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Rosuvastatin use increases plasma fibrinolytic potential: a randomised clinical trial

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Abstract

We conducted a study to assess the effect of rosuvastatin use on fibrinolysis in patients with previous venous thromboembolism (VTE). This was a *post hoc* analysis within the STAtins Reduce Thrombophilia (START) study (NCT01613794). Plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, plasminogen activator inhibitor-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) were measured before and after four weeks of rosuvastatin or no treatment in participants with prior confirmed VTE, after ending anticoagulant therapy. In the non-rosuvastatin group (n = 121), plasma fibrinolytic potential and individual fibrinolysis parameters did not change at the end of the study versus the baseline, whereas in the rosuvastatin group (n = 126), plasma fibrinolytic potential increased: the mean clot lysis time decreased by 8·75 min (95% CI −13·8 to −3·72), and plasmin inhibitor levels and TAFI activity were lower at the end of the study (−0·05 U/ml; 95% CI −0·07 to −0·02 and −4·77%; 95% CI −6·81 to −2·73, respectively). PAI-1 levels did not change and fibrinogen levels were 0·17 g/l (95% CI 0·04–0·29) higher. In participants with prior VTE, rosuvastatin use led to an increased fibrinolytic potential compared with non-statin use. Our findings support the need for further studies on the possible role for statins in the secondary prevention of VTE.

Keywords: venous thromboembolism, fibrinolysis, blood coagulation tests, fibrin clot lysis time, HMG-CoA reductase inhibitors.

HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitors, more commonly known as statins, exert cardiovascular protective effects which are independent of LDL-cholesterol lowering, including antithrombotic effects.[1] A meta-analysis of 36 studies, including 23 randomised studies, showed a 15% risk reduction of statin use on first venous thromboembolism (VTE) events compared to placebo or no treatment.[2] Antithrombotic effects seem to be most robust for rosuvastatin.[3] However, the exact mechanism behind the antithrombotic effects of statins is not completely unraveled.[4,5] The fibrinolytic system, which plays an important role in dissolving thrombi, is likely to contribute to the antithrombotic effects. In two large observational studies, hypofibrinolytic activity has been linked to an increased risk of VTE.[6,7] Also, statin use has been associated with lower levels of D-dimer and other fibrin degradation products (FDPs).[8] During fibrinolysis, the inactive proenzyme plasminogen is converted by tissue plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA) to the active enzyme plasmin.[9,10] Several inhibitors of fibrinolysis are known, such as plasminogen activator inhibitor-1 (PAI-1), which can inhibit these converting enzymes. The activated plasmin degrades fibrin into FDPs, and is inhibited by plasmin inhibitor. Additionally, thrombin converts fibrinogen into fibrin, but also activates thrombin-activatable fibrinolysis inhibitor (TAFI). A better understanding of the exact effect of statins on fibrinolysis will help in determining the position of statins in the treatment of prevention of (recurrent) VTE without increased risk of haemorrhage.

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To test the effects of rosuvastatin use on fibrinolysis, we performed a *post hoc* analysis of the START (STAtins Reduce Thrombophilia) study. Plasma fibrinolytic potential as well as fibrinogen, plasmin inhibitor (also called α2-antiplasmin), PAI-1 and TAFI were determined in blood samples before and after four weeks of rosuvastatin or no treatment in a population suspected to be hypercoagulable. Notably, D-dimer levels have been measured and analysed previously.[11]

Materials and methods

Study design

This was a *post hoc* analysis within the STAtins Reduce Thrombophilia (START) study, a collaboration of three anticoagulation clinics in the Netherlands (Star-shl in Rotterdam, the Leiden Anticoagulation Clinic and Atalmedial, Hoofddorp), of which the design was previously described (clinical trials.gov NCT01613794).[11] This study was performed in accordance with the Declaration of Helsinki, all participants gave written informed consent and the study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands. The START trial was performed to evaluate the antithrombotic effects of statins.

Participants were included if they were 18 years or older with a prior (initial or recurrent) confirmed symptomatic proximal deep vein thrombosis or pulmonary embolism. Also, they should have stopped their vitamin K antagonist treatment for one month (to allow anticoagulant drugs to wear off), as decided by their treating physician. Before inclusion, we did not check if the ultrasound or CTPA was negative for VTE. Reasons for exclusion were current use of statins or other lipid lowering drugs, or contraindications for rosuvastatin use. All those participants included were randomised to 28 days of treatment with rosuvastatin 20 mg, or no study medication. Blood samples were collected during the randomisation visit and at the end of the study period.

Measurements

Blood sampling by venipuncture was performed using the Vacutainer system (Becton Dickinson), containing sodium citrate (final concentration 0·109 mol/l). Participants' blood was drawn between 8:00 a.m. and 3:00 p.m., and at the same hour of the day at the end of the study for logistical reasons, but also because of the circadian rhythm of especially clot lysis time and PAI-1.[12–14] Within 3 h, blood samples were centrifuged at 2500 ***g*** for 15 min at 18°C and stored afterwards at −80°C in our biobank.

To study the plasma fibrinolytic potential, the plasma clot lysis time was measured by experienced technicians as described previously.[14] Platelet-poor plasma was diluted 1·7 times in a buffer (25 mmol/l Hepes, 137 mmol/l sodium chloride, 3·5 mmol/l potassium chloride, 1% (w/v) BSA, pH 7·4) at room temperature, and the diluted plasma (85 µl) was added to wells of a microtitre plate containing 15 µl of a reaction mixture. The reaction mixture contained the following components (with the final concentrations in the assay): tissue factor (TF, Innovin, 1000 times diluted, Dade Behring, Marburg, Germany), calcium chloride (17 mmol/l), t-PA (30 ng/ml, Actilyse, Boehringer Ingelheim, Ingelheim am Rhein, Germany) and phospholipid vesicles (10 µmol/l, Rossix, Mölndal, Sweden). After mixing the diluted plasma with the reaction mixture on a plate shaker, each well was covered with 50 µl paraffin oil (Merck, Darmstadt, Germany) and the microtitre plate was placed into the pre-heated chamber of the microplate reader (Victor™, PerkinElmer, Waltham, MA, USA). The optical density at 405 nm was measured every minute for 300 min at 37°C. The clot lysis time was measured in duplicate (time from the midpoint of minimum turbidity to maximum turbidity, to the midpoint of maximum turbidity to minimum turbidity). These midpoints were calculated using the Shiny app.[15,16] The intra- and inter-assay variation coefficients from our laboratory were previously shown to be 3·5% and 6·5–8%, respectively.[14,17] PAI-1 activity was determined using a TriniLIZE PAI-1 activity bio-immunoassay (BIA), according to the instructions of the manufacturer (Tcoag, Wicklow, Ireland). Fibrinogen levels (Thrombin Reagent, Siemens) were measured using the von Clauss method, plasmin inhibitor levels using a chromogenic assay with reported units converted from % to U/ml (Stachrom, Stago,100% = 1 U/ml) and TAFI activity using the Pefakit® chromogenic assay in plasma (Pentapharm, Aesch, Switzerland) on a Sysmex CS5100 coagulation analyser (Siemens Healthcare Diagnostics B.V.). In this Pefakit assay, the amounts of TAFI in each plasma sample were activated by exogenous thrombin and thrombomodulin. These test results are therefore independent of the coagulation factors in the sample.

Study aim and endpoints

The primary aim of the present study was to assess the effect of rosuvastatin use on fibrinolysis and fibrinolysis parameters. The secondary aim was to evaluate the impact of each individual fibrinolysis parameter on overall plasma fibrinolytic potential after rosuvastatin use.

The primary endpoints of this study were plasma fibrinolytic potential and levels of fibrinogen, plasmin inhibitor, PAI-1 and TAFI before and after rosuvastatin and non-rosuvastatin use. The secondary endpoints of this study were the regression coefficient and the explained variance of the individual fibrinolysis parameters on a change in the plasma fibrinolytic potential after rosuvastatin use.

Data analysis

The general characteristics of the participants are reported as means and ranges. For the primary endpoint, mean levels with 95% Confidence Interval (CI) of the plasma fibrinolytic potential and fibrinolysis parameters were calculated at the time of randomisation and at the end of the study period. All the

parameters were normally distributed and we compared the mean values obtained at baseline and after four weeks of treatment. Because more men were randomised to non-rosuvastatin use and participants in this non-rosuvastatin group were older compared to the rosuvastatin users, we decided *a priori* to additionally perform an adjusted analysis for sex and age by methods of linear regression.

We furthermore prespecified a subgroup analysis in the group that did not report any signs or symptoms of an infection during the study, because inflammation leads to a procoagulant state and consequently increased fibrinolysis.[18,19] Additionally, we performed a subgroup analysis comparing participants after an unprovoked or provoked first VTE, because an etiology including hypercoagulability and recurrence rate of VTE is expected to be different among these groups.[20] A two-sided *P*-value of 0·05 or lower was considered to indicate statistical significance.

For the secondary endpoint, results of the individual participants on change in plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, PAI-1 and TAFI were standardised by calculating *Z*-scores in order to compare the relative strength of the various fibrinolysis parameters on plasma fibrinolytic potential. This *Z*-score is calculated for an observation in a participant as the number of standard deviations (SD) from the mean. To determine the relative impact on change of the various fibrinolysis parameters on the change in the plasma fibrinolytic potential, simple and also multiple linear regression analyses were performed in the rosuvastatin group. The standardised regression coefficient for a fibrinolysis parameter indicates the increase or decrease in SDs of the plasma fibrinolytic potential, when that particular fibrinolysis parameter increases per SD and all other variables in the model are unchanged. The R^2 was calculated, denoting (when multiplied by 100%) the explained variation of change in plasma fibrinolytic potential in rosuvastatin users by the individual, or the combined change in fibrinolysis parameters. All data was analysed using 'IBM SPSS Statistics for Windows, version 25'. Reporting of this study is in accordance with the CONSORT statement and the broader EQUATOR guidelines.[21]

Results

Study population

Between December 2012 and December 2016, a total of 255 participants were randomised: 131 assigned to the rosuvastatin group, and 124 to the non-rosuvastatin group. From 126 participants of the rosuvastatin group, and from 121 participants of the non-rosuvastatin group, blood samples were available for analysis of plasma fibrinolytic potential, fibrinogen, plasmin inhibitor, PAI-1 and TAFI. In one participant from the rosuvastatin group and in two participants from the non-rosuvastatin group, PAI-1 levels could not be measured due to technological failure. Mean age was lower in rosuvastatin users (57 years, range 19–82) compared to non-rosuvastatin users (59 years, range 21–81) and the percentage of male participants was lower in rosuvastatin users (54%) compared to non-rosuvastatin users (69%) Table 1. Other baseline characteristics were balanced. In the rosuvastatin group, mean cholesterol levels were reduced by 1·96 mmol/l (95% CI 1·83–2·09) compared to 0·19 mmol/l (95% CI 0·10–0·27) in the non-rosuvastatin group, indicating good adherence to the rosuvastatin treatment.

The effect of rosuvastatin use on fibrinolysis

We found that in the rosuvastatin group, clot lysis time as indicator of the plasma fibrinolytic potential decreased by 8·75 min (95% CI −13·8 to −3·72), mean fibrinogen levels were 0·17 g/l higher (95% CI 0·04–0·29), plasmin inhibitor levels were 0·05 U/ml lower (95% CI −0·07 to −0·02) and TAFI activity was 4·77% lower (95% CI −6·81 to −2·73) at the end of study as compared with baseline, but PAI-1 levels did not differ (mean change −1·01 IU/ml; 95% CI −3·90 to 1·89). (Fig 1 and Table S1). In the non-rosuvastatin group, plasma fibrinolytic potential and individual fibrinolysis parameters did not change during follow-up (mean change clot lysis time −3·65 min, 95% CI −10·7 to 3·39). When we compared the change in plasma fibrinolytic potential during follow-up between the rosuvastatin and the non-rosuvastin group, the change in plasma fibrinolytic potential tended to be less for rosuvastatin users than for non-rosuvastatin users

Table 1. Baseline characteristics.

	Rosuvastatin-users (n = 126)	Non-rosuvastatin users (n = 121)
Age (years)	57 (19–82)	59 (21–81)
Male sex	68 (54)	84 (69)
Body mass index (kg/m^2)	27·4 (19·2–43·5)	27·7 (17·2–43·2)
Current smoking	18 (14)	17 (14)
Hypertension	24 (19)	21 (17)
Diabetes	3 (2)	0 (0)
Baseline cholesterol (mmol/l)	5·61 (2·95–8·99)	5·59 (3·33–7·89)
Recurrent venous thrombosis	10 (8)	8 (7)
Unprovoked venous thromboembolism	57 (45)	64 (53)
Provoked venous thromboembolism	69 (55)	57 (47)
Provoked by		
Surgery/trauma/ immobilisation	32 (25)	31 (26)
Travel >4 h	22 (18)	14 (12)
Oestrogen use (% in women)	24 (41)	14 (38)
Pregnancy/puerperium (% in women)	0 (0)	2 (5)
Malignancy	2 (2)	8 (7)

Categorical variables are denoted as n (%) and continuous variables as mean (range).

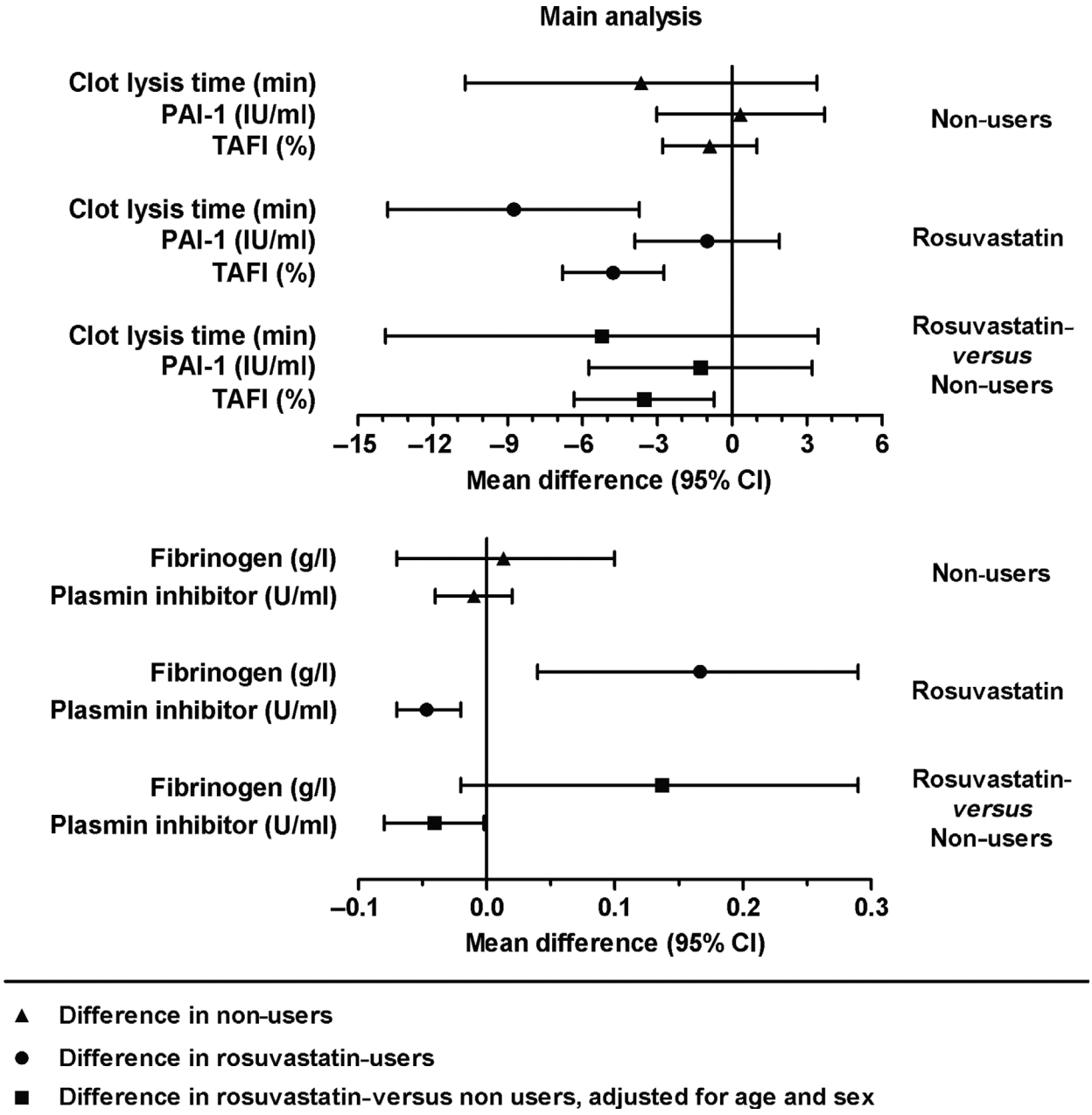

Figure 1. Effects of rosuvastatin on measures of fibrinolysis.

after adjustment for sex and age (−5·79 min, 95% CI −14·5 to 2·91) (Fig 1 and Table S1). For the fibrinolysis parameters, the plasmin inhibitor levels and TAFI concentration were both lower at the end of the study in rosuvastatin users compared to non-rosuvastatin users (−0·04 U/ml, 95% CI −0·08 to −0·003 and −3·78%,95% CI −6·63 to −0·92, respectively). The mean change in fibrinogen and PAI-1 levels did not differ between groups.

In the subgroup analysis of patients who did not report any symptoms or signs of infection (n = 239), results in both groups were similar to the main analysis. When we compared results of participants after an unprovoked or provoked VTE, the mean change in plasma fibrinolytic potential appeared larger in the group of rosuvastatin users after a provoked VTE as compared to the unprovoked group (−11·3 min; 95% CI −19·0 to −3·59, compared to −5·67 min; 95% CI −11·9 to 0·57). For fibrinogen and TAFI, change in levels or concentration at the end of the study in rosuvastatin users was also more evident in the subgroup after provoked VTE (Table S2).

Table 2. Mean impact in change in plasma fibrinolytic potential with one SD increase in fibrinolytic parameter between baseline and end of study in rosuvastatin users.

	Simple model*		Multiple model†	
	β (95%CI)	R^2	β (95%CI)	R^2
Change in fibrinogen (g/l)	−0·02 (−0·20 to 0·16)	0·00	−0·04 (−0·22 to 0·14)	0·05
Change in plasmin inhibitor (U/ml)	0·18 (0·002 to 0·35)	0·03	0·18 (0·003 to 0·36)	
Change in plasminogen activator inhibitor-1(PAI-1) (IU/ml)	0·001 (−0·18 to 0·18)	0·00	0·04 (−0·14 to 0·21)	
Change in thrombin activatable fibrinolysis inhibitor (TAFI) (%)	−0·13 (−0·31 to 0·04)	0·02	−1·26 (−0·30 to 0·05)	

SD, standard deviation; CI, confidence interval.

*In each of the four different single models, plasma fibrinolytic potential was the dependent variable, and only one of the fibrinolytic parameters was the independent variable.

†Plasma fibrinolytic potential was the dependent variable and all four fibrinolytic parameters simultaneously were independent variables.

The impact of individual fibrinolysis parameters on change in plasma fibrinolytic potential in rosuvastatin users

Among the fibrinolysis parameters, the only change in plasmin inhibitor was associated with change in plasma fibrinolytic potential in rosuvastatin users (Table 2). The regression coefficient of this association was 0·18 (95% CI 0·002–0·352). The explained variance in this model was 3% (R^2 0·03). Including all the fibrinolytic parameters in a multiple regression model increased the explained variance of change in plasma fibrinolytic potential to 5% (R^2 0·05).

Discussion

Our randomised study in those participants with prior VTE who stopped anticoagulant treatment showed that four weeks of 20 mg/day rosuvastatin use led to an increased plasma fibrinolytic potential, whereas non-use did not lead to increased fibrinolysis. The difference in change in fibrinolytic potential in users versus non-users did, however, not reach statistical significance. Among the fibrinolysis parameters, plasmin inhibitor and TAFI decreased during the use of rosuvastatin, and the changes in rosuvastatin users compared to non-users also differed significantly. Fibrinogen levels were increased after four weeks of rosuvastatin use, but PAI-1 levels did not change (Figure S1). The variance in change in plasma fibrinolytic potential between the baseline and the end of the study was explained for 3% by the change in plasmin inhibitor and not by other individual fibrinolysis parameters. Notably, there is a relationship between clot formation and lysis,[22] suggesting that increased fibrinolytic potential by rosuvastatin could be secondary to decreased coagulation. However, we also found decreased levels of TAFI and plasmin inhibitor, which support a direct pro-fibrinolytic effect of rosuvastatin. The changes in fibrinolytic potential and the individual fibrinolysis parameters in rosuvastatin users could be clinically relevant, though this should be investigated in prospective larger studies.

This is the first study in participants with prior VTE, showing that 28 days use of rosuvastatin increases plasma fibrinolytic potential and lowers plasma levels of TAFI and plasmin inhibitor. Direct effects on plasma fibrinolytic potential were also measured earlier after a very short use (three days) of atorvastatin in a non-randomised study.[23] Another before-after study evaluated plasma fibrinolytic potential after simvastatin therapy, and presented a mean shortening in clot lysis time of 12·9 min after a three-month treatment with simvastatin 40 mg.[24] Reduced plasma fibrinolytic potential (i.e. longer clot lysis times) has been shown to be associated with a higher risk of VTE.[3,6,7] Our findings corroborate fibrinolytic activity being increased by rosuvastatin and that it might therefore decrease the risk of recurrent VTE.

Results on the individual fibrinolytic parameters are primarily in line with the results on plasma fibrinolytic potential. Specifically, lower levels of plasmin inhibitor and TAFI will lead to reduced inhibition of fibrinolysis, consequently resulting in shorter clot lysis times and therefore increased global fibrinolytic potential. Decreased TAFI levels were also previously observed in 35 patients with hyperlipidaemia after eight weeks of treatment with simvastatin and in another study in which 44 patients with hypercholesterolaemia were treated with atorvastatin.[25,26] Studies on plasmin inhibitor are scarce with only one comparable study on 24 patients with primary dyslipidaemia treated with simvastatin, and 18 patients treated with pravastatin for 12 weeks.[27] In the simvastatin group, plasmin inhibitor did not change after treatment, but in the pravastatin group the levels decreased. Remarkably, in our study PAI-1 levels did not change after rosuvastatin use even though we expected this fibrinolytic parameter to have a high impact on the plasma fibrinolytic potential.[28] Fibrinogen levels were also unexpectedly higher after rosuvastatin use in our study. Since fibrinogen is associated with pro-inflammatory and pro-coagulants effects, one would expect that statins would decrease fibrinogen levels. In a meta-analysis of 14 other studies, including patients with high cholesterol or stable coronary disease, no effect of statin treatment on PAI-1 levels and fibrinogen was found either.[29] For rosuvastatin specifically, a reduction in fibrinogen levels after six months of treatment was shown in 24 patients with rheumatoid arthritis and in 100 patients with metabolic

syndrome, but in another study, fibrinogen levels did not change after three months in 17 patients with type 2 diabetes.[30–32] D-dimer levels, a FDP generated in the blood clot during fibrinolysis *in vivo*, were already measured in the START study. Measured D-dimer levels were higher at the end of the study in non-rosuvastatin users, but remained unchanged in the rosuvastatin group.[11] After withdrawal of anticoagulant treatment, D-dimer levels normally increase, which is called the rebound phenomenon.[33,34] D-dimer levels are the result of the amount of available fibrin and the fibrinolytic potential. Rosuvastatin is expected to lower fibrin levels by reducing clotting factors and by its anti-inflammatory effects, and to increase the fibrinolytic potential.[9,11,35] Because of the lack of increase in D-dimer levels in rosuvastatin users during follow-up, we expect that the effect of lowering fibrin levels is stronger than the effect of increasing fibrinolytic potential.

Interestingly, in the subgroup analysis results on increased plasma fibrinolytic potential, decreased plasmin inhibitor and TAFI seemed to be more robust after a provoked VTE than unprovoked VTE. Karasu *et al.*, on the other hand, reported that provoked VTE showed a 1·5-fold increased risk of VTE in the presence of hypofibrinolysis, whereas for unprovoked VTE, hypofibrinolysis was associated with a higher 2·1-fold increased risk.[36] This contradiction might be explained by a different or stronger additional impact of rosuvastatin use on hypofibrinolysis in patients after provoked VTE rather than unprovoked VTE, or a reduced impact in our study for performing this subanalysis.

To evaluate the impact of each individual fibrinolysis parameter on overall plasma fibrinolytic potential after rosuvastatin use, we performed an additional regression analysis. Results suggest minor impact of change of individual fibrinolysis parameters with only a small significant impact of plasmin inhibitor. This was unexpected, because in another study, TAFI and PAI-1 levels explained the majority of the variance in clot lysis time.[28] However, in our analysis we only evaluated the change in parameters according to the change in plasma fibrinolytic potential.

Some aspects of this study warrant comment. The START study was open label and we noticed a difference in the distribution of sex and age between the groups. However, since we evaluated fibrinolysis parameters and we decided *a priori* to correct our analysis for these possible confounders, it is unlikely that both factors influenced our results. Another aspect is that we only tested the effect of rosuvastatin, a hydrophilic type of statin. Hydrophilic statins, such as pravastatin and rosuvastatin, have different properties to lipophilic statins, such as simvastatin, atorvastatin and fluvastatin. Cellular uptake of hydrophilic types of statins, for example, is only present in hepatocytes but not in extrahepatic cells, whereas lipophilic statins penetrate cell membranes and enter cells in any organ.[37] The exact effect on fibrinolysis might therefore be different for hydrophilic statins than for rosuvastatin. Nevertheless, our findings on increased fibrinolysis after rosuvastatin treatment underline the suggestion that statins could be prescribed to patients with prior VTE who are considered to be at high risk of anticoagulation-related bleeding.

In conclusion, we found that in participants with prior VTE who stopped anticoagulant treatment, four weeks of rosuvastatin use led to an increased plasma fibrinolytic potential, decreased plasmin inhibitor and TAFI and higher fibrinogen levels compared to non-statin users, whereas PAI-1 levels did not change. Variance in change in plasma fibrinolytic potential could only be explained for a small part by change in plasmin inhibitor and not by the other individual fibrinolysis parameters. This increase in fibrinolytic potential and formerly reported anticoagulant effects after rosuvastatin treatment support the need for further studies on the possible role for statins in the secondary prevention of VTE.

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Conflict of interest

None of the authors reports a conflict of interest with regard to this manuscript.

Author contributions

SS-G, FWGL, WML, FJMvdM, DCR and MJHAK designed the study. JSB and WML collected patient data. MPMdeM and DCR contributed in essential analysis and interpretation of all laboratory measurements. SS-G analysed the data which was verified by MPMdM, TvG, WML, DCR, JV and MJHAK and interpreted by all other authors. SS-G wrote the first draft of the manuscript, which was reviewed, modified and approved by all other authors.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Schematic overview of effects of rosuvastatin 20 mg/day on fibrinolysis.

Table S1. Effects of rosuvastatin on measures of fibrinolysis.

Table S2. Effects of rosuvastatin use on measures of fibrinolysis, separated into subgroups of participants with unprovoked or provoked venous thromboembolism.

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