


## ORIGINAL ARTICLE

# Effects of simvastatin on tissue factor pathway of blood coagulation in STATCOPE (Simvastatin in the prevention of COPD exacerbations) trial

A. Koneti Rao<sup>1</sup>  | Fabiola Del Carpio-Cano<sup>1</sup> | Sumalaxmi Janapati<sup>1</sup> | Huaqing Zhao<sup>2</sup> | Helen Voelker<sup>3</sup> | Xiaoning Lu<sup>2</sup> | Gerard Criner<sup>4</sup> | the NIH COPD Clinical Research Network, the Canadian Institute of Health Research Investigators

<sup>1</sup>Sol Sherry Thrombosis Research Center, Department of Medicine, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

<sup>2</sup>Department of Clinical Sciences, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

<sup>3</sup>Department of Biostatistics, University of Minnesota, Minneapolis, MN, USA

<sup>4</sup>Department of Thoracic Medicine and Surgery, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

## Correspondence

A. Koneti Rao, Sol Sherry Thrombosis Research Center and the Department of Medicine, Lewis Katz School of Medicine at Temple University, MRB-204A, 3400 N Broad Street, Philadelphia, PA 19140, USA.  
Email: koneti@temple.edu

## Funding information

Supported by grants from the National Heart, Lung, and Blood Institute of the National Institutes of Health (U10 HL074407, U10 HL074408, U10 HL074409, U10 HL074416, U10 HL074418, U10 HL074422, U10 HL074424, U10 HL074428, U10 HL074431, U10 HL074439, and U10 HL074441) and the Canadian Institutes of Health Research (115074); STATCOPE ClinicalTrials.gov number, NCT01061671.

## Abstract

**Background:** Statins are widely used to lower lipids and reduce cardiovascular events. In vitro studies and small studies in patients with hyperlipidemias show statins inhibit tissue factor (TF) and blood coagulation mechanisms. We assessed the effects of simvastatin on TF and coagulation biomarkers in patients entered in STATCOPE, a multicenter, randomized, placebo-controlled trial of simvastatin (40 mg daily) versus placebo on exacerbation rates in patients with chronic obstructive pulmonary disease (COPD).

**Methods:** In 227 patients (114 simvastatin, 113 placebo; mean [ $\pm$  standard error of the mean] age  $62 \pm 0.53$  years, 44.5% women) we measured (baseline, and 6 and 12 months): whole blood membrane TF-procoagulant activity (TF-PCA) and plasma factors VIIa, VII, VIII, fibrinogen, TF antigen, tissue factor pathway inhibitor (TFPI), thrombin-antithrombin complexes (TAT), and D-dimer. We excluded patients with diabetes, cardiovascular disease, and those taking or requiring a statin.

**Results:** In the statin group, there was a small increase in TF-PCA (from  $25.18 \pm 1.08$  to  $30.36 \pm 1.10$  U/ml;  $p = .03$ ) over 12 months; factors VIIa and VIII, fibrinogen, TAT, and D-dimer did not change. Plasma TFPI (from  $52.4 \pm 1.75$  to  $44.7 \pm 1.78$  ng/ml;  $p < .0001$ ) and FVIIc ( $1.23 \pm 0.04$  to  $1.15 \pm 0.03$  U/ml;  $p = .03$ ) decreased and correlated with total cholesterol levels. No changes in biomarkers were observed with placebo.

**Conclusions:** In contrast to previous studies on statins, in COPD patients without diabetes, cardiovascular disease, or requiring a statin treatment, simvastatin (40 mg per day) did not decrease TF or factors VIIa and VIII, fibrinogen, TAT, or D-dimer. The decreases in TFPI and factor VII reflect the decrease in serum lipids.

## KEYWORDS

blood coagulation, chronic obstructive pulmonary disease, simvastatin, statin, tissue factor pathway

## Essentials

- Studies in vitro and in patients with hyperlipidemias show statins inhibit tissue factor and coagulation mechanisms.
- We studied effects of simvastatin (40 mg daily, 12 months) vs placebo on TF and coagulation biomarkers in 227 COPD patients.
- Simvastatin did not decrease membrane-bound TF, or plasma factor VIIa, factor VIII, fibrinogen, TAT or D-dimer.
- Simvastatin decreased plasma TFPI and factor VII, which correlated with decreases in lipids.

## 1 | INTRODUCTION

Statins are widely used to lower lipids and reduce cardiac mortality and morbidity in the primary and secondary prevention of cardiovascular disease.<sup>1,2</sup> In addition to lipid-lowering, other effects attributed to statins include anti-inflammatory effects and inhibition of the blood coagulation mechanisms.<sup>3,4</sup> Tissue factor (TF) is a membrane-bound protein expressed on monocytes and other cells and is the physiological initiating mechanism for blood coagulation.<sup>5</sup> Circulating TF in blood is thrombogenic<sup>5,6</sup> and levels are elevated in disorders associated with increased risk of thrombotic events, including diabetes mellitus,<sup>7,8</sup> cardiovascular disease,<sup>9</sup> stroke,<sup>10</sup> sickle cell disease,<sup>11</sup> and chronic obstructive pulmonary disease (COPD).<sup>12</sup>

Several relatively small studies have reported that statins inhibit expression of TF and coagulation mechanisms, often with conflicting findings.<sup>4</sup> This evidence comes from studies studying the effects of statins in vitro on monocytes from subjects with hypercholesterolemia or cardiovascular disease<sup>4,13,14</sup> and studies using monocytes stimulated with lipopolysaccharide (LPS),<sup>13,14</sup> which acutely upregulates TF. *In vivo* evidence has come from animal models with hyperlipidemia.<sup>15,16</sup> In some studies, statin effects were studied in human subjects administered LPS<sup>17</sup> or in the blood from skin bleeding time wounds of patients with cardiovascular disease and/or hypercholesterolemia<sup>18-21</sup> in which simvastatin was shown to inhibit activation of prothrombin, factor V, and factor XIII and enhance factor Va inactivation.<sup>19,21</sup> Some authors have suggested that the effects of statins on the coagulation mechanisms are unrelated to their lipid-lowering effects.<sup>4,16</sup> Statins have also been reported to decrease plasma coagulation factors, including FVII, FVIII, and fibrinogen; markers of thrombin generation; and tissue factor pathway inhibitor (TFPI) in hypercholesterolemic patients.<sup>4,20,22,23</sup> One study showed that fibrin clots in patients with COPD, including those with hyperlipidemia and diabetes mellitus, are denser and resistant to lysis than those from subjects matched for age, sex, and cardiovascular risk factors; these aspects were improved by simvastatin.<sup>24</sup> However, the effects of statins on blood coagulation mechanisms and tissue factor remain unclear, particularly in the absence of hyperlipidemia or LPS activation of monocytes.

COPD patients have an increased incidence of cardiovascular disease<sup>25</sup> and venous thromboembolism (VTE).<sup>26</sup> We have previously shown that circulating TF procoagulant activity (TF-PCA) is elevated in patients with quiescent COPD.<sup>12</sup> Exacerbations of COPD have been reported to increase lung and systemic inflammation,

which has been associated with an increased risk of acute myocardial infarction, congestive heart failure, pulmonary embolism, cardiac arrhythmias, stroke, and VTE.<sup>27-29</sup> Some of these events, such as VTE, pulmonary embolism, congestive heart failure, and acute myocardial infarction, share clinical presentations with COPD exacerbations, which are difficult for clinicians to discern.<sup>30</sup> Based on retrospective and small studies reporting statins decrease exacerbations, hospitalizations, and mortality in patients with COPD,<sup>31</sup> the efficacy of simvastatin to prevent COPD exacerbations was studied in a large multicenter, randomized, trial of simvastatin (40 mg daily) versus placebo (STATCOPE).<sup>32</sup> In STATCOPE, simvastatin did not affect exacerbation rates in moderate-to-severe COPD patients at high risk for exacerbations.<sup>32</sup> However, we postulated that statins may have a beneficial effect on ameliorating the procoagulant blood biomarker profile of patients with moderate to severe COPD that were prone to exacerbations.

Herein we report the effects of simvastatin (40 mg daily) on TF and plasma coagulation biomarkers and their relationships to COPD exacerbation rates in patients studied in STATCOPE. In STATCOPE, patients with diabetes mellitus or cardiovascular disease and those who were taking statins or required statins were excluded, providing a unique group to study statin effects.

## 2 | METHODS

### 2.1 | Study design

We measured coagulation biomarkers in 227 patients (114 simvastatin, 113 placebo) enrolled in STATCOPE and from whom sequential blood samples were available at baseline and 6 and 12 months. Details regarding STATCOPE study design have been previously reported.<sup>32</sup>

In STATCOPE, 885 moderate-to-severe COPD patients (mean  $\pm$  standard error of the mean [SEM]) age  $62 \pm 0.53$  years, 44% women) from 45 centers were randomized to treatment with simvastatin (40 mg daily) or placebo for 12 to 36 months. Patients were eligible if they were 40 to 80 years of age, had COPD (defined by a forced expiratory volume in 1 s of  $<80\%$  and a ratio of forced expiratory volume in 1 s to forced vital capacity of  $<70\%$ ), smoking history of 10 or more pack-years, were receiving supplemental oxygen or treatment with glucocorticoids or antibiotic agents, or had an emergency department visit or hospitalization for COPD within the

past year. Patients with diabetes or cardiovascular disease and those who were taking statins or who required statins on the basis of Adult Treatment Panel III criteria were excluded.

## 2.2 | Biomarker assays

Blood was collected into one-tenth volume of 3.2% sodium citrate from outpatients following fasting for 12 h. Aliquots (1 ml) were frozen for whole blood TF-PCA assay. Blood samples were centrifuged (2000g, 30 min, room temperature) within 60 min of blood draw. Plasma was harvested immediately, frozen as multiple aliquots, and stored at  $-80^{\circ}\text{C}$ . They were shipped on dry ice to the Sol Sherry Thrombosis Research Center, Temple University, Philadelphia. All biomarker assays were performed in blinded manner with respect to the treatment group.

TF-PCA was measured in whole-blood cell lysates from blood collected into one-tenth volume of sodium citrate as previously described<sup>11,33</sup> with a two-stage clotting assay using recombinant human FVIIa (Sekisui Diagnostics, LLC, human factor X; Haematologic Technologies Inc.), and pooled normal human plasma (George King Bio-medical, Inc.) containing phospholipid vesicles. The TF-PCA assay measures cell-bound and microparticle-associated TF in lysed whole blood. HemosIL RecombiPlastin 2G (Instrumentation Laboratory) was used as a standard.

Coagulation biomarkers were measured in plasma harvested by centrifugation from blood collected into one-tenth volume of 3.2% sodium citrate.<sup>33</sup> Coagulation factor VII (FVIIIC), factor VIII (FVIIC), and fibrinogen were measured by standard clotting assays.<sup>33</sup> Plasma factor VIIa (the activated form of FVII) activity was measured by a commercially available assay (STACLOT VIIa-rTF, Diagnostica Stago Inc.). Plasma thrombin-antithrombin complexes (Enzygnost TAT micro, Siemens Healthcare Diagnostics), D-dimer (IMUCLONE D-dimer, Sekisui Diagnostics, LLC.), tissue factor antigen (IMUBIND Tissue Factor, Sekisui Diagnostics, LLC.), and TFPI (IMUBIND total TFPI, American Diagnostica GmbH.) were measured using ELISAs.

The methods for the measurements of serum lipids, C-reactive protein (CRP), blood sugar, and HbA1c have been described.<sup>32</sup>

## 2.3 | Statistical analysis

Descriptive statistics were generated and compared between groups, including demographics, baseline levels of coagulation biomarkers, lipid, and CRP levels. Log transformations were employed for biomarkers (TF-PCA, FVIIa, TAT, D-dimer, TF antigen) with skewed distribution to achieve normality. Continuous data are expressed as mean and standard deviation and categorical data are expressed as frequency and percentage. Patient baseline characteristics and coagulation biomarkers were compared by Pearson chi-square test for categorical variables and two-sample *t* test for continuous variables. A linear mixed-effects model was used to examine the effect of statin and placebo on the coagulation biomarker

levels change over time, and the relationship to cancer and smoking status at baseline. Two-sample *t*-test was used to examine the relationship between the coagulation marker at 12 months and the presence or absence of exacerbation at 12 months. Pearson's correlation coefficients were calculated to examine the relationships among biomarkers, between biomarker levels and lipid levels at baseline, and between biomarker levels at 12 months and the number of acute exacerbations. In addition, Pearson's correlation coefficients were used to examine relationships between biomarkers using the average of the levels at three time points in each patient and separated by treatment groups. All statistical analyses were performed with the use of SAS software, version 9.4 (SAS Institute Inc.).

## 3 | RESULTS

Demographic and baseline clinical characteristics were similar in the statin and placebo groups (Table 1). These were also similar in patients in whom the coagulation biomarkers were measured, and in the patients in whom these measurements were not performed in the parent STATCOPE study.

### 3.1 | Coagulation biomarkers

Baseline levels of coagulation biomarkers were comparable between the placebo and statin groups. Over the 12-month follow-up mean TF-PCA levels minimally increased in the statin group (from  $25.18 \pm 1.08$  to  $30.36 \pm 1.10$  U/ml;  $p = .03$ ) but not in the placebo group (Figure 1). Plasma TFPI ( $p < .0001$ ) and FVIIIC ( $p = .03$ ) decreased in the statin group but were unchanged in the placebo group. Other markers (fibrinogen, FVIIC, FVIIa, TAT, D-dimer) did not change over time in the

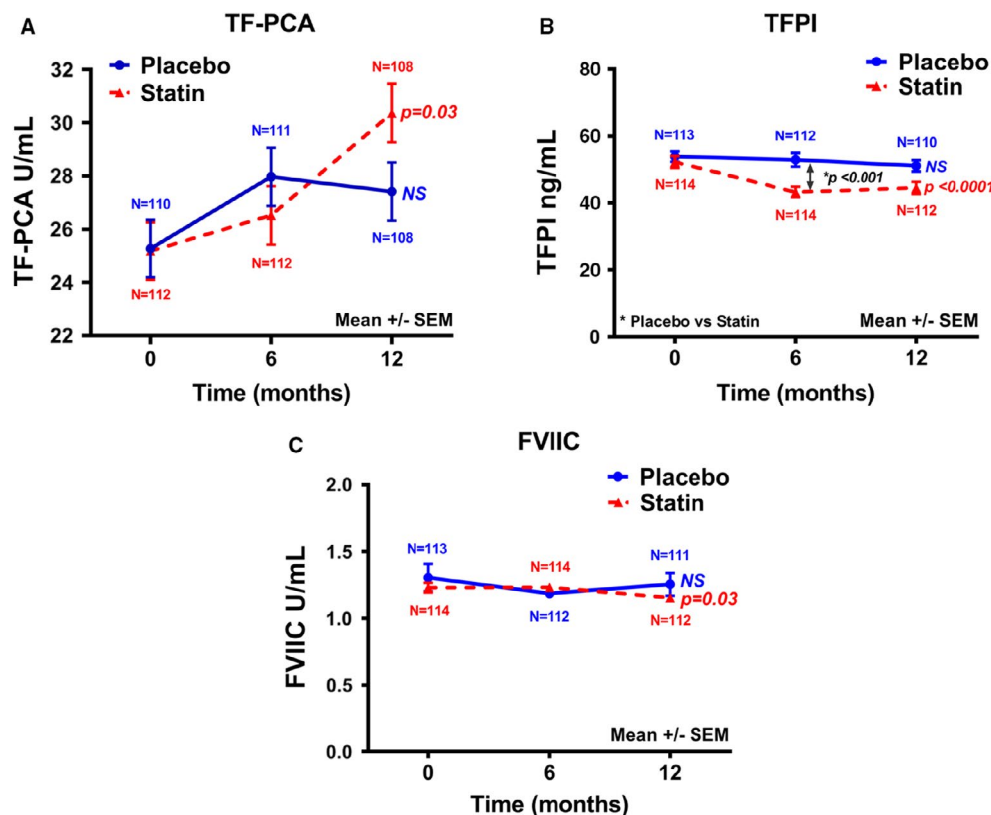
TABLE 1 Demographics of the patients<sup>a</sup>

Characteristic	Simvastatin (N = 114)	Placebo (N = 113)
Age, y	62.8 $\pm$ 8.8	62.0 $\pm$ 8.2
Sex, n (%) female	47 (41.2%)	54 (47.8%)
Black, n (%)	24 (21.1%)	21 (18.6%)
FEV <sub>1</sub> , % of predicted value	44.4 $\pm$ 17.0	42.3 $\pm$ 18.0
Smoking history, pack-year	47.0 $\pm$ 23.6	51.4 $\pm$ 28.1
Current smoking status, n (%)	38 (33.3%)	40 (35.4%)
Body mass index, kg/m <sup>2</sup>	26.8 $\pm$ 5.8	27.7 $\pm$ 8.1
High blood pressure, n (%)	48 (42.1%)	40 (35.4%)
History of clots, n (%)	4 (3.5%)	2 (1.8%)
Cirrhosis, n (%)	2 (1.8%)	1.0 (0.9%)
Cancer, n (%)	8 (7.0%)	15 (13.3%)

Note: There were no significant differences between the simvastatin and the placebo groups.

Abbreviation: FEV<sub>1</sub>, forced expiratory volume in 1 s.

<sup>a</sup>Shown are means  $\pm$  SD.



**FIGURE 1** Effect of simvastatin and placebo on (A) tissue factor procoagulant activity (TF-PCA), (B) tissue factor pathway inhibitor (TFPI), and (C) factor VII coagulant activity (FVIIC). Shown are mean  $\pm$  SEM for patients ( $n = 108$ – $113$ ) in the placebo and the simvastatin ( $n = 108$ – $114$ ) groups. The  $p$  values shown are for change over time in the placebo or statin group. TFPI levels were different between placebo and statin groups ( $p < .0001$ ) and this is shown. TF-PCA and FVIIC levels were not different between the two groups

statin group. In the placebo group, there was no change in any biomarker. TFPI levels were lower at 6 and 12 months in the statin compared with the placebo group (Figure 1); other biomarkers were similar between groups. There were 23 patients (15 placebo, eight statin) with known cancer at baseline (Table 1). Among the coagulation biomarkers, only FVIIa was lower in the cancer group ( $3.96 \pm 0.83$  mU/mL,  $n = 23$ ) compared with those without cancer ( $4.28 \pm 0.10$  mU/mL,  $n = 200$ ) ( $p = .02$ ). A multivariable analysis performed taking into account the presence of cancer did not reveal a significant effect on the change of coagulation biomarkers between placebo and statin group.

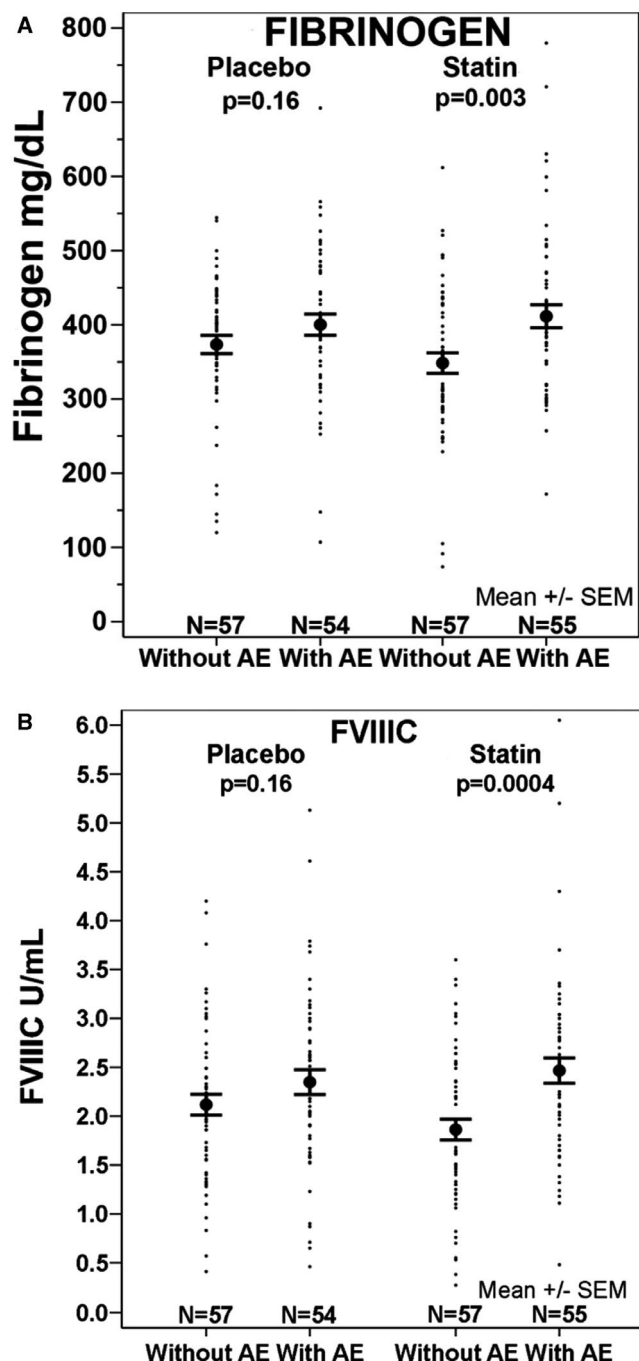
### 3.2 | Relationship of coagulation biomarkers and COPD exacerbations

In the statin group, fibrinogen ( $p = .003$ ) and FVIIC ( $p = .0004$ ) were higher in those who reported exacerbations over the 1-year postrandomization in STATCOPE compared with those without exacerbation; this was not observed in the placebo group (Figure 2). In the placebo group, there were weak correlations between TF-PCA ( $r = .19$ ;  $p = .05$ ), TAT ( $r = -.20$ ,  $p = .04$ ), and D-dimer ( $r = -.20$ ,  $p = .03$ ) levels and the number of acute exacerbations. In the statin group, there was no relationship between the number of exacerbations and biomarker levels.

Because of the potential effects of smoking on disease progression and coagulation parameters, we examined the relationship between the coagulation factor levels and smoking status at enrollment; 33.3% and 35.45% of patients were current smokers in the statin and placebo groups, respectively. The data were analyzed combining patients in the two treatment groups by repeated measures analyses; only plasma D-dimer ( $p = .007$ ) and TAT ( $p = .037$ ) were different between current smokers versus nonsmokers (Figure 3). The change in these biomarkers over time was not statistically significant between smokers versus nonsmokers. Plasma D-dimer was lower at all three time points in current smokers compared with nonsmokers. TAT levels were higher in current smokers at baseline and no different at 6 and 12 months compared with nonsmokers (Figure 3). These findings indicate the effect of smoking status on these parameters and suggest there may be differential effects of smoking on TAT and D-dimer levels.

### 3.3 | Relationships between blood coagulation biomarker levels

We explored the relationships between baseline levels of blood coagulation biomarkers, TF-PCA, plasma fibrinogen, and FVIIC, all



**FIGURE 2** (A) Plasma fibrinogen and (B) factor VIII (mean  $\pm$  SEM) at 12 months in patients with and without acute exacerbation (AE) at 1 year in the placebo or statin groups

of which are acute phase reactants (Table 2). Combining both the statin and placebo groups, there was a positive relationship between membrane-bound TF-PCA with plasma fibrinogen ( $r = .41$ ,  $p < .0001$ ); and between fibrinogen and FVIII ( $r = .27$ ,  $p < .0001$ ). TF-PCA ( $r = .19$ ,  $p = .004$ ) and fibrinogen ( $r = .39$ ,  $p < .0001$ ) levels correlated with CRP levels. TF is a membrane protein and, in this study, we measured it in membranes (TF-PCA) and in plasma (TF antigen). There was no correlation between plasma TF antigen with TF-PCA, plasma FVIII, or plasma fibrinogen. Plasma TF correlated with FVIIa ( $r = .19$ ,  $p = .002$ ). Plasma FVIII correlated with FVIIa ( $r = .24$ ,

$p < .001$ ), and TAT correlated with D-dimer ( $r = .21$ ,  $p = .001$ ). FVIII ( $r = .19$ ,  $p = .004$ ), and FVIIa ( $r = .21$ ,  $p = .001$ ) correlated with TAT levels. In addition, we examined the above relationships using the average of the levels at three time points in each patient, separated by treatment groups, and found the relationships to be the similar (Table S1).

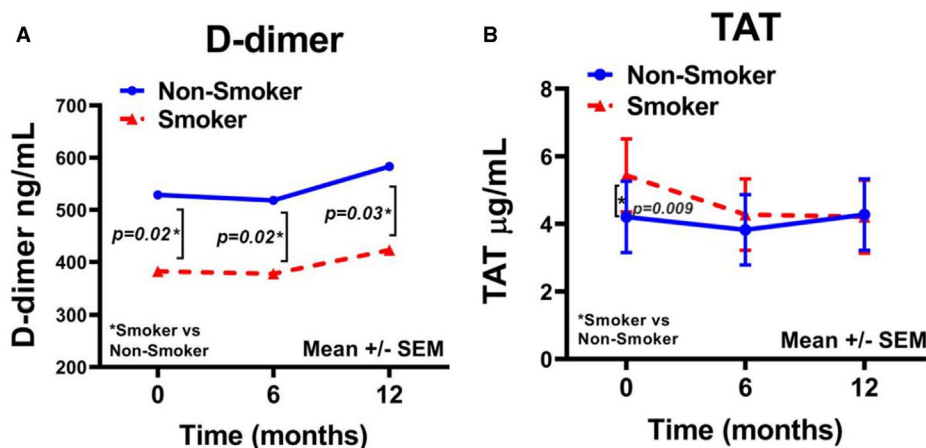
### 3.4 | Relationship between coagulation biomarkers and serum lipids, HbA1c, and CRP

Baseline levels of total cholesterol (mean  $\pm$  standard deviation:  $193.4 \pm 37.0$  vs.  $195.4 \pm 36.7$  mg per deciliter,  $p = .44$ ), low-density lipoprotein (LDL) cholesterol (mean:  $112.0 \pm 29.0$  vs.  $114.0 \pm 29.0$  mg/dl,  $p = .62$ ) and high-density lipoprotein (HDL) cholesterol (mean:  $61.5 \pm 22.4$  vs.  $63.0 \pm 26.3$  mg/dl,  $p = .99$ ), and triglycerides (mean:  $98.8 \pm 57.5$  vs.  $92.2 \pm 49.8$  mg/dl,  $p = .32$ ) were similar in the statin and placebo groups. At 1 year, LDL cholesterol ( $79.8 \pm 31.7$  vs.  $105.4 \pm 28.6$  mg/dl;  $p < .0001$ ) and total cholesterol ( $164.2 \pm 39.4$  vs.  $189.1 \pm 39.4$  mg/dl;  $p < .0001$ ) were lower in the statin group compared to the placebo group; HDL cholesterol levels were similar ( $65.4 \pm 27.6$  vs.  $62.9 \pm 24.5$  mg/dl [ $p = .57$ ]). APOA1, triglycerides, and glucose levels at baseline and 12 months and HbA1c measured at baseline were not different between the groups (data not shown) (Table S2). Baseline CRP levels were similar in both treatment groups (Table S2) and did not change over time in either group.

We explored the relationship between baseline coagulation biomarker and serum lipid levels, combining both groups (Table 3). There was a weak relationship of plasma FVIII with total cholesterol ( $r = .18$ ,  $p = .007$ ) and APOA1 ( $r = .14$ ;  $p = .03$ ); and of plasma FVIIa with HDL ( $r = .18$ ,  $p = .009$ ) and APOA1 ( $r = .15$ ,  $p = .02$ ) levels. A stronger relationship was noted between plasma TFPI and total cholesterol ( $r = .47$ ,  $p < .0001$ ), LDL ( $r = .44$ ,  $p < .0001$ ), HDL ( $r = .18$ ,  $p = .006$ ), and APOA1 ( $r = .22$ ,  $p = .001$ ). There was a weak relationship between FVIII and HDL ( $r = .17$ ;  $p = .009$ ) and APOA1 ( $r = .15$ ;  $p = .02$ ). No relationships were observed between TF-PCA and serum lipid levels. TF-PCA ( $r = .16$ ;  $p = .02$ ) and FVIII ( $r = .21$ ,  $p = .002$ ) correlated with HbA1c at baseline. CRP levels correlated with TF-PCA ( $r = .19$ ,  $p = .004$ ), fibrinogen ( $0.39$ ,  $p < .0001$ ), and D-dimer ( $r = .20$ ,  $p = .002$ ). In addition, we examined the above relationships using an average of the three timed measurements in each patient and separated by treatment groups and found the same relationships (Table S3).

## 4 | DISCUSSION

In this large, multicenter, placebo-controlled trial in moderate-to-severe COPD patients at risk for exacerbations of COPD but without cardiovascular disease, diabetes mellitus, or an indication for a statin, we found that simvastatin (40 mg daily) administered for 12 months did not decrease circulating TF-PCA, but was associated with a small increase in TF-PCA levels (Figure 1). In addition, we



**FIGURE 3** (A) Plasma D-dimer and (B) TAT (mean ± SEM) in current smokers ( $n = 78$ ) and nonsmokers ( $n = 149$ ) at baseline. The data were analyzed combining patients in the statin and placebo groups

**TABLE 2** Correlations between baseline levels of coagulation markers

Biomarker		TF-antigen	FVIIC	FVIIa	FVIIIIC	Fibrinogen	TAT	D-dimer	TFPI
TF-PCA	<i>r</i>	-.05	.08	-.003	.27	.41	-.05	.05	.04
	<i>p</i>	.48	.23	.97	<.0001	<.0001	.50	.47	.56
TF antigen	<i>r</i>		.09	.19	.008	-.03	.07	.15	.03
	<i>p</i>		.19	.002	.91	.61	.30	.02	.63
FVIIC	<i>r</i>			.24	-.06	-.11	.19	-.07	.08
	<i>p</i>			<.001	.34	.09	.004	.33	.24
FVIIa	<i>r</i>				-.01	-.12	.21	-.04	.04
	<i>p</i>				.89	.06	.001	.60	.58
FVIIIIC	<i>r</i>					.30	.02	.10	.11
	<i>p</i>					<.0001	.74	.12	.10
Fibrinogen	<i>r</i>						-.16	-.05	.09
	<i>p</i>						.02	.44	.2
TAT	<i>r</i>							.21	.07
	<i>p</i>							.001	.32
D-dimer	<i>r</i>								.13
	<i>p</i>								-.049

Note: Shown are Pearson's correlations coefficients (*r*) and *p* values, and includes patients from placebo and statin groups ( $N = 222$ -227). Shown in bold are correlations that were significant; *p* values are in italics.

Abbreviations: TF, tissue factor; TF-PCA, tissue factor procoagulant activity, TAT, thrombin antithrombin complexes; TFPI, tissue factor pathway inhibitor.

observed no decrease in plasma fibrinogen, FVIIC, FVIIa, and markers of thrombin generation with the use of statins as reported by others.<sup>4,20,22,23</sup> There was a decrease at 12 months in plasma FVIIC in the statin group (Figure 1), which has been observed in some but not other studies.<sup>4</sup> FVII binds to lipid membranes and the decrease may reflect the lower lipid levels in the statin group. We observed a relationship between FVIIC with total cholesterol and LDL levels. Moreover, the decrease in plasma FVII may reflect increased binding to membrane-bound TF and removal from plasma secondary to the increase in TF-PCA. We have observed a decline in plasma FVIIC associated with an increase in TF-PCA in healthy human volunteers during hyperglycemic clamps.<sup>33</sup> Plasma TFPI decreased in the statin group (Figure 1), as also observed in previous studies.<sup>4</sup> TFPI in plasma circulates bound to lipids<sup>34</sup>; the decline likely reflects the

lipid-lowering effect of statin. In the present study, basal TFPI levels correlated with total, LDL, and HDL cholesterol levels.

Our results differ from reports that statins decrease expression of TF and blood coagulation biomarkers. Most studies were small in size and, more importantly, focused on patients with hypercholesterolemia and/or patients with or at high risk for cardiovascular disease.<sup>4,20,22</sup> Hypercholesterolemia is associated with upregulated monocytes (a source of TF) and blood coagulation mechanisms.<sup>4,9,16</sup> Thus, an unrecognized lipid-lowering statin effect may be a confounding factor in studies where a statin was administered to patients with hyperlipidemia or diabetes mellitus. In STATCOPE, patients with cardiovascular disease, diabetes mellitus, and those with an indication statin therapy were excluded<sup>32</sup>; this enabled us to define the effects of simvastatin unencumbered by the effects



TABLE 3 Correlation between baseline levels of coagulation biomarkers and lipids, HbA1c, blood glucose, and CRP

Biomarker		TF-PCA	TF-antigen	FVIIC	FVIIa	FVIIIC	Fibrinogen	TAT	D-dimer	TFPI
Total cholesterol	<i>r</i>	.007	.06	<b>.18</b>	.07	.05	.05	-.01	.01	<b>.47</b>
	<i>p</i>	.91	.37	<b>.007</b>	.33	.47	.45	.88	.86	<b>&lt;.0001</b>
LDL	<i>r</i>	.04	-.03	.12	-.05	-.08	.11	.03	.08	<b>.44</b>
	<i>p</i>	.59	.70	.07	.46	.21	.12	.66	.23	<b>&lt;.0001</b>
HDL	<i>r</i>	-.06	.11	.07	<b>.17</b>	<b>.17</b>	-.05	-.04	-.08	<b>.18</b>
	<i>p</i>	.42	.11	.29	<b>.008</b>	<b>.009</b>	.47	.51	.24	<b>.006</b>
Triglycerides	<i>r</i>	.05	.03	.12	-.04	-.004	.001	-.02	-.003	.02
	<i>p</i>	.50	.63	.07	.55	.95	.99	.81	.96	.77
APOA1	<i>r</i>	-.09	<b>.15</b>	<b>.14</b>	<b>.15</b>	<b>.15</b>	-.07	-.02	-.05	<b>.22</b>
	<i>p</i>	.17	<b>.03</b>	<b>.03</b>	<b>.02</b>	<b>.02</b>	.31	.72	.45	<b>.001</b>
HbA1c	<i>r</i>	.16	.09	.08	.02	.21	.08	.11	.10	-.01
	<i>p</i>	<b>.02</b>	.18	.28	.80	<b>.001</b>	.23	.08	.12	.81
Blood glucose	<i>r</i>	-.06	-.08	-.03	-.09	-.07	-.03	-.08	.02	-.06
	<i>p</i>	.39	.25	.68	.16	.30	.68	.25	.74	.37
CRP	<i>r</i>	.19	.06	-.02	-.08	.05	<b>.39</b>	-.02	<b>.20</b>	-.05
	<i>p</i>	<b>.004</b>	.038	.83	.25	.48	<b>&lt;.0001</b>	.80	<b>.002</b>	.44

Note: Shown are Pearson's correlation coefficients (*r*) and *p*-values and includes patients from placebo and statin group combined (*N* = 220–226). Shown in bold are correlations that were significant; *p* values are shown in italics.

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TF-PCA, tissue factor procoagulant activity; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

of increased lipids and glucose, or manifest atherothrombotic disease, all of which are associated with elevation in TF and coagulation biomarkers.<sup>4,5,8,9,12</sup> The strengths of this study include the relatively large sample size, the prospective design and the presence of a placebo group.

Previous studies have suggested that statins have anti-inflammatory effects.<sup>3,4</sup> In our study, TF-PCA levels correlated with plasma fibrinogen, FVIIC, and CRP (Tables 2 and 3), three recognized acute-phase reactants indicating that TF-PCA also functions as an acute-phase reactant. We found no decrease in the statin group in fibrinogen, FVIII, TF-PCA, or CRP, which is particularly relevant in COPD because it is an intense pro-inflammatory state. Moreover, plasma fibrinogen and FVIIC were higher in those with COPD exacerbations at 1 year compared with those without (Figure 2), indicating ongoing inflammation. Interestingly, this was observed only in the statin group and the reasons are unclear. In our moderate to severe COPD patients, simvastatin did not decrease CRP levels (current study) or the rate of acute exacerbations.<sup>32</sup> In the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial of apparently healthy persons without hyperlipidemia but with elevated high-sensitivity CRP levels, rosuvastatin significantly reduced CRP levels and the incidence of major cardiovascular events.<sup>35</sup> The lack of effect in our study may be related to the ongoing, greater inflammatory state in COPD patients compared with the healthy people in the JUPITER trial, in addition to the difference in the statin used.

In the statin group, there was a small increase in circulating TF-PCA associated with a decrease in its primary inhibitor TFPI. Elevated levels of circulating TF-PCA are thrombogenic<sup>5</sup> and

reported in patients with diseases associated with increased predisposition to thrombosis.<sup>8–11,33</sup> The STATCOPE trial included patients with moderate-to-severe COPD at increased risk for exacerbations based on their prior exacerbation history and/or the need for use of home oxygen therapy, but without known cardiovascular disease or an indication for a statin. TF is both prothrombotic and pro-inflammatory.<sup>5</sup> COPD patients have an increased predisposition to cardiovascular events.<sup>25,36</sup> In the STATCOPE trial, there were no differences in the fatal or nonfatal cardiovascular events between the placebo and statin groups.<sup>32</sup> Further studies are needed to clarify the impact of the statin-induced changes in TF and coagulation mechanisms on clinical thrombotic events in patients with moderate to severe COPD who are prone to increased exacerbation risk.

Our study has some limitations. The participants had moderate-to-severe airway disease and it is unclear whether the observation that simvastatin did not decrease TF-PCA levels would apply to patients with less pulmonary impairment or those treated with a higher simvastatin dose. Moreover, the findings may not be applicable to a broader population with hyperlipidemias and other comorbidities.

In conclusion, in contrast to previous studies on statins, in COPD patients without diabetes, cardiovascular disease, or requiring a statin treatment, simvastatin (40 mg/day) did not decrease TF or factors VIIa and VIII, fibrinogen, TAT, or D-dimer. The decreases observed in TFPI and factor VII reflect the decrease in serum lipids.

## ACKNOWLEDGMENTS

The National Institutes of Health COPD Clinical Research Network investigators and the Canadian Institute of Health Research Investigators participated in the parent STATCOPE trial.

## CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare with respect to this manuscript.

## AUTHOR CONTRIBUTIONS

A. Koneti Rao and Gerard Criner contributed to the concept and design, analysis and interpretation of data, and writing of the manuscript; Fabiola Del Carpio-Cano performed the assays and contributed to the writing of the manuscript; Sumalaxmi Janapati performed the assays; Huaqing Zhao, Helen Voelker, and Xiaoning Lu contributed to the analyses and interpretation of the data, and contributed to the manuscript.

## ORCID

A. Koneti Rao  <https://orcid.org/0000-0002-3078-7778>

## REFERENCES

- Brugts JJ, Yetgin T, Hoeks SE, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ*. 2009;338:b2376.
- Ross SD, Allen IE, Connelly JE, et al. Clinical outcomes in statin treatment trials: a meta-analysis. *Arch Intern Med*. 1999;159:1793-1802.
- Oesterle A, Laufs U, Liao JK. Pleiotropic effects of statins on the cardiovascular system. *Circ Res*. 2017;120:229-243.
- Undas A, Brummel-Ziedins KE, Mann KG. Statins and blood coagulation. *Arterioscler Thromb Vasc Biol*. 2005;25:287-294.
- Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38:709-725.
- Giesen PLA, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: another view of thrombosis. *Proc Natl Acad Sci USA*. 1999;96:2311-2315.
- Singh A, Boden G, Homko C, Gunawardana J, Rao AK. Whole-blood tissue factor procoagulant activity is elevated in type 1 diabetes: effects of hyperglycemia and hyperinsulinemia. *Diabetes Care*. 2012;35(6):1322-1327.
- Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. *J Clin Endocrinol Metab*. 2007;92:4352-4358.
- Sambola A, Osende J, Hathcock J, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation*. 2003;107:973-977.
- Gentile NT, Vaidyula VR, Kanamalla U, DeAngelis M, Gaughan J, Rao AK. Factor VIIa and tissue factor procoagulant activity in diabetes mellitus after acute ischemic stroke: impact of hyperglycemia. *Thromb Haemost*. 2007;98:1007-1013.
- Key NS, Slungaard A, Dandele L, et al. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. *Blood*. 1998;91:4216-4223.
- Vaidyula VR, Criner GJ, Grabianowski C, Rao AK. Circulating tissue factor procoagulant activity is elevated in stable moderate to severe chronic obstructive pulmonary disease. *Thromb Res*. 2009;124:259-261.
- Bruni F, Puccetti L, Pasqui AL, et al. Different effect induced by treatment with several statins on monocyte tissue factor expression in hypercholesterolemic subjects. *Clin Exp Med*. 2003;3:45-53.
- Ferro D, Basili S, Alessandri C, Cara D, Violi F. Inhibition of tissue-factor-mediated thrombin generation by simvastatin. *Atherosclerosis*. 2000;149:111-116.
- Aikawa M, Rabkin E, Sugiyama S, et al. An HMG-CoA reductase inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases and tissue factor in vivo and in vitro. *Circulation*. 2001;103:276-283.
- Owens AP, Passam FH, Antoniak S, et al. Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. *J Clin Invest*. 2012;122:558-568.
- Steiner S, Speidl WS, Pleiner J, et al. Simvastatin blunts endotoxin-induced tissue factor in vivo. *Circulation*. 2005;111:1841-1846.
- Undas A, Brummel-Ziedins KE, Potaczek DP, et al. Atorvastatin and quinapril inhibit blood coagulation in patients with coronary artery disease following 28 days of therapy. *J Thromb Haemost*. 2006;4:2397-2404.
- Undas A, Celinska-Lowenhoff M, Brummel-Ziedins KE, Brozek J, Szczeklik A, Mann KG. Simvastatin given for 3 days can inhibit thrombin generation and activation of factor V and enhance factor Va inactivation in hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2005;25:1524-1525.
- Undas A, Celinska-Löwenhoff M, Domagala TB, et al. Early antithrombotic and anti-inflammatory effects of simvastatin versus fenofibrate in patients with hypercholesterolemia. *Thromb Haemost*. 2005;94:193-199.
- Undas A, Brummel KE, Musial J, Mann KG, Szczeklik A. Simvastatin depresses blood clotting by inhibiting activation of prothrombin, factor V, and factor XIII and by enhancing factor Va inactivation. *Circulation*. 2001;103:2248-2253.
- Sanguigni V, Pignatelli P, Lenti L, et al. Short-term treatment with atorvastatin reduces platelet CD40 ligand and thrombin generation in hypercholesterolemic patients. *Circulation*. 2005;111:412-419.
- Adams NB, Lutsey PL, Folsom AR, et al. Statin therapy and levels of hemostatic factors in a healthy population: the Multi-Ethnic Study of Atherosclerosis. *J Thromb Haemost*. 2013;11:1078-1084.
- Undas A, Kaczmarek P, Sladek K, et al. Fibrin clot properties are altered in patients with chronic obstructive pulmonary disease. Beneficial effects of simvastatin treatment. *Thromb Haemost*. 2009;102:1176-1182.
- Andre S, Conde B, Fragoso E, Boléo-Tomé JP, Areias V, Cardoso J. COPD and cardiovascular disease. *Pulmonology*. 2019;25:168-176.
- Barba R, Zapatero A, Marco J, et al. Venous thromboembolism in COPD hospitalized patients. *J Thromb Thrombolysis*. 2012;33:82-87.
- Moua T, Wood K. COPD and PE: a clinical dilemma. *Int J Chron Obstruct Pulmon Dis*. 2008;3(2):277-284.
- Børvik T, Brækkan SK, Enga K, et al. COPD and risk of venous thromboembolism and mortality in a general population. *Eur Respir J*. 2016;47:473-481.
- Akpınar EE, Hosgun D, Akpınar S, Atac GK, Doganay B, Gulhan M. Incidence of pulmonary embolism during COPD exacerbation. *J Bras Pneumol*. 2014;40:38-45.
- Beghe B, Verduri A, Roca M, Fabbri LM. Exacerbation of respiratory symptoms in COPD patients may not be exacerbations of COPD. *Eur Respir J*. 2013;41(4):993-995.
- He Y, Li X, Gasevic D, et al. Statins and multiple noncardiovascular outcomes: umbrella review of meta-analyses of observational studies and randomized controlled trials. *Ann Intern Med*. 2018;169:543-553.
- Criner GJ, Connett JE, Aaron SD, et al. Simvastatin for the prevention of exacerbations in moderate-to-severe COPD. *N Engl J Med*. 2014;370:2201-2210.
- Vaidyula VR, Rao AK, Mozzoli M, Homko C, Cheung P, Boden G. Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. *Diabetes*. 2006;55:202-208.
- Mast AE. Tissue factor pathway inhibitor: multiple anticoagulant activities for a single protein. *Arterioscler Thromb Vasc Biol*. 2016;36:9-14.
- Ridker PM, Danielson E, Fonseca FAH, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195-2207.



36. Curkendall SM, deLuise C, Jones JK, et al. Cardiovascular disease in patients with chronic obstructive pulmonary disease, Saskatchewan Canada cardiovascular disease in COPD patients. *Ann Epidemiol*. 2006;16:63-70.

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Rao AK, Del Carpio-Cano F, Janapati S, et al. Effects of simvastatin on tissue factor pathway of blood coagulation in STATCOPE (Simvastatin in the prevention of COPD exacerbations) trial. *J Thromb Haemost*. 2021;19:1709–1717. <https://doi.org/10.1111/jth.15282>