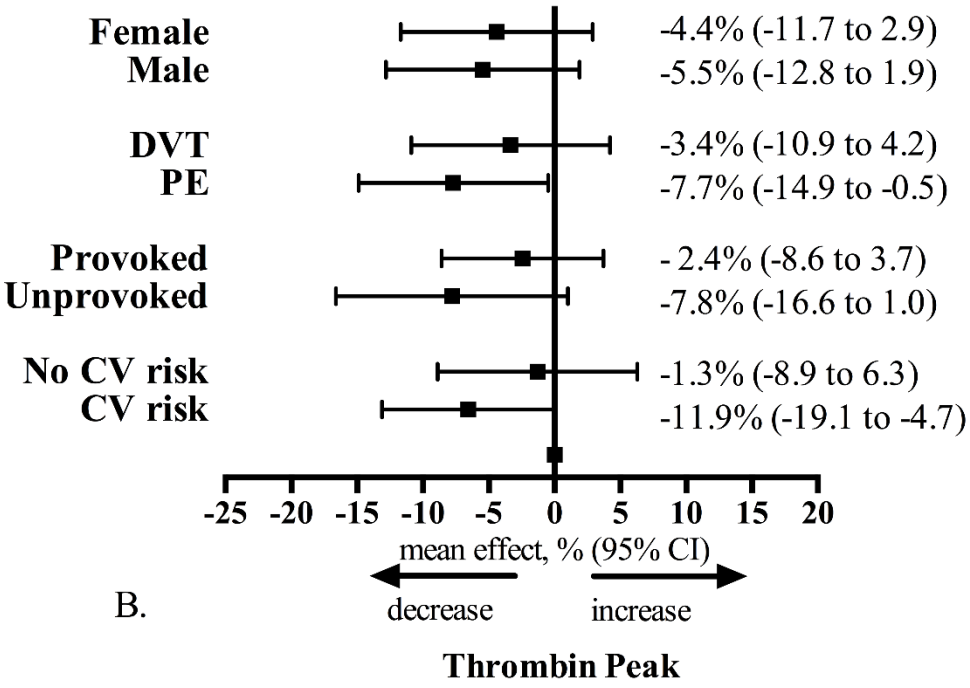


Figure 4. Relative effect of rosuvastatin treatment on thrombin generation potential by subgroups. The relative effect of rosuvastatin treatment on endogenous thrombin potential (A) and on thrombin peak (B) in pre-specified subgroups: sex (female/male), type of VTE (DVT/PE), VTE classification (provoked/unprovoked) and presence of cardiovascular (CV) risk factors (no CV risk/CV risk) compared with non-statin treatment.