Supplemental Material to Zolcinski et al. ” Effects of atorvastatin on plasma fibrin clot properties in apparently healthy individuals and patients with previous venous thromboembolism” (Thromb Haemost 2012; 107.5)

Patients

The inclusion criterion for the control group was low cardiovascular risk according to the guidelines developed by European Society of Cardiology. Subjects with signs of venous insufficiency were excluded from the control group. Angina, acute thrombosis, diabetes, hemorrhagic diathesis, autoimmune diseases, hypo- or hyperthyroidism, acute inflammation, use of any medication (apart from antihypertensive drugs) and severe comorbidities e.g. cancer were the exclusion criteria for both groups.

Overweight was defined as body mass index (BMI) of 25–29.9 kg/m^2. Arterial hypertension was diagnosed in subjects with diastolic blood pressure above 90 mmHg, but less than 100 mmHg and/or systolic pressure in the range of 140-160 mmHg at least 2 times or when individuals were treated with antihypertensive medications. Hypertensive subjects with mild arterial hypertension were eligible. Current smoking was defined as smoking of 5 or more cigarettes daily. Family history of VTE or coronary artery disease was established when at least one first-degree relative of a subject declared a previous documented episode of any of these diseases.

In VTE patients genotyping for factor (f)V Leiden (A1691G) and prothrombin (G20210A) polymorphism was performed. Lupus anticoagulant, anticardiolipin and anti-β_2-glycoprotein I antibodies of IgG and IgM isotype, antithrombin (AT), protein C, free protein S and fVIII were assayed.

Statistical analysis

Data were shown as mean±SD or median and interquartile range (IQR) as appropriate. Normal distribution was tested with the Shapiro-Wilk test. Independence of categorical variables was verified by Chi-square test. The correlation analysis was performed with ranked Spearman test. Comparisons between VTE patients and controls were carried out by unpaired t-test for normally distributed independent variables, paired t-test for normally distributed dependent variables, whereas for non-normally distributed variables nonparametric tests were used: Kruskall-Wallis test for independent and Wilcoxon test for dependent variables. Analysis of covariance (ANCOVA) was performed on fibrin clot parameters between groups (before and after treatment), as covariates we considered the possible random differences in fibrinogen and CRP concentrations. The minimal sample size for interventional study was computed with respect to a 2-tailed Student t test for independent groups since standard deviation of paired differences was not known; we considered the following: (1) mean K_s, $t_{50\%}$ and their respective SDs from the previous study;[10] (2) expected 25% increase in mean K_s and 20% decrease in mean $t_{50\%}$ and (3) type 1 error probability $\alpha=0.05$ and power $1-\beta=0.90$; this resulted in n=20 for K_s and n=14 for $t_{50\%}$. Twenty-eight patients were recruited, exceeding the minimal computed sample size for both variables.

Suppl. Table 1: Characteristics of patients with venous thromboembolism (VTE) and controls.

	VTE patients (n=28)	Controls (n=25)	P
age (yrs)	43.9 ±11.1	42.4 ±10.5	0.60
male gender, n (%)	14 (56%)	14 (50%)	0.67
BMI (kg/m^2)	28.3 (24.8-30.5)	24.4 (23.0-27.8)	0.04
current smoking, n (%)	9 (32%)	6 (24%)	0.52
arterial hypertension, n (%)	6 (21%)	4 (16%)	0.62
administration of ACEI, n (%)	3 (11%)	3 (12%)	0.89

family history of CAD, n (%)	6 (21%)	5 (20%)	0.91
family history of VTE, n (%)	4 (14%)	0 (0%)	0.05

Values are given as mean ± SD or median (interquartile range) or percentage. BMI, body mass index; ACEI, angiotensin-converting enzyme inhibitors; CAD, coronary artery disease.

Suppl. Table 2: Fibrin clot parameters at the baseline and after 3 days of atorvastatin 40 mg/day in venous thromboembolism (VTE) patients depending on the presence or absence of residual venous thrombosis (RVT). Day 0 denotes the day before atorvastatin and day 3 denotes the last day of atorvastatin administration.

Variable	**RVT present (n=9)**	**RVT absent (n=19)**	**P**
K_s (10^{-9} cm^2)			
Day 0	6.03 ±0.9	7.18 ±1.4	0.03
Day 3	7.85 ±0.6	8.68 ±0.8	
ΔK_s	1.8 ±0.9	1.5 ±1.4	0.54
lag phase (s)			
Day 0	41.2 ±2.4	44.5 ±2.8	0.005
Day 3	42.7 ±4.8	46.6 ±3.2	
Δlag phase	1.5 ±5.4	2.1 ±4.4	0.70
ΔAbs (405nm)			
Day 0	0.93 ±0.04	0.86 ±0.06	0.005
Day 3	0.83 ±0.09	0.85±0.08	
ΔΔAbs	0.10 ±0.07	0.01 ±0.07	0.005
$t_{50\%}$ (min)			
Day 0	10.5 ±1.1	9.2 ±1.4	0.03
Day 3	8.5 ±1.1	7.4 ±1.1	
$\Delta t_{50\%}$	2.1 ±0.7	1.8 ±1.5	0.61
CLT (min)			

Day 0	93.3 ±12.7	87.3 ±13.7	0.3
Day 3	93.7 ±5.5	69.9 ±14.2	
ΔCLT	-0.4 ±12.5 *	17.4 ±6.4	<0.0001
D-D $_{max}$ (mg/L)			
Day 0	4.4 ±0.56	4.0 ±0.33	0.02
Day 3	4.2 ±0.37	3.9 ±0.45	
ΔD-D $_{max}$	0.4 ±0.7	0.1 ±0.5	0.37
D-D$_{rate}$ (mg/L/min)			
Day 0	0.067 ±0.007	0.068 ±0.004	0.7
Day 3	0.070 ±0.005	0.078 ±0.006	
ΔD-D $_{rate}$	0.003 ±0.01	0.009 ±0.01	0.04

Values are given as mean ± SD. RVT – residual venous thrombosis. * CLT increased after atorvastatin in patients with RVT. Abbreviations (see Table 1 in manuscript).