

**ORIGINAL ARTICLE**

# Influence of rosuvastatin on apolipoproteins and coagulation factor levels: Results from the STatin Reduce Thrombophilia trial

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**Abstract**

**Background:** The STAtins Reduce Thrombophilia trial showed that, in patients with prior venous thrombosis, rosuvastatin decreased various coagulation factor levels.

**Objectives:** Here, we investigated the hypothesis that statins decrease coagulation factor levels through shared mechanisms of synthesis or regulatory pathways with apolipoproteins.

**Methods:** We measured the levels of apolipoprotein (Apo)A-I, A-II, A-IV, (a), B-100, B-total, C-I, C-II, C-III, and E in patients (n = 126) randomized to 28 days of rosuvastatin use. We assessed the association between apolipoproteins and coagulation factors at baseline using linear regression. The mean difference in apolipoprotein levels between baseline and after 28 days of rosuvastatin use was determined through linear regression, adjusting for age, sex, and body mass index. Coagulation factors were added to this model to determine if the lowering of apolipoproteins by rosuvastatin was linked with coagulation factor levels.

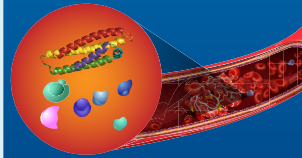
**Results:** At baseline, levels of all apolipoproteins, except Apo(a), were positively associated with FVII, FIX, and FXI. Apolipoproteins levels, except for ApoA-I, A-IV, and Apo(a), were decreased after 28 days of rosuvastatin. ApoB-100 showed the largest mean decrease of -0.43 g/L (95% CI = -0.46 to -0.40). The decrease in ApoC-I and C-III levels was associated with a decrease in FVII, whereas the decrease in apoA-II, B-100, and B-total was associated with a decrease in FXI. The decrease in apolipoproteins was neither associated with FVIII or vWF decrease nor with endogenous thrombin potential changes.

**Conclusions:** Rosuvastatin decreases the level of several apolipoproteins, but this decrease was associated only with a decrease in FVII and XI and not with FVIII/vWF.

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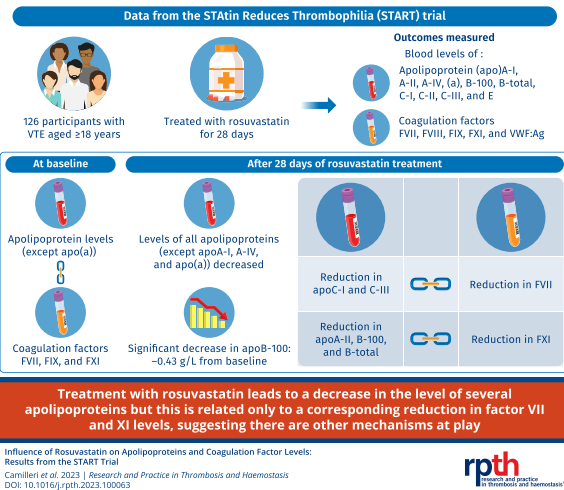
## Mechanisms Behind Rosuvastatin-Mediated Decrease in Coagulation Factors

The risk of venous thromboembolism (VTE) can be influenced by coagulation factors and apolipoprotein levels, and statins may modulate the levels of both



While statins like rosuvastatin reduce the levels of coagulation factors, the mechanism behind this effect and its relationship with apolipoproteins are not clear

Does modulation of apolipoproteins drive the statin-related lowering of coagulation in blood?



### KEYWORDS

apolipoproteins, blood coagulation factors, hydroxymethylglutaryl-CoA reductase inhibitor, rosuvastatin calcium, venous thrombosis

### Essentials

- Rosuvastatin has been shown to decrease coagulation factors through an unknown mechanism in the STATin Reduce Thrombophilia.
- We investigated if apolipoproteins are associated with statin-related lowering of coagulation.
- Rosuvastatin-related decrease in apolipoproteins was associated only with a decrease in FVII and XI.
- Rosuvastatin decreases coagulation factors through other mechanisms than via apolipoproteins.

## 1 | INTRODUCTION

Dyslipidemia is a well-known risk factor for arterial thrombosis [1] and is associated with an increased risk for venous thromboembolism (VTE) [2,3], although the mechanism for this association is not yet established. A possible explanation is the presence of confounding factors, ie, lifestyle and comorbidities, such as obesity and diabetes [4,5]. Lipids modulate the expression and function of hemostatic [6,7] and inflammatory [8] factors, which may also lead to hemostatic changes that increase the risk of VTE. Moreover, growing evidence suggests that statins, the cholesterol-lowering medication widely used for cardiovascular disease prevention, could be an alternative treatment for VTE prophylaxis due to their potential antithrombotic properties [9].

Results of the Statins Reduce Thrombophilia (START) trial showed that rosuvastatin improves the coagulation profile of patients with previous venous thrombosis by reducing levels of coagulation factors VII, VIII, XI, von Willebrand factor (vWF) [10], and thrombin generation [11]. These results are concordant with previous observational studies showing that statin use is associated with reduced coagulation factor levels [12,13]. This corroborates the theory that rosuvastatin may influence coagulation directly. However, the underlying mechanism behind this pleiotropic effect is not established.

Apolipoproteins are lipid-binding proteins that form lipoproteins as part of lipid transport. Furthermore, apolipoproteins interact with lipoprotein receptors and lipid transport proteins, mediating lipoprotein uptake and clearance. In clinical studies, apolipoprotein levels have been associated with levels of coagulation factors [14] and have been shown to be predictive for both venous and arterial cardiovascular events [14–16]. Statins, beyond the well-known inhibition of the synthesis of low-density lipoprotein (LDL) cholesterol, also exert an effect on the level of apolipoproteins, decreasing the level of apolipoproteins B and C and mildly increasing the level of apolipoprotein A-I [17,18]. Apolipoprotein levels may explain the association between statins, hemostatic changes, and VTE risk.

We hypothesized that statins decrease coagulation parameters through their effect on apolipoprotein levels via possible shared mechanisms of synthesis or regulatory pathways. Therefore, the 3 objectives of this study were to (1) assess whether apolipoprotein and coagulation factors levels are associated at baseline (before statin treatment) in a population with previous VTE; (2) study the effect of 28 days of rosuvastatin treatment on apolipoprotein levels; (3) assess whether changes in apolipoprotein levels after rosuvastatin use are associated with changes in coagulation factor levels. By focusing on these objectives, we aimed to gain insight into the mechanism of lowering coagulation factors through statin treatment.

## 2 | METHODS

### 2.1 | Study population

We used data from the multicenter, randomized, controlled, open-label START trial. Details of the trial have been described previously [10]. Briefly, a total of 255 participants aged 18 years or older, with a confirmed episode of proximal deep vein thrombosis or pulmonary embolism and who were allowed to stop anticoagulant treatment, were included between December 2012 and December 2016. Patients were enrolled from 3 anticoagulation clinics in the Netherlands. Reasons for exclusion were statin use, other lipid-lowering drugs, or a contraindication to rosuvastatin use. After informed consent, participants' demographic and clinical characteristics, including acquired risk factors for thrombosis, were registered through a questionnaire. At randomization, participants were allocated to receive either rosuvastatin 20 mg once daily or no study medication, with 131 assigned to the rosuvastatin-therapy group and 124 to the non-rosuvastatin-therapy group. The duration of follow-up was 28 days. Blood samples were collected 1 month after the end of vitamin K antagonist treatment at the randomization visit (baseline) and at the end of follow-up (28 days later). All blood collections were performed between 08:00 and 15:00, and plasma and serum were immediately stored at  $-80^{\circ}\text{C}$  after centrifuge within 3 hours of venipuncture.

In this study, we included all participants assigned to the treatment group of the START trial, ie, 131 patients allocated to rosuvastatin therapy.

### 2.2 | Variables tested

Levels of apolipoprotein (apo) A-I, A-II, A-IV, (a), B-100, B-total, C-I, C-II, C-III and E were measured on unfrozen serum samples. To determine the apolipoprotein profile, a mass spectrometric method was developed for multiplexed quantification of apolipoproteins [14,19].

All coagulation-related laboratory measurements (FVII, FVIII, FIX, FXI, vWF:Ag) were analyzed on the ACL-Top 700 Analyzer (Instrumentation Laboratory). FVIII, FIX, and FXI levels were measured using modified activated partial thromboplastin time assays with immunodepleted plasmas. Similarly, FVII was determined using a modified prothrombin time assay. vWF antigen was measured using an automated latex-enhanced immunoassay with the HemosIL vWF:Ag Reagent Kit [10].

The thrombin generation potential was assessed by the Calibrated Automated Thrombogram (Diagnostica Stago) according to the specifications of the manufacturer [20]. The fluorescent signal representing generated thrombin was monitored in a Fluoroskan Ascent fluorometer (Thermo Fisher Scientific), and the parameters were calculated with the Thrombinoscope software (Thrombinoscope BV). Endogenous thrombin potential (ETP) was the parameter determined through the thrombin generation assay [11,15].

### 2.3 | Statistical analysis

First, to answer whether apolipoproteins are associated with coagulation markers at baseline (before rosuvastatin treatment), we used linear regression models, including apolipoprotein levels as the independent variables and coagulation factor levels and ETP as the outcome variables. Coagulation factor levels and ETP were considered continuous variables, whereas apolipoprotein levels were considered in separate models as continuous variables and per SD increase. Apolipoprotein levels were tested to assess their normal distribution, and Apo(a) levels were not normally distributed and were log-transformed to achieve normal distribution. The assumption of equal variance was checked by plotting the residuals against the fitted values, and homoscedasticity was confirmed for each model.

Next, to assess the change in apolipoprotein levels after rosuvastatin therapy, we evaluated the difference between apolipoprotein levels at baseline (before rosuvastatin treatment) and after 28 days of rosuvastatin treatment. Mean levels and standard deviations of all prespecified apolipoproteins were calculated at baseline and the end of the study period. We calculated the absolute mean difference with 95% CI between the 2 measurements by means of a paired Student's sample *t*-test. As the Apo(a) levels were not normally distributed, we calculated the mean ratio between the 2 Apo(a) measurements by back-transforming the log-transformed mean Apo(a) difference, considering that the difference between the 2 logarithms is the logarithm of their ratio. Relative mean differences were calculated by subtracting the baseline value from the 28-day value, dividing this value by the baseline value, and multiplying the result by 100%.

Finally, we used multivariable linear regression to investigate the association between the difference in coagulation parameters and the difference in apolipoproteins, both calculated subtracting the levels before from the levels after rosuvastatin treatment. In each model, the difference between apolipoprotein levels before and after rosuvastatin treatment was included as an independent variable, and the difference between coagulation parameters was the dependent variable. Apolipoprotein differences were considered as both per SD lowering and as a continuous variable. For the analysis of the vWF, we decided *a priori* to add a sensitivity analysis to exclude those patients who developed an infection during follow-up, similar to the START trial [10]. The assumption of equal variance was verified by plotting the residuals against the fitted values, and homoscedasticity was confirmed in each model.

For all aforementioned analyses, we performed a complete-case analysis; therefore, we only used the cases for which the measurements were available and were not missing. All analyses were performed with R Studio, R version 3.4.4 (<http://www.R-project.org/>).

## 3 | RESULTS

### 3.1 | Study population

Between December 2012 and December 2016, a total of 255 patients were randomized, with 131 assigned to the rosuvastatin-therapy

group. Two participants randomized to rosuvastatin did not start the trial, and 3 did not complete the study (2 due to a recurrent VTE event and one due to another serious adverse event) [10]. Compliance with rosuvastatin during the trial was assessed by asking the patient to take the first tablet in the presence of an investigator and by measuring cholesterol levels. Only one patient stopped rosuvastatin treatment because of admission to the hospital for acute asthma exacerbation.

The mean age of the 126 participants included in the current analysis was 57 years (range = 19–82 years), and the majority (68, 54%) were men (Table 1). At least 1 cardiovascular risk factor was present in 89 (71%) patients, with overweight and obesity being the most prevalent in 54 (43%) and 29 (23%) patients, respectively. Measures for all apolipoproteins failed due to technical failures in 2 samples collected at the end of the study; moreover, measurement of Apo(a) failed in 1 sample collected before and 1 after rosuvastatin treatment, leading to the exclusion of these measurements from the analysis.

### 3.2 | Hemostatic factors and apolipoprotein levels at baseline

At baseline, levels of all apolipoproteins, except for Apo(a), were positively associated with levels of FVII, FIX, and FXI (Table 2 and Supplementary Table 1). Levels of ApoA-I were most strongly associated with levels of FVII (7.0 IU/dL per SD increase, 95% CI = 2.8–11.2), whereas levels of ApoBs and ApoCs were positively associated with levels of FIX and FXI. Higher levels of ETP were associated with higher Apo(a) levels (47.2 nM min per SD increase, 95% CI = 1.9–92.4) but not with the other apolipoproteins. Similarly, higher levels of FVIII and vWF were associated only with higher ApoA-I levels (5.8 IU/dL, 95% CI = 0.4–11.2 and 9.2, 95% CI: 1.7–16.6 per SD increase, respectively).

### 3.3 | Mean apolipoprotein levels at baseline and 28 days after randomization

At the end of the study, mean levels of all apolipoproteins, except ApoA-I, A-IV, and Apo(a), decreased in comparison to baseline levels (Figure 1). ApoB-100 and ApoB-total showed the largest decrease, both with an absolute mean difference of  $-0.43$  g/L (95% CI =  $-0.46$  to  $-0.40$ ). ApoC-I, C-II, and C-III also decreased, as well as ApoA-II, albeit to a smaller extent compared to the other apolipoproteins. In contrast, ApoA-I and ApoA-IV levels were similar to the baseline levels. Apo(a) levels were increased at the end of the study, with an absolute mean ratio of 1.10 (95% CI = 1.02–1.19), which represents the ratio between Apo(a) mean levels after and before rosuvastatin treatment, ie, a 10% increase in levels.

**TABLE 1** Demographic and clinical characteristics at baseline.

	Rosuvastatin users (n = 126)
General	
Age (y)	57 (19–82)
Men	68 (54)
BMI (kg/m <sup>2</sup> )	27 (19–44)
Baseline cholesterol (mmol/L)	5.6 (2.9–8.9)
Antiplatelet use	5 (4)
Venous thrombosis characteristics	
Type of venous thrombosis	
Deep vein thrombosis	72 (57)
Pulmonary embolism	54 (43)
Unprovoked	57 (45)
Provoked by	69 (55)
Surgery/trauma/immobilization	32 (25)
Travel >4 h	22 (18)
Estrogen use (% in women)	24 (41)
Pregnancy/puerperium (% in women)	0 (0)
Malignancy	2(2)
Recurrent venous thrombosis	10 (8)
Cardiovascular risk factors	
Absent	37 (29)
Present	89 (71)
Current smoking	18 (14)
Hypertension	24 (19)
Diabetes	3 (2)
Overweight	54 (43)
Obesity	29 (23)

BMI, body mass index.

### 3.4 | Association between changes in apolipoprotein levels and coagulation factors after rosuvastatin treatment

The decrease in levels of ApoC-I and C-III was associated with a decrease in levels of FVII (5.2 IU/dL, 95% CI = 1.7–8.8 and 6.7 IU/dL, 95% CI = 2.8–10.5 per SD decrease, respectively), whereas the decrease in levels of ApoA-II, B-100, and B-total was associated with a decrease in levels of FXI and FIX (Table 3 and Supplementary Table 2). The decrease in levels of ApoE was associated with a decrease in levels of vWF (5.7 UI/dL, 95% CI = 1.9–9.7 per SD decrease). Moreover, a decrease in levels of ApoC-I, C-II, and C-III was associated with an increase in levels of FVIII and with a decrease in vWF levels. Apolipoprotein decrease was not associated with changes in ETP after rosuvastatin treatment.

**TABLE 2** Increment of hemostatic parameters and ETP per SD increment of apolipoprotein at baseline, before rosuvastatin treatment<sup>a</sup>.

Apolipoproteins (95% CI)	Factor VII (IU/dL)	Factor VIII (IU/dL)	Factor IX (IU/dL)	Factor XI (IU/dL)	vWF (IU/dL)	ETP (nM*min)
ApoA-I (SD = 0.28 g/L)	7.0 (2.8 to 11.2)	5.8 (0.4 to 11.2)	4.5 (1.0 to 7.9)	3.1 (-0.8 to 7.1)	9.2 (1.7 to 16.6)	-13.1 (-61.5 to 35.3)
ApoA-II (SD = 56.2 mg/dL)	2.9 (-1.5 to 7.5)	4.1 (-1.5 to 9.8)	3.9 (0.2 to 7.5)	3.0 (-1.0 to 7.1)	6.7 (-0.9 to 14.3)	2.6 (-47.7 to 52.9)
ApoA-IV(SD = 53.8 mg/dL)	5.8 (1.8 to 9.8)	3.2 (-1.8 to 8.4)	3.4 (0.03 to 6.6)	2.0 (-1.7 to 5.7)	1.0 (-6.3 to 8.4)	-19.7 (-65.1 to 25.7)
Ln Apo(a)	-2.1 (-6.3 to 1.9)	3.2 (-1.9 to 8.5)	2.2 (-1.2 to 5.6)	0.5 (-3.3 to 4.2)	2.8 (-4.4 to 10.0)	47.2 (1.9 to 92.4)
ApoB-100 (SD = 0.25 g/L)	2.1 (-2.0 to 6.4)	3.4 (-1.9 to 8.6)	5.6 (2.3 to 8.9)	7.8 (4.2 to 11.3)	0.9 (-6.4 to 8.1)	3.8 (-42.7 to 50.4)
ApoB (SD = 0.25 g/L)	1.8 (-2.3 to 6.0)	2.1 (-2.9 to 7.3)	4.9 (1.7 to 8.1)	6.8 (3.3 to 10.4)	1.0 (-6.0 to 7.9)	10.3 (-35.1 to 55.8)
ApoC-I (SD = 5.57 mg/dL)	4.9 (0.5 to 9.5)	4.6 (-1.0 to 10.3)	4.6 (1.0 to 8.3)	4.3 (0.2 to 8.4)	6.6 (-1.5 to 14.7)	-9.1 (-41.7 to 59.9)
ApoC-II (SD = 28.1 mg/dL)	3.0 (-1.3 to 7.4)	0.2 (-5.3 to 5.6)	5.5 (2.0 to 8.9)	4.8 (1.0 to 8.7)	0.9 (-6.7 to 8.6)	35.6 (-12.3 to 83.5)
ApoC-III (SD = 36.5 mg/dL)	4.7 (0.2 to 9.1)	-1.6 (-7.2 to 4.1)	7.5 (4.1 to 11.0)	7.1 (3.2 to 11.0)	-4.2 (-12.3 to 3.9)	39.2 (-10.2 to 88.5)
ApoE (SD = 12.2 mg/dL)	1.6 (-3.2 to 6.5)	5.7 (-0.3 to 11.8)	3.8 (-0.2 to 7.7)	4.3 (-0.04 to 8.7)	7.6 (-1.0 to 16.3)	6.9 (-47.3 to 61.0)

<sup>a</sup>The regression coefficient and 95% CI represent the unit of increase in coagulation factors at baseline by 1 SD increase in apolipoproteins. A positive coefficient indicates that coagulation factors increase as apolipoprotein increases. For example, a 1 SD (0.28 g/L) increase in ApoA-I is associated with a 7.0 IU/dL increase in the FVII level. ETP, endogenous thrombin potential; vWF, von Willebrand factor.

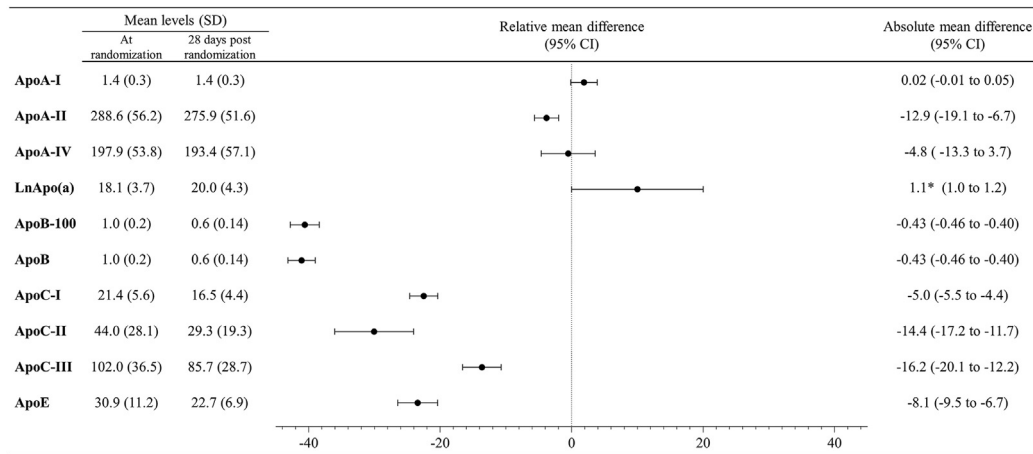
## 4 | DISCUSSION

The results of our study showed that, at baseline, levels of all apolipoproteins, except Apo(a), were positively associated with the levels of coagulation factors FVII, FIX, and FXI. After 28 days of treatment with rosuvastatin, mean levels of all apolipoproteins noticeably decreased, except for ApoA-I, A-IV, and Apo(a). Most importantly, our main findings on the relationship between the changes in apolipoproteins and hemostatic factors after rosuvastatin treatment, compared with baseline, showed that the decrease in ApoB-100 and B-total levels was mainly associated with a decrease in FIX and FXI levels. Furthermore, ApoC-I and C-III decreases were associated with a decrease in FVII levels.

Previous studies have shown a positive association between apolipoproteins and levels of coagulation factors [14,16,21]. In the MEGA study [14], several apolipoproteins (ie, ApoC-I, C-II, C-III, and E) were associated with levels of FVII, FIX, and FXI in control individuals without venous thromboembolism, which is in line with our results. A positive association of ApoA-I and B with vitamin-K dependent coagulation factors and coagulation inhibitors was also previously reported in MEGA control participants [16]. In contrast to our findings, the MEGA study reported higher levels of ApoC-III and E that were associated with higher levels of FVIII and vWF, which was not confirmed by our results [14]. Furthermore, our results showed that ApoC-III levels were strongly associated with FVII levels. This correlation is consistent with the results of a population-based study in which ApoC-III levels strongly correlated with activated FVII-antithrombin complex [21]. Collectively, our findings were mostly concordant with previous reports, providing additional evidence of an association between apolipoproteins and coagulation factor levels that could reflect the existence of several shared mechanisms of synthesis or common regulatory pathways of apolipoproteins and coagulation factors.

Mean levels of all apolipoproteins, except for ApoA-I and Apo(a), markedly decreased at the end of our study. Statins, particularly rosuvastatin, have previously been shown to decrease the levels of ApoB, C-I, C-II, C-III, and E [17,22-24]. Studies on the effect of statins on ApoA-I have reported mixed results, with some studies reporting a slight increase [23,25] and some reporting no effect [26], similar to our results. Statins are also known to increase levels of both lipoprotein(a) and Apo(a) [27,28]; therefore, our results are consistent with the known effect of rosuvastatin on apolipoprotein levels.

Our main hypothesis was that coagulation factors and apolipoproteins could share a common regulatory mechanism and that the observed effect of rosuvastatin on coagulation factors could be explained by its effect on apolipoprotein synthesis. Our results remain unclear on this issue. For instance, ApoB100 and ApoB-total levels showed the maximum decrease during rosuvastatin treatment but were associated only with FXI decrease. The lowering of ApoB100 and ApoB-total was not associated with vWF and FVIII, the coagulation factors that showed the maximum decrease in the START trial. Decrease in ApoC-I, C-II, and C-III was instead associated with an increase in FVIII. Apolipoprotein decrease was also not associated



**FIGURE 1** Mean difference in apolipoprotein levels at the time of randomization and 28 days post-randomization to rosuvastatin<sup>a</sup> Mean ratio calculated with back transformation of the natural logarithm. Missing values: measurements of all apolipoprotein failed due to technical reasons in 2 samples collected at the end of the study. Measurements for Apo(a) failed due to technical reasons in 2 additional samples (one at randomization, one at the end of the study).

with change in ETP, the thrombin generation parameter representing the total amount of thrombin generated.

Multiple hypotheses might explain these different associations. The negative association between ApoC-I, C-II, C-III, and FVIII could be explained by the mechanism by which ApoCs interact with the LDL receptor-related protein-1 (LRP-1) and the LDL receptor (LDLR). LRP-1 and LDLR contribute to the hepatic clearance and to the variation in FVIII plasma levels through binding with FVIII [29]. Laboratory studies have shown that deficiency of LRP-1 and LDLR leads to elevated FVIII levels and a prolonged FVIII half-life [30,31]. ApoC-I and C-III inhibit the binding of lipoproteins to LRP-1 and LDLR [32]; therefore, decreased levels of ApoC-I and C-III facilitate the binding between lipoprotein and LRP-1 and LDLR. This could lead to a lower number of free receptors for binding FVIII. Thus, decreased levels of ApoC might increase the competition between lipoproteins and FVIII for the same receptor and could lead to higher levels of FVIII due to lower degradation mediated by the binding with LDLR and LRP-1.

Moreover, our results showed that apolipoproteins were mainly associated with FVII, FIX, and FXI. This could be explained by the fact that these clotting factors are mainly produced in the liver [33–35]. vWF is principally produced and stored in the endothelial cells [36], and FVIII, albeit synthesized both in endothelial and sinusoidal cells of the liver, is rapidly cleared when not bound to vWF in the plasma circulation. Therefore, its levels are strictly dependent on vWF concentration [37]. It seems plausible that the strongest association between apolipoproteins and coagulation factors can be seen between those factors that are mainly produced by the liver, such as FVII, FIX, and FXI, and not seen for those that are mainly regulated by the endothelium, such as vWF and FVIII. This hypothesis is supported by *in vivo* studies showing that apolipoproteins and coagulation genes share common transcription factors, such as hepatocyte nuclear factor 4 $\alpha$  [38], which are known to be modulated by statins [39]. Similarly, epidemiological studies have shown that apolipoproteins are mainly

associated with hepatocyte- and not with endothelial-derived coagulation factors [14,16].

Instead of a shared mechanism of synthesis, the association could potentially be explained by a modulatory effect of apolipoproteins on coagulation activity. Previous studies have shown that lipoproteins, such as very low density lipoprotein and LDL, support the prothrombinase complex and thrombin generation [40,41]. This effect might be mediated by surface phospholipids or apolipoproteins through the support of protein-protein or protein-lipid interactions [40,41]. Therefore, statins could influence coagulation by reducing the influence of apolipoproteins or phospholipids on clotting activation. Other *in vivo* and *in vitro* studies demonstrated that statin could additionally downregulate tissue factor expression [42–44], but this effect was largely independent of their lipid-lowering mechanism [43].

Furthermore, studies have demonstrated that the pleiotropic effects of statins are largely independent of their mechanism of action on hepatocytes and are mainly related to the drug effect on endothelial cells and platelets or inflammation modulation [45,46]. In the START trial, FVIII was the main coagulation factor that decreased in rosuvastatin users. At the same time, a reduction in FVII and FXI was also observed, but FIX did not change (the latter being an unpublished result). Therefore, altogether our results suggest that the improvement of coagulation parameters observed in rosuvastatin users is not only related to the rosuvastatin effect on apolipoproteins levels, although apolipoproteins could have shared mechanisms of regulation with liver-produced factors such as FVII and FXI. Rosuvastatin may also induce changes in coagulation factors through its pleiotropic effects on platelets and endothelial cells. However, this hypothesis is beyond the scope of our study as we presently have no information on platelets and endothelial cells in the START trial [10].

Several aspects of this study warrant a comment. We measured a complete panel of apolipoproteins through a mass spectrometry method for multiplexed quantitation. In contrast to immunoassay for

**TABLE 3** Association between differences in apolipoprotein levels (on the SD scale) and differences between coagulation factor levels and ETP, before and after rosuvastatin treatment<sup>a</sup>.

Delta apolipoproteins (95%CI)	Delta FVII (IU/dL)	Delta FVIII (IU/dL)	Delta FIX (IU/dL)	Delta FXI (IU/dL)	Delta vWF (IU/dL)	Delta ETP (nM min)
Delta ApoA-I (SD = 0.17 g/L)	5.1 (1.4 to 8.8)	4.0 (-0.7 to 8.8)	2.3 (-1.0 to 5.5)	0.9 (-2.6 to 4.5)	1.5 (-2.2 to 5.2)	-30.0 (-81.7 to 21.6)
Delta ApoA-II (SD = 34.98 mg/dL)	1.8 (-2.1 to 5.7)	3.5 (-1.4 to 8.3)	2.8 (-0.4 to 6.1)	4.2 (0.7 to 7.7)	3.5 (-0.6 to 7.6)	-5.4 (-58.3 to 47.4)
Delta ApoA-IV (SD = 47.67 mg/dL)	6.9 (3.4 to 10.5)	-2.5 (-7.1 to 2.1)	-0.4 (-3.5 to 2.7)	-0.5 (-3.9 to 2.8)	-2.7 (-6.4 to 1.0)	-13.1 (-63.2 to 36.9)
Delta Ln Apo(a)	-1.1 (-5.6 to 4.5)	-5.2 (-10.8 to 0.4)	1.1 (-2.7 to 4.9)	-0.2 (-3.9 to 4.3)	-0.5 (-4.8 to 3.9)	43.4 (-17.4 to 104.2)
Delta ApoB100 (SD = 0.17 g/L)	1.9 (-1.7 to 5.6)	3.4 (-1.1 to 7.9)	2.6 (-0.4 to 5.6)	6.1 (3.0 to 9.3)	3.1 (-0.3 to 6.7)	-10.7 (-59.6 to 38.3)
Delta ApoB (SD = 0.17 g/L)	2.2 (-1.4 to 5.7)	2.2 (-2.2 to 6.7)	2.9 (0.01 to 5.9)	5.1 (2.0 to 8.2)	3.3 (-0.2 to 6.9)	9.5 (-38.9 to 57.8)
Delta ApoC-I (SD = 3.17 mg/dL)	5.2 (1.7 to 8.8)	-2.8 (-7.4 to 1.7)	-0.7 (-3.7 to 2.4)	0.9 (-2.4 to 4.3)	1.7 (-2.1 to 5.5)	-20.1 (-69.2 to 28.9)
Delta ApoC-II (SD = 15.55 mg/dL)	0.7 (-3.0 to 4.5)	-2.7 (-7.3 to 2.0)	-0.6 (-3.7 to 2.6)	-0.05 (-2.9 to 4.0)	2.4 (-1.5 to 6.3)	-12.3 (-63.0 to 38.3)
Delta ApoC-III (SD = 22.34 mg/dL)	6.7 (2.8 to 10.5)	-1.9 (-6.9 to 3.1)	2.6 (-0.8 to 5.9)	1.9 (-1.7 to 5.6)	1.3 (-2.8 to 5.5)	12.5 (-41.8 to 66.8)
Delta ApoE (SD = 7.75 mg/dL)	1.5 (-2.5 to 5.6)	1.8 (-3.2 to 7.0)	0.3 (-3.1 to 3.7)	3.3 (-0.4 to 6.9)	5.7 (1.9 to 9.7)	-15.3 (-70.4 to 39.7)

<sup>a</sup>The regression coefficient and 95% CI represent the unit of increase in the difference of coagulation factors from baseline by 1 SD increase in the difference of apolipoproteins. A positive coefficient indicates that both differences follow the same direction. For example, 1 SD decrease (-3.17 mg/dL) of ApoC-I from baseline is associated with 5.1 IU/dL decrease in the FVII level from baseline. ETP, endogenous thrombin potential; vWF, von Willebrand factor.

apolipoprotein, which is not standardized for ApoC and ApoE, the quantitative proteomics test allows standardization of unequivocally characterized apolipoproteins through selection of multiple peptides which are representative of each apolipoprotein. The analytical performance (ie, the desirable maximum imprecision of the test) was derived from the known biological variation of apolipoprotein, as clinical outcome data were unavailable [19,47]. This method was developed according to Clinical and Laboratory Standards Institute guidelines and is intended for diagnostics purposes. The method development, including the specification of the analytical performance and the analytical validation, has been described elsewhere [19,32,48].

The crossover design, in which each subject acts as their own control, allowed us to eliminate the potential effect of fixed confounders (such as sex, genetic factors, or race/ethnicity). However, due to the lack of a control group, we cannot exclude that our findings may be an effect of time. Examples of such effects over time are lifestyle or diet changes that could contribute to the modification in the levels of apolipoprotein. However, we believe it is unlikely that our finding could be attributed to chance or to an effect of time on apolipoprotein levels due to the short follow-up of 28 days, and the observed associations were consistent with previous literature [17,23,26,49]. As for lifestyle or diet changes, previous studies have shown that their contribution to the modification of lipid levels is minimal without concomitant statin therapy [50,51]. Moreover, there is a possibility that some findings (or their strength) resulted from chance because of the substantial number of statistical tests. However, in this case, we believe that the issue of multiple testing was limited because we did not base our conclusions on statistical significance testing. Nonetheless, confirmation of our results in future studies is needed.

In conclusion, rosuvastatin decreased several apolipoprotein levels, which was associated only with the decrease in liver-derived coagulation factors VII and XI and not with FVIII and vWF.

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## AUTHOR CONTRIBUTIONS

E.C., W.M.L., S.C.C., and N.v.R. designed the research. E.C. and N.v.R. analyzed the data. E.C. and N.v.R. wrote the manuscript. E.C., W.M.L., N.v.R., S.C.C., B.J.M.v.V., J.S.B., M.J.H.A.K., F.W.L., F.J.v.d.M., and C.M.C. revised the paper for important intellectual content. All authors read and approved the final version of the paper.

## RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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#### SUPPLEMENTARY MATERIAL

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