The impact of statin therapy on plasma levels of von Willebrand factor antigen

Systematic review and meta-analysis of randomised placebo-controlled trials

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Summary

Increased plasma levels of von Willebrand factor antigen (vWF:Ag) are associated with high risk of coronary artery disease. The effect of statin therapy on vWF:Ag levels remains uncertain. Therefore the aim of this meta-analysis was to evaluate the effect of statin therapy on vWF:Ag Levels. A systematic multiple-database search was carried out to identify randomized controlled trials (RCTs) that investigated the effect of statins on plasma vWF:Ag levels. Random-effect meta-analysis of 21 treatment arms revealed a significant decrease in plasma vWF:Ag levels following statin therapy (SMD: –0.54, 95 %CI: –0.87, –0.21, p=0.001). In subgroup analyses, the greatest effect was observed with simvastatin (SMD: –1.54, 95 %CI: –2.92, –0.17, p=0.028) and pravastatin (SMD: –0.61, 95 %CI: –1.18, –0.04, p=0.035), but not with fluvastatin (SMD: –0.34, 95 %CI: –0.69, 0.02, p=0.065), atorvastatin (SMD: –0.23, 95 %CI: –0.57, 0.11, p=0.179) and rosuvastatin (SMD: –0.20, 95 % CI: –0.71, 0.30, p=0.431). The lowering effect of statins on plasma vWF:Ag levels was greater in the subset of studies lasting ≥ 12 weeks (SMD: –0.70, 95 %CI: –1.19, –0.22, p=0.005) compared with that of studies lasting < 12 weeks (SMD: –0.34, 95 %CI: –0.67, 0.003, p=0.052). Finally, low-intensity statin therapy was not associated with a significant reduction in vWF:Ag levels (SMD: –0.28, 95 %CI: –0.82, 0.27, p=0.320), but a significant effect was observed in high-intensity statin trials (SMD: –0.66, 95 %CI: –1.07, –0.24, p=0.002). This meta-analysis of available RCTs demonstrates a significant reduction in plasma vWF:Ag levels following statin therapy.

Keywords

von Willebrand Antigen, statins, cardiovascular diseases, atherosclerosis, thrombosis

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Introduction

Von Willebrand factor (vWF) is a glycoprotein that is released into circulation from vascular endothelial cells and megakaryocytes. vWF is involved in platelet adhesion, coagulation factor VIII binding and transport, and formation and deposition of thrombus (1, 2). Circulating vWF is nearly entirely of endothelial source. Elevated vWF concentrations have been shown to be associated with endothelial dysfunction and/or injury (3). After secretion, vWF undergoes a process of dimerisation and multimerisation with larger multimer size offering a greater biological activity of vWF than small ones in terms of platelet adhesion (2). The large vWF multimers are cleaved into smaller and significantly less procoagulant forms by a metalloproteinase, named a *Disintegrin and Metalloprotease with TrompoSpondin motif repeats 13* (ADAMTS13) (4). VWF:antigen (vWF:Ag) is generally evaluated utilising either LIA (latex-immunoassay) or ELISA (enzyme linked immunosorbent assay) techniques (5). In screening for abnormal vWF associated disorders, vWF:Ag levels are commonly measured (6).

It has been shown that several genetic and non-genetic factors could influence the plasma levels of vWF. ABO blood groups, race and gender difference affect the levels of vWF antigen, higher

values being observed in African-Americans than Caucasians (7) and lower levels in blood group 0 individuals than non-0 individuals (8, 9). The CHARGE consortium analysed 23,000 European-ancestry individuals from five studies: the Atherosclerosis Risk in Communities (ARIC) Study (10), the Cardiovascular Health Study (CHS) (11), the Framingham Heart Study (FHS) (12), the Rotterdam Study (13), and the British 1958 Birth Cohort (14). The investigators noticed eight genetic variations associated with vWF:Ag levels and five genetic variations associated with FVIII activity (FVIII:c) (15). Other genes encoding three specific receptors: stabilin-2, LRP1 (also known as CD91), and CLEC4M (also known as CD299) have also been suggested as determinants of vWF plasma levels. Furthermore, single nucleotide polymorphisms (SNPs) in the vWF gene lead to a great variability in plasma vWF concentrations (16). Besides genetic factors, age, stress, physical activity, diet, hypernatremia (17), hormones (18), inflammation (19), hypoxia (20), shear stress (21) and pregnancy might affect plasma concentration and/or activity of vWF (22).

The levels of vWF have been evaluated in several randomised controlled trials (RCTs) (23). Some studies have showed that statin therapy decreases plasma vWF:Ag levels (24, 25), but other studies yielded inconsistent data. The regulation of statin-induced alterations to vWF secretion and metabolism in humans is not fully clarified. Beyond their cholesterol-lowering effects, a number of pleiotropic effects of statins, including antithrombotic actions (26, 27), have been ascribed to statin; however, the relevance of these effects remain a matter of debate (28, 29). We set out to evaluate the impact of statin therapy on circulating vWF:Ag levels using a systematic review and meta-analysis of RCTs.

Material and methods

Search strategy

This study was designed according to the guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement (30). SCOPUS (http://www.scopus.com) and Medline (http://www.ncbi.nlm.nih.gov/pubmed) databases were searched using the following search terms in titles and abstracts (also in combination with MESH terms): (atorvastatin OR simvastatin OR rosuvastatin OR fluvastatin OR pravastatin OR pitavastatin OR lovastatin OR cerivastatin OR "statin therapy" OR statins OR statin) AND ("von Willebrand" OR „Willebrand" OR vWF OR „factor VIII") AND (placebo). The wild-card term "*" was used to increase the sensitivity of the search strategy. No language restriction was used in the literature search. The search was limited to studies in human. The literature was searched from inception to January 31, 2015.

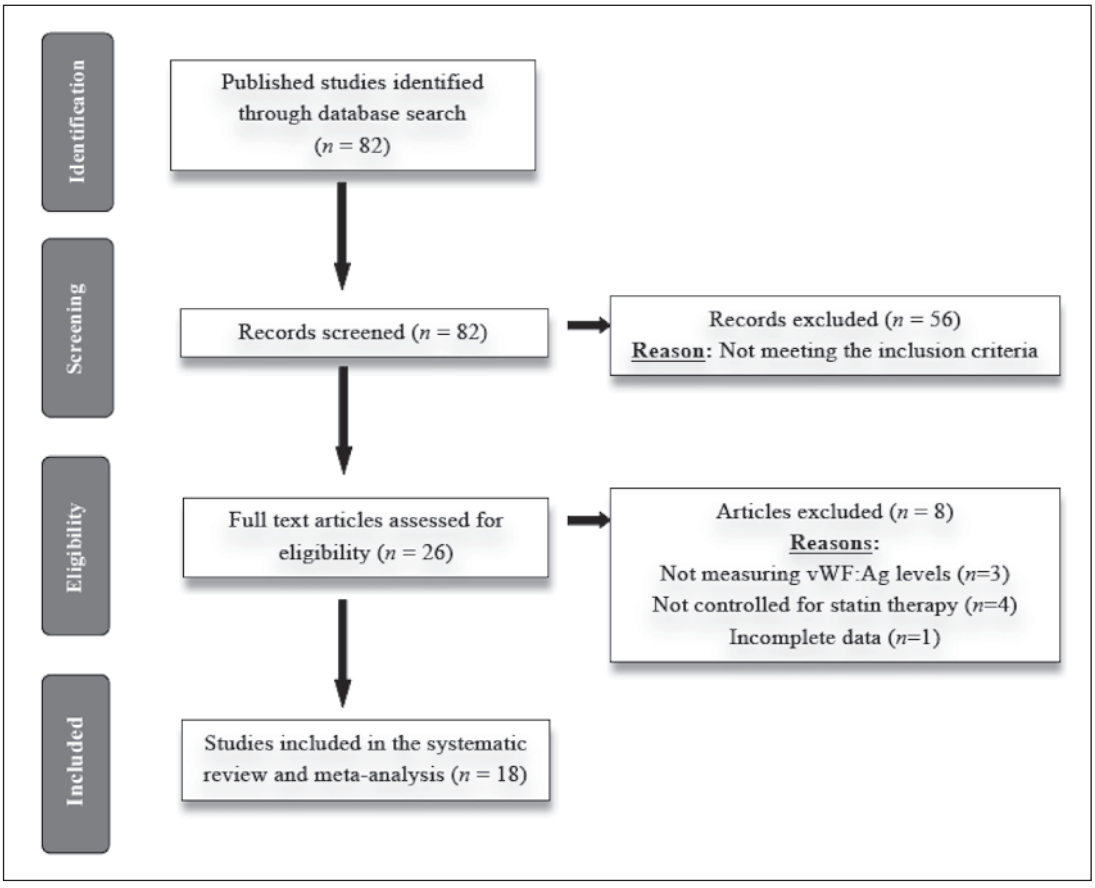

Figure 1: Flow chart of the number of studies identified and included into the meta-analysis.

Table 1: Demographic characteristics and baseline parameters of the studies selected for analysis.

Study		Abou Raya et al. (48)	Almquist et al. (49)		Ambrosi et al. (50)	Barreto et al. (51)	Blann et al. (52)	Casey et al. (53)	Hjelstuen et al. (54)	Kondur-acka et al. (55)	Krysiak & Okopien (56)
Year		2007	2012		2000	2008	2001	2007	2007	2008	2013
Location		Egypt	Sweden		France	Brazil	United Kingdom	Ireland	Norway	Poland	Poland
Design		Randomised, placebo-controlled parallel group trial	Randomised double-blind cross-over group trial		Randomised, double-blind, placebo controlled, crossover group trial	Randomised double-blind placebo-controlled parallel group trial	Randomised double-blind placebo-controlled parallel group trial	Randomised double-blind placebo-controlled cross-over group trial	Randomised 2×2 factorial intervention trial	Randomised placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial
Duration of trial		6 months	20 weeks		8 weeks	6 months	4 months	2 weeks	12 months	6 months	90 days
Inclusion criteria		Patients fulfilling the ACR preliminary classification criteria for Systemic Sclerosis	Patients aged 18–80 yrs, with DM type 1 or 2 and an estimated glomerular filtration rate (eGFR) of 15–59 mL/min × 1.73 m^2, i. e. CKD stages 3–4, (DM-CKD group) or >75 mL/min × 1.73 m^2 (DM-only group).		Heart transplant recipients with plasma cholesterol above 5.5 mmol/l and a time since transplantation higher than 6 months	Adolescents and adults with pulmonary arterial hypertension either idiopathic or associated with congenital heart disease, in the absence or presence of hypoxemia (resting SpO2 of ≥90 or <90 %, respectively)	Patients with peripheral artery disease proved by Doppler/ultrasound stenosis of the iliac and/or femoral arteries and/or stenosis of a carotid artery, and total cholesterol between 5.5 and 7.5 mmol/l (210 to 290 mg/ml).	Young male patients with type 1 diabetes free from any evidence of clinical vascular disease, normoalbuminuria, non-smokers, no medication apart from appropriate insulin doses, and no dyslipidaemia.	Men between 40 and 75 yrs of age on drug treatment for essential hypertension, being overweight, defined as BMI between 25 and 35 kg/m2, and having a sedentary lifestyle, defined as less than 1 hour of regular exercise a week.	Consecutive Caucasians with long-duration (>10 years) type1DM, without CHD and AH	Subjects (30–70 yrs old) with peripheral artery stenosis, isolated hypertriglyceridemia (triglyceride levels between 200 and 500 mg/dl) and a family history of coronary artery disease. Other inclusion criteria: plasma total cholesterol<200 mg/dl, and LDL cholesterol <130 mg/dl.
Statin form		atorvastatin	simvastatin#		fluvastatin	rosuvastatin	pravastatin	pravastatin	fluvastatin	atorvastatin	simvastatin
Statin intervention		40 mg/day	40mg/day		40 mg/day	10 mg/day	40 mg/day	40 mg/day	40 mg/day	40 mg/day	40 mg/day
Participants	Case	20	21*	18**	20	30	17	9	42	154	20
	Control	20				30	15		41	50	19
Age (years)	Case	59.9 ± 10.1	64 ± 7*	67 ± 6**	59.1 ± 9	34.6 ± 12.3	65 ± 8	22 ± 3.8	55.8 ± 7.9	36.3 ± 8.3	49 ± 5
	Control	58.7 ± 9.2				33.7 ± 11.1	61 ± 12		57.599.2		47 ± 4
Male (%)	Case	22.5	61.9*	55.5**	90.0	40.0	58.82	100.0	100.0	45.09	60.0
	Control	12.5					60.0		100.0		63.0
BMI (kg/m^2)	Case	NR	NR*	NR**	NR	NR	NR	25.1 ± 2.2	29.1 ± 2.6	25.5 ± 6	28.1 ± 2.2
	Control	NR				NR	NR	25.5 ± 1.7	29.4 ± 2.2		27.9 ± 2.6
hs-CRP (mg/l)	Case	3.79 ± 1.8	1.1(0.7; 3.6) *	1.9(1.3; 4.7) **	NR	NR	425 (216–694)	NR	NR	1.39 (0.70–2.80)	NR
	Control	3.85 ± 1.4				NR	217 (77–583)	NR	NR	1.62 (0.75–3.10)	NR
Total cholesterol (mg/dl)	Case	198.3 ± 25.5	216.16 ± 38.6*	231.6 ± 38.6**	214.23 ± 38.6	NR	250.9 ± 23.16	150.54 ± 11.58	226.97 ± 28.95	NR	168 ± 16
	Control	189.4 ± 25.9			270.97 ± 50.18	NR	235.46 ± 23.16	162.12 ± 15.44	232.37 ± 37.83	NR	164 ± 15

Krysiak et al. (57)	Liu et al. (58)	Lynch et al. (59)	McCarey et al. (60)	Sadik et al. (61)	Tan et al. (62)	Tehrani et al. (63)	Van de Ree et al. (64)	Ordulu et al. (25)
2011	2009	2005	2004	2010	1999	2013	2003	2008
Poland	China	USA	United Kingdom	United Kingdom	Hong Kong	Sweden	Netherlands	Turkey
Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised double-blind placebo-controlled crossover group trial	Randomised double-blind placebo-controlled parallel-group trial	Randomised placebo-controlled parallel-group trial
90 days	12 weeks	14 days	6 months	8 weeks	12 weeks	2 months	30 weeks	2 weeks
Persons aged 20–75 yrs screened for the presence of asymptomatic atherosclerosis by the sonographic assessment of common carotid intima-media thickness with primary isolated hypercholesterolemia, defined as plasma total cholesterol >200 mg/dL, LDL cholesterol >130 mg/dL, and triglycerides <150 mg/dL.	Patients with idiopathic dilated cardiomyopathy with: (1) age ≥18 and ≤70 years; (2) stable NYHA functional class II or III congestive heart failure of unknown cause; (3) left ventricle ejection fraction (LVEF)<40 % by radionuclide angiography; (4) sinus rhythm and (5) high-quality ultrasonography images	Patients presenting within 48 hours of aneurysmal subarachnoid hemorrhage	Patients aged 18–80 yrs with active rheumatoid arthritis (ACR criteria) despite ongoing disease-modifying antirheumatic drug therapy	Patients with systemic sclerosis aged between 18 and 75 yrs	Patients aged between 35 and 70 years with type 2 diabetes mellitus	Patients aged between30 and 70 yrs with type 1 diabetes and elevated levels of low-density lipoprotein (LDL) (>2.5 mmol/L) and/or total cholesterol (>4.5 mmol/L)	Patients aged 45–75 with type 2 DM and a typical diabetic dyslipidemia	Consecutive patients who were admitted to the coronary care unit with the diagnosis of unstable angina and non-ST elevation acutemyocardial infarction
simvastatin	atorvastatin	simvastatin	atorvastatin	atorvastatin	fluvastatin	atorvastatin	atorvastatin	atorvastatin
40 mg/day	10 mg/day	80 mg/day	40 mg/day	20 mg/day	20 mg/day and 40 mg/day§§	80 mg/day	10 mg/day or 80 mg/day	10 mg/day or 80 mg/day
25	32	19	58	18	37	40	69	20
24	32	20	58	18	20		61	20
53.9 ± 3.5	49.4 ± 6.4	65 (48–73)	55.5 ± 11.8	60 (43–73)§	58 ± 6	44(39–61)	59.7 ± 7.6	61.10 ± 10.92
52.4 ± 2.2	51.9 ± 5.2	47 (41–53)	56.5 ± 10.0	56 (39–65)§	60 ± 8		58.6 ± 7.5	65.95 ± 10.76
56.0	68.75	15.79	12.07	16.67	24.32	50.0	62.0	60.0
54.2	62.5	15.0	15.52	11.11	35.0		48.0	40.0
27.9 ± 2.6	20.89 ± 3.27	NR	NR	NR	25.9 ± 5.6	25 ± 3	29.9 ± 3.8	27.61 ± 3.04
28.6 ± 2.3	21.22 ± 2.37	NR	NR	NR	24.4 ± 2.8		32.2 ± 6.1	28.92 ± 3.82
NR	7.69 ± 2.20	NR	11.6 ± 2.95	3.1 (1.5–6.4)	NR	NR	NR	5.26 ± 3.24
NR	7.13 ± 2.34	NR	13.18 ± 3.62$	1.7 (0.6–3.4)	NR		NR	6.3 ± 2.87
255.0 ± 13.8	192.61 ± 33.58	NR	196.09 ± 31.27	104.22 (96.5–119.66)	260 ± 31	185.28 ± 19.3	227.74 ± 34.74	NR
247.2 ± 10.9	193.39 ± 25.86	NR	198.02 ± 38.21	115.8 (100.36–123.52)-	255 ± 24		231.6 ± 30.88	NR

Table 1: Continued

Study		Abou Raya et al. (48)	Almquist et al. (49)		Ambrosi et al. (50)	Barreto et al. (51)	Blann et al. (52)	Casey et al. (53)	Hjelstuen et al. (54)	Kondur-acka et al. (55)	Krysiak & Okopien (56)
LDL-C (mg/dl)	Case	112.6 ± 21.4	131.5 ± 30.88*	146.68 ± 38.6**	NR	NR	165.98 ± 23.16	65.62 ± 30.88	148.61 ± 28.95	119.66 (111.55–138.9)	96 ± 8
	Control	111.8 ± 20.9				NR	165.98 ± 19.3	88.78 ± 27.02	150.93 ± 35.9	119.66 (111.55–135.1)	95 ± 8
HDL-C (mg/dl)	Case	59.9 ± 19.6	50.18 ± 15.44*	50.18 ± 15.44**	NR	NR	44.78 ± 8.88	57.9 ± 7.72	48.25 ± 11.19	57.9 (46.32–69.48)	47 ± 5
	Control	60.1 ± 19.5				NR	43.62 ± 8.11	61.76 ± 7.72	49.79 ± 13.12	59.83 (42.46–67.94)	46 ± 5
Trig-lyce-rides (mg/dl)	Case	NR	168.15 ± 123.9*	168.15 ± 115.05 **	165.49 ± 70.8	NR	194.7(150.45–221.25)	123.9 ± 53.1	154.87 ± 82.3	94.69 (70.8–118.5)	298 ± 40
	Control	NR			200.01 ± 97.35	NS	132.75(106.2–247.8)	132.75 ± 97.35	160.18 ± 102.66	97.35 (71.68–128.3)	287 ± 35
Glucose (mg/dl)	Case	NR	NR*	NR**	NR	NR	NR	275.4 ± 154.8	NR	100.8 ± 23.4	91 ± 4
	Control	NR				NR	NR	343.8 ± 248.4	NR	102.6 ± 21.6	90 ± 4
SBP (mmHg)	Case	NR	131 ± 13*	143 ± 19**	NR	NR	149 ± 19	95 ± 2	141.8 ± 12.3	125.2 ± 12.6	132 ± 8
	Control	NR				NR	157 ± 24	96 ± 4	140.4 ± 15.3	124.7 ± 12.4	131 ± 8
DBP (mmHg)	Case	NR	72 ± 9*	70 ± 11**	NR	NR	81 ± 11	80 ± 4	90.5 ± 7.4	74.3 ± 11.5	85 ± 4
	Control	NR				NR	84 ± 21	90 ± 4	88.6 ± 9.0	75.6 ± 12.0	87 ± 4

Values are expressed as mean ± SD or median (25–75 percentiles). ABBREVIATIONS: BMI: body mass index; NR: not reported; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; hs-CRP: high-sensitivity C-reactive protein; BMI: body mass index; eGFR: estimated glomerular filtration rate; CKD: concomitant chronic kidney disease; CHD: coronary heart disease; AH: arterial hypertension; ACR: American College of

Study selection

Original studies were included if they met the following criteria: i) randomised placebo-controlled trial with either parallel or cross-over design, ii) investigating the impact of statin therapy on plasma/serum concentrations of vWF:Ag, iii) treatment duration of at least two weeks, iv) presentation of sufficient information on vWF:Ag concentrations at baseline and at the end of follow-up in each group or providing the net change values.

Exclusion criteria were i) non-randomised trials, ii) lack of an appropriate control group for statin treatment arm(s), iii) observational studies with case-control, cross-sectional or cohort design, iv) trials that recruited subjects receiving stable statin therapy, iv) measurement of factor VIII coagulant activity or ristocetin cofactor activity instead of vWF:Ag, and iv) lack of sufficient information on baseline or follow-up vWF:Ag concentrations.

Data extraction

Eligible studies were reviewed and the following data were abstracted: 1) first author's name; 2) year of publication; 3) study location; 4) study design; 5) number of participants in the statin and control (in case of randomised design) groups; 5) age, gender and body mass index (BMI) of study participants; 6) baseline levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, high-sensitivity C-reactive protein (hs-CRP) and glucose; 7) systolic and

Krysiak et al. (57)	Liu et al. (58)	Lynch et al. (59)	McCarey et al. (60)	Sadik et al. (61)	Tan et al. (62)	Tehrani et al. (63)	Van de Ree et al. (64)	Ordulu et al. (25)
182.0 ± 10.5	110.78 ± 13.9	NR	120.05 ± 29.34	NR	187 ± 25	119.66 ± 19.3	142.82 ± 34.74	120.99 ± 39.83
174.9 ± 8.9	111.94 ± 11.58	NR	129.70 ± 34.35	NR	186 ± 20		146.68 ± 30.88	96.07 ± 27.50
46.4 ± 4.2	NR	NR	52.50 ± 18.14	NR	44 ± 12	46.32 (46.32–54.0)	40.53 ± 10.04	NR
47.2 ± 4.2	NR	NR	50.95 ± 50.57	NR	42 ± 8		40.5 ± 8.11	NR
121.6 ± 10.2	147.79 ± 16.81	NR	118.59 ± 53.98	NR	148(129–167)	61.95 ± 26.55	221.25 ± 79.65	NR
120.2 ± 9.8	155.76 ± 22.12	NR	123.01 ± 87.61	NR	132(107–156)		230.1 ± 79.65	NR
NR	NR	NR	NR	NR	149.4 ± 39.6	NR	185.4 ± 55.8	NR
NR	NR	NR	NR	NR	140.4 ± 45		189 ± 64.8	NR
NR	115 ± 16	NR	NR	NR	137 ± 13	130 ± 15	NR	134.50 ± 22.99
NR	113 ± 15	NR	NR	NR	139 ± 12		NR	136.50 ± 18.64
NR	67 ± 6	NR	NR	NR	81 ± 5	74 ± 8	NR	78.40 ± 13.32
NR	63 ± 7	NR	NR	NR	79 ± 6		NR	84.25 ± 11.27

Rheumatology; #treatment arms were simvastatin + ezetimibe 10 mg and simvastatin + placebo; *denotes DM-only group; **denotes DM-CKD group; §data expressed as median and range; §§ patients received fluvastatin 20 mg/day for 6 weeks and 40 mg/day for the subsequent 6 weeks; $expressed as geometric means (95 % confidence intervals).

diastolic blood pressures; and 8) data regarding baseline and follow-up concentrations of vWF:Ag.

Quality assessment

According to the Cochrane Collaboration (31), a specific tool for assessing risk of bias in each included study comprises judgment of specific features of the study. This involves assessing the risk of bias as 'low risk', as 'high risk or as 'unclear risk'. The last category indicates either lack of information or uncertainty over the potential for bias. There are seven analysed domains comprising: sequence generation (selection bias), allocation sequence concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias) and other potential sources of bias.

Quantitative data synthesis

Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, Englewood, NJ, USA) (32). Net changes in measurements (change scores) were calculated as follows: measure at end of follow-up – measure at baseline. Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = square root $[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 - (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient (R) = 0.5. If the outcome measures were reported in median and inter-quartile range, mean and standard SD values

Table 2: Assessment of risk of bias in the included studies using Cochrane criteria.

Study, year	Ref.	Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other potential threats to validity
Abou Raya et al. 2007	(48)	U	U	H	H	L	L	L
Almquist et al. 2012	(49)	U	L	L	U	L	L	L
Ambrosi et al. 2000	(50)	U	L	L	U	L	L	L
Barreto et al. 2008	(51)	L	L	L	L	L	L	L
Blann et al. 2001	(52)	U	L	U	U	U	U	L
Casey et al. 2007	(53)	U	U	U	U	U	U	L
Hjelstuen et al. 2007	(54)	U	U	H	H	L	L	L
Konduracka et al. 2008	(55)	U	U	H	H	L	L	L
Krysiak & Okopien 2013	(56)	U	U	U	L	L	L	L
Krysiak et al. 2011	(57)	U	U	U	U	L	L	L
Liu et al. 2009	(58)	U	U	U	U	L	L	L
Lynch et al. 2005	(59)	U	U	L	L	L	L	L
McCarey et al. 2004	(60)	L	L	L	L	L	L	L
Sadik et al. 2010	(61)	L	L	L	L	L	L	L
Tan et al. 1999	(62)	U	U	U	U	U	L	L
Tehrani et al. 2013	(63)	U	U	U	U	L	L	L
Van de Ree et al. 2003	(64)	U	U	U	U	L	L	L
Ordulu et al. 2008	(25)	U	U	U	U	L	L	L

L: low risk of bias; H: high risk of bias; U: unclear risk of bias.

were estimated using the method described by Hozo et al. (33). To convert interquartile range (IQR) into Min-Max range, the following equations were used: A = median + 2 × (Q_3 – median) and B = median – 2 × (median – Q_1), where A, B, Q_1 and Q_3 are upper and lower ends of the range, upper and lower ends of the IQR, respectively.

A random-effects model (using DerSimonian-Laird method) and the generic inverse variance method were used to compensate for the heterogeneity of studies in terms of demographic characteristics of populations being studied and also differences in study design and type of statin being studied (34). Heterogeneity was quantitatively assessed using I^2 index. Effect sizes were expressed as standardised mean difference (SMD) and 95% confidence interval (CI) owing to the methodological differences in vWF:Ag assay. Subgroup analyses were conducted to evaluate the effect of different statins, treatment duration, and intensity of treatment (based on the American Heart Association/American College of Cardiology lipid guidelines) (35) recommendation of on the pooled estimate. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using leave-one-out method, i.e. removing one study each time and repeating the analysis.

Meta-regression

Random-effects meta-regression was performed using unrestricted maximum likelihood method to evaluate the association between calculated SMD and potential moderators including duration of treatment with statins and magnitude of LDL-C reduction by statin therapy.

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, and Begg's rank correlation and Egger's weighted regression tests. Duval & Tweedie "trim and fill" and "fail-safe N" methods were used to adjust the analysis for the effects of publication bias (36).

Results

The initial screening for potential relevance removed the articles whose titles and/or abstracts were obviously irrelevant. Among the 26 full-text articles assessed for eligibility, eight studies were excluded because: not measuring vWF:Ag levels (n=3), not controlled for statin therapy (n=4) and having incomplete data (n=1)

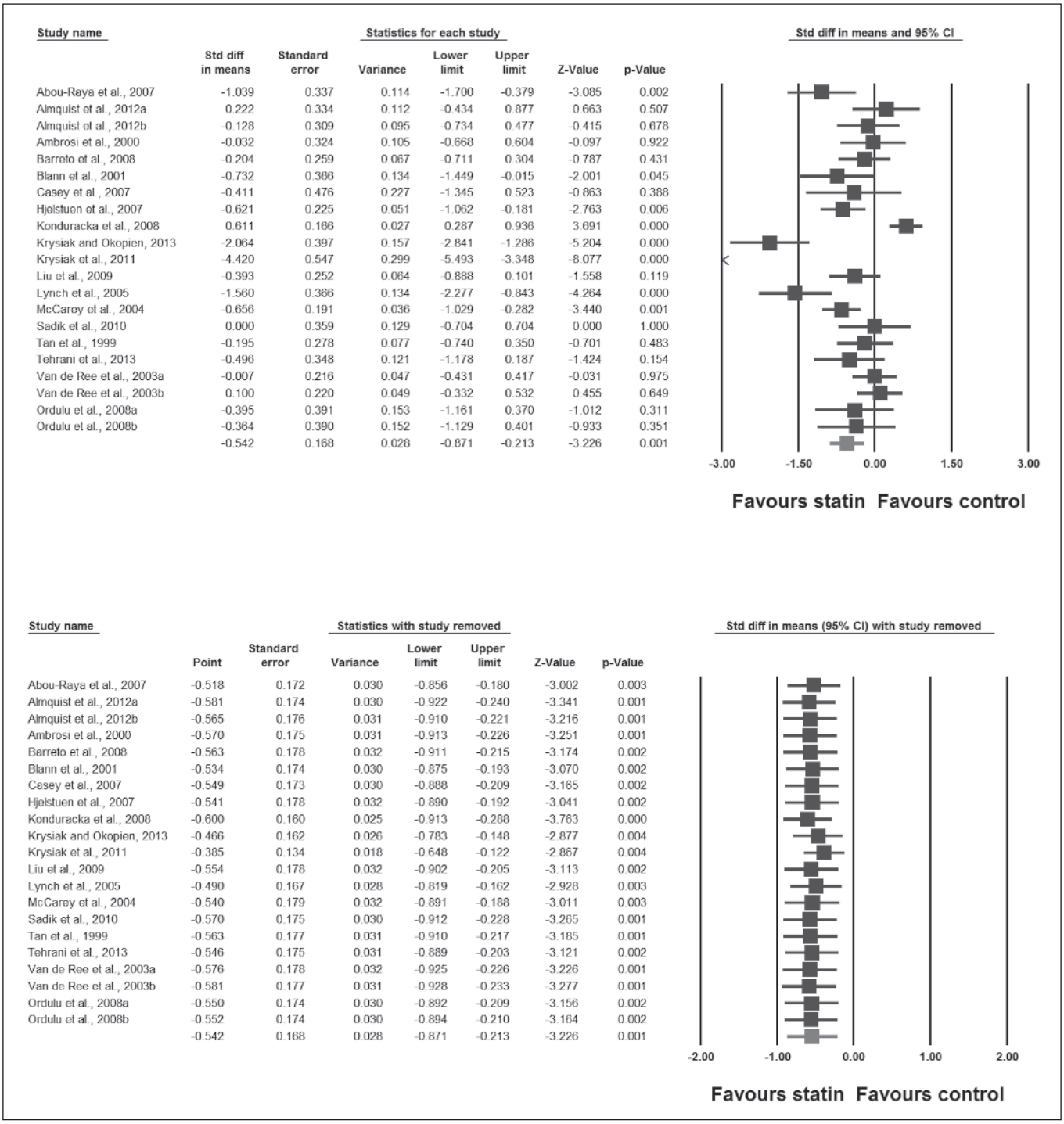

Figure 2: Forest plot displaying standardised mean difference and 95 % confidence intervals for the impact of statin therapy on plasma vWF:Ag levels. Lower plot shows leave-one-out sensitivity analysis.

(►Figure 1). After final assessment, 18 trials achieved the inclusion criteria and entered the final meta-analysis.

Characteristics of included studies

A total of 18 studies were included in this analysis. 1434 participants were randomised, of whom 755 were allocated to statin intervention groups, 143 to other treatment groups (statins + ezetimibe or statins + lifestyle changes) and 536 to placebo group. The number of participants in these trials ranged from nine to 204. Included studies were published between 1999 and 2013, and were conducted in Poland (n=3), United Kingdom (n=3), Sweden (n=2), Egypt, France, Brazil, Ireland, Norway, China, United States, Hong Kong, the Netherlands and Turkey. The following sta-

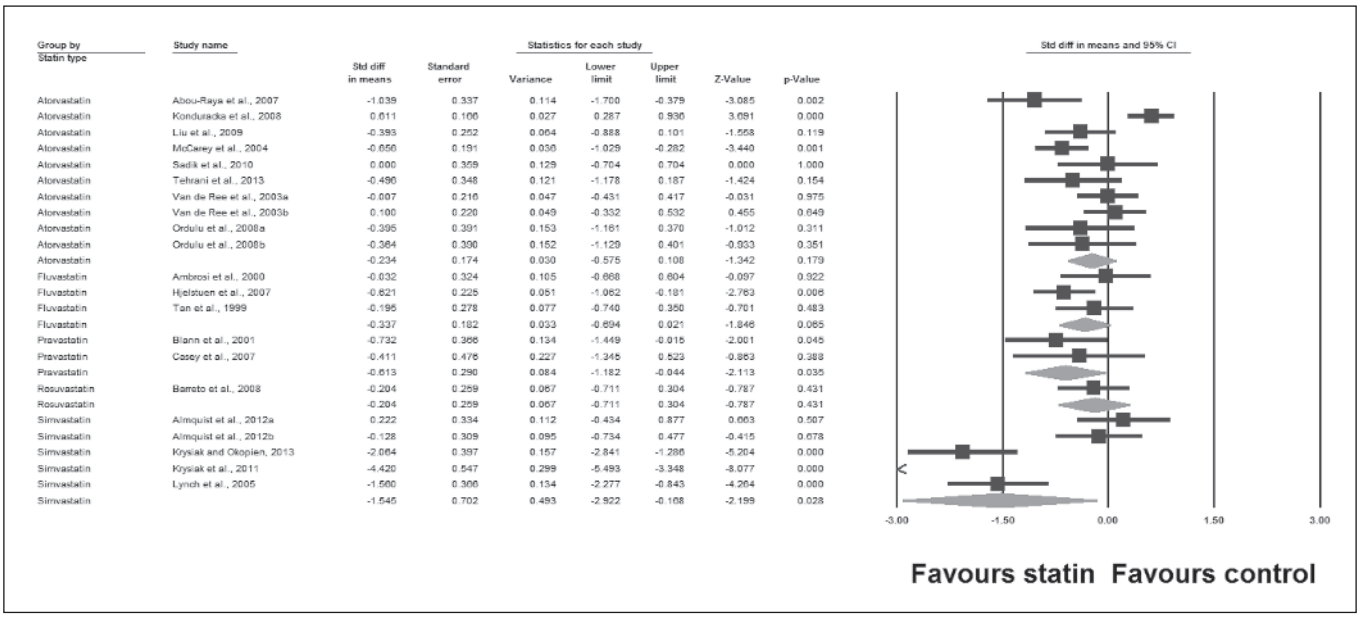

Figure 3: Forest plot displaying standardised mean difference and 95 % confidence intervals for the impact of different statins on plasma vWF:Ag levels.

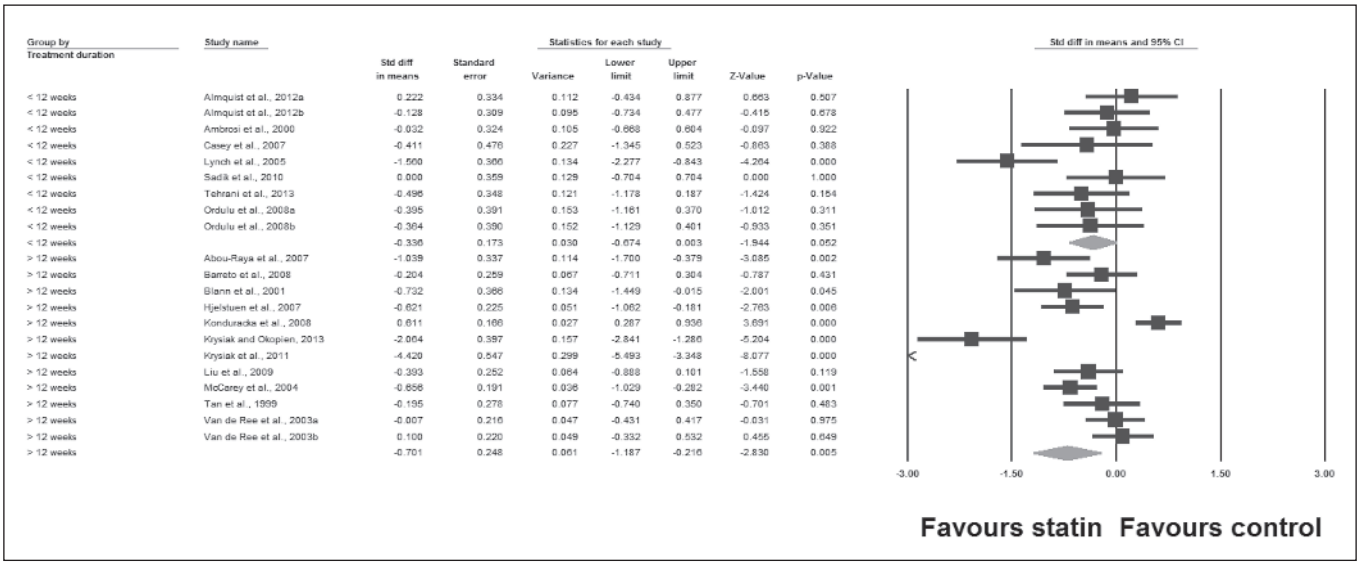

Figure 4: Forest plot displaying standardised mean difference and 95 % confidence intervals for the impact of statin therapy on plasma vWF:Ag levels in trials with treatment durations of < 12 weeks (upper plot) and ≥ 12 weeks (lower plot).

tin doses were administered in the included trials: 10 mg to 80 mg/day atorvastatin, 40 mg to 80 mg/day simvastatin, and 20 mg to 40 mg/day fluvastatin, 10 mg/day rosuvastatin, and 40 mg/day pravastatin. Duration of statin intervention ranged between two weeks and 12 months. Thirteen trials were designed as parallel-group; four as cross-over studies and one study was a 2×2 factorial intervention trial. Demographic and baseline parameters of the included studies are shown in ►Table 1.

The risk assessment of bias

The systematic assessment of bias in the analysed studies, including seven domains according to Cochrane criteria, is presented in ►Table 2.

Effect of statin therapy on plasma vWF:Ag levels

Random-effect meta-analysis of 21 treatment arms revealed a significant decrease in plasma vWF:Ag levels following statin therapy

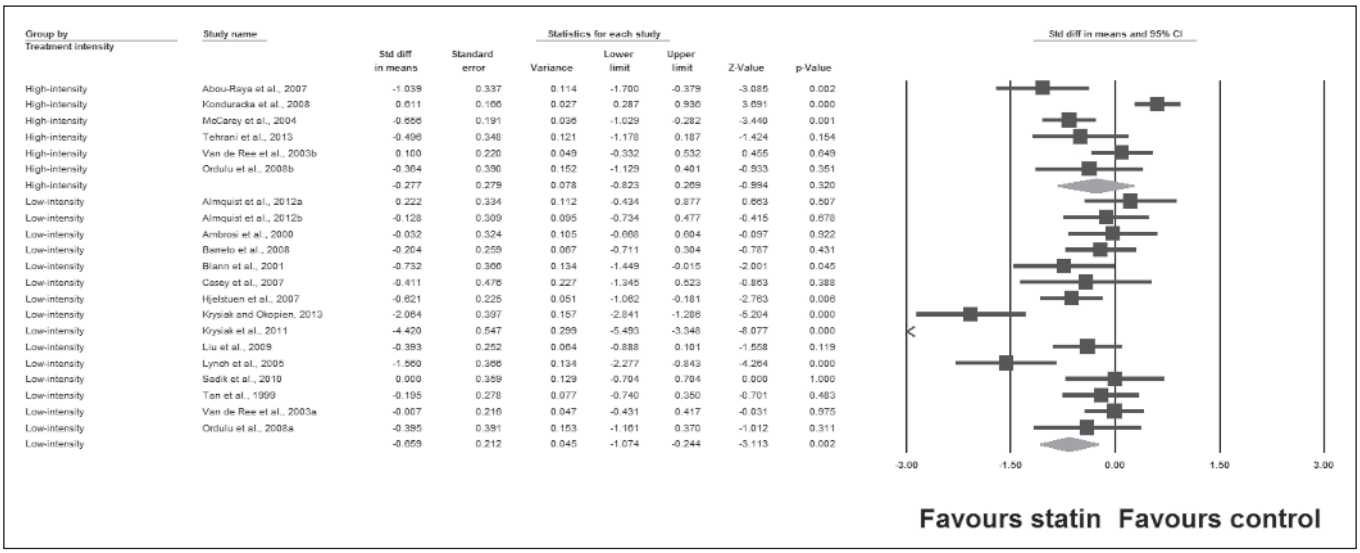

Figure 5: Forest plot displaying standardised mean difference and 95 % confidence intervals for the impact of high- and low-intensity statin therapy on plasma vWF:Ag levels.

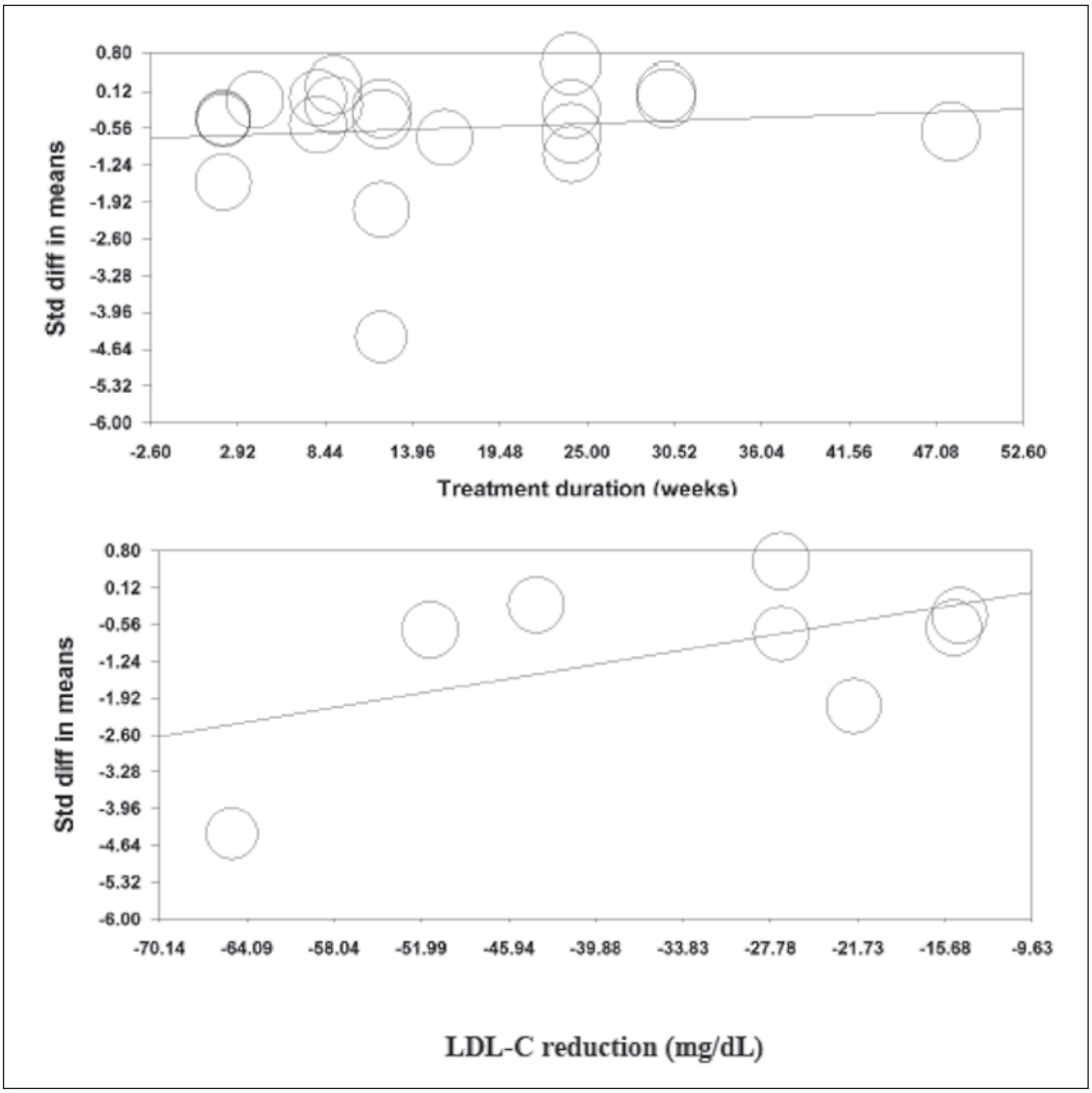

Figure 6: Meta-regression plots of the association between mean changes in plasma vWF:Ag levels with duration of statin therapy (upper plot) and magnitude of LDL-C reduction (lower plot).

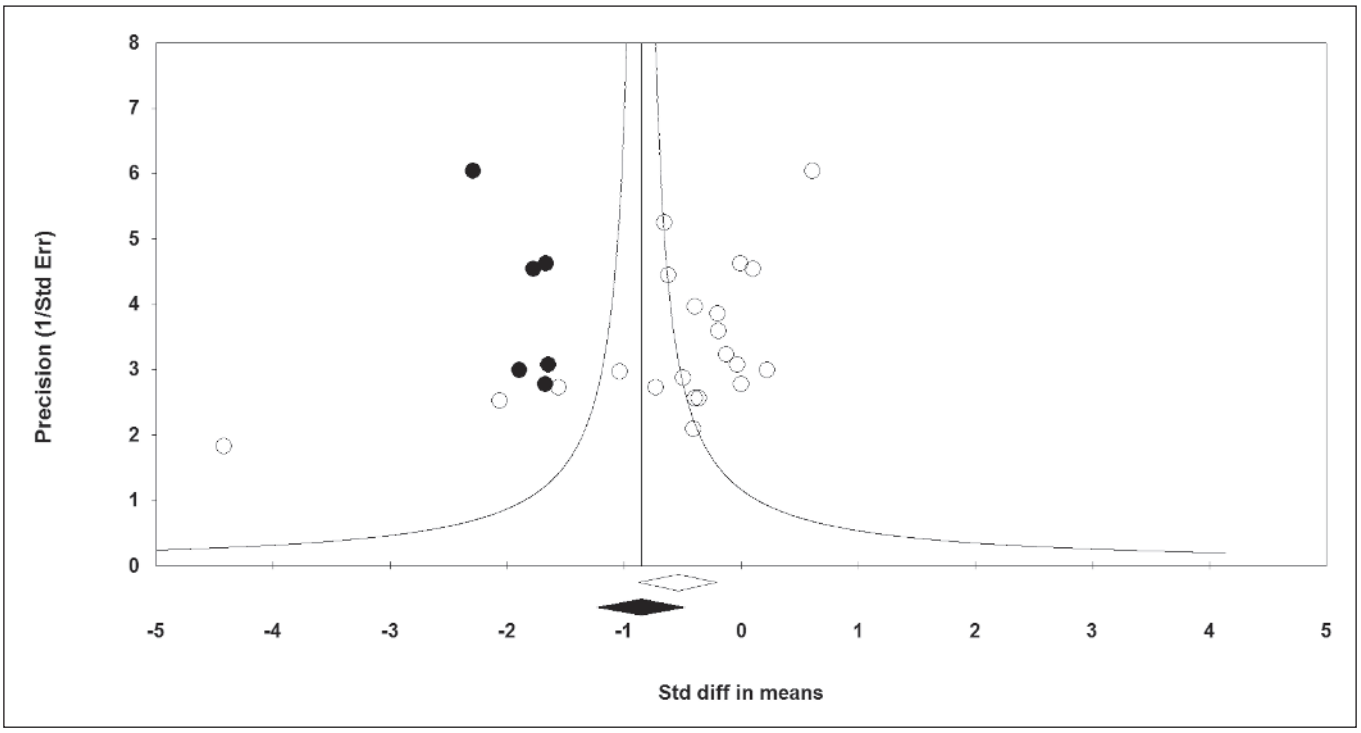

Figure 7: Funnel plot detailing publication bias in the studies reporting the impact of statin therapy on plasma vWF:Ag levels. Open diamond represents observed effect size; closed diamond represents imputed effect size.

(SMD: –0.54, 95% CI: –0.87, –0.21, p = 0.001). This effect size was robust and removing each of the included treatment arms from analysis did not change statistical significance of the pooled estimate (►Figure 2). In subgroup analysis, the greatest effect was observed with simvastatin (SMD: –1.54, 95% CI: –2.92, –0.17, p = 0.028), followed by pravastatin (SMD: –0.61, 95% CI: –1.18, –0.04, p = 0.035), but not for fluvastatin (SMD: –0.34, 95% CI: –0.69, 0.02, p = 0.065), atorvastatin (SMD: –0.23, 95% CI: –0.57, 0.11, p = 0.179) and rosuvastatin (SMD: –0.20, 95% CI: –0.71, 0.30, p = 0.431) (►Figure 3). The lowering effect of statins on plasma vWF:Ag levels was greater in the subset of studies lasting ≥12 weeks (SMD: –0.70, 95% CI: –1.19, –0.22, p = 0.005) compared with that of studies lasting <12 weeks (SMD: –0.34, 95% CI: –0.67, 0.003, p = 0.052) where the significance was borderline (►Figure 4). Finally, high-intensity statin therapy was associated with a significant reduction in vWF:Ag levels (SMD: –0.66, 95% CI: –1.07, –0.24, p = 0.002), while no effect was observed in low-intensity statin treatment (SMD: –0.28, 95% CI: –0.82, 0.27, p = 0.320) (►Figure 5).

Meta-regression

Random-effects meta-regression showed that changes in plasma vWF:Ag levels are independent of treatment duration (slope: 0.01; 95% CI: –0.02, 0.04; p = 0.565). However, a marginally significant positive association was observed between changes in plasma vWF:Ag and LDL-C levels (slope: 0.04; 95% CI: –0.004, 0.09; p = 0.071) following statin therapy (►Figure 6).

Publication bias

The funnel plot of the study precision (inverse standard error) by effect size (SMD) was asymmetrical and indicated potential publication bias (►Figure 7). This observation was confirmed by results of Begg's rank correlation (Kendall's Tau with continuity correction = –0.32, z = 2.02, two-tailed p-value = 0.043) and Egger's linear regression (intercept = –5.25, standard error = 1.60; 95% CI = –8.61, –1.90, t = 3.28, df = 19, two-tailed p = 0.004) tests. Using trim and fill correction, six potentially missing studies were imputed on the left side of funnel plot, leading an imputed SMD of –0.85 (95% CI: –1.23, –0.48) was suspected. Fail-safe N test suggested that 224 missing studies would be needed to bring the calculated SMD down to a non-significant value.

Discussion

To our knowledge, the current systematic review and meta-analysis is the first to assess the effects of statin therapy on plasma vWF:Ag levels and presents a comprehensive synthesis of outcomes from RCTs. Random-effects meta-analysis of 21 treatment arms revealed a significant decrease in plasma vWF:Ag levels following statin therapy. This effect size was robust in sensitivity analysis. In subgroup analysis, the greatest effect was observed with simvastatin, followed by pravastatin, fluvastatin, atorvastatin and rosuvastatin. Also, a greater effect was observed with high-intensity compared with low-intensity statin therapy. These findings

are of clinical interest since von Willebrand factor might be a new useful biomarker in cardiovascular disease (CVD) (37), since elevated levels of vWF indicate impaired endothelial function and increased risk of thrombotic manifestations, which could contribute to increased CV risk (1).

Plasma vWF:Ag levels are recognised to be enhanced in a variety of clinical settings, such as rheumatoid arthritis and vasculitis (38), hyperthyroidism, arterial hypertension, diabetes (39, 40) and stroke (41). It is still debated whether vWF itself plays a pathogenetic role in the process of atherogenesis. Despite the fact that many experimental studies suggested that vWF can contribute to the progression of atherosclerosis (42, 43), numerous human studies did not validate this observation (44). Moreover, a recent meta-analysis suggested that vWF plays no causal role in the progression of atherosclerosis, but the development of atherosclerosis is associated with increased vWF levels which could contribute to arterial thrombosis (45).

Nevertheless, scientific societies do not recommend the screening of vWF levels for evaluating the risk of thromboembolic disease (41) and this is only partly related to the fact that measuring vWF:Ag is not fully standardised (41). Moreover, factors that are involved in the regulation of plasma vWF levels (46, 47) are poorly understood.

The present meta-analysis has limitations. There were only few eligible RCTs, and most of them included a relatively small number of participants. Furthermore, the included studies were heterogeneous concerning the characteristics of patients and study design. The studies included did not provided the values for different genetic and non-genetic factors which may account for the variability of vWF:Ag values.

In conclusion, this meta-analysis of available RCTs showed a significant reduction in plasma vWF:Ag levels following statin therapy. In light of this finding, it might be considered that vWF concentrations could represent a new potential target for statin therapy. However, larger and well-designed studies are required to verify our findings, and to determine whether the reduced vWF:Ag–associated effects of statins can protect against atherosclerosis and cardiovascular events.

Author contributions

AS – designed the study, made the statistical analysis, corrected the draft of the paper; CS – designed the study, made the literature search, drafted the manuscript; SU – made the statistical analysis, drafted the manuscript; DPM, AU, GYHL, VB, KKR, GFW, GKH, JR, JJPK – corrected the draft of the paper and the revised version; MB – designed the study, made the literature search, drafted the manuscript, prepared the revised version, submitted the paper.

Conflicts of interest

None declared.

Abbreviations

AMI = acute myocardial infarction (AMI); CI = confidence interval; CV = cardiovascular; CVD = cardiovascular disease; CMA = Comprehensive Meta-Analysis; ELISA = enzyme linked immunosorbent assay; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; LIA = latex-immunoassay; LDL-C= low-density lipoprotein cholesterol; RCTs = randomized controlled trials; SD = standard deviation; SMD = standardized mean difference; SNPs = single nucleotide polymorphisms; VWF = von Willebrand factor; vWF:Ag = VWF:antigen.

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