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Atorvastatin on Inflammatory Markers (AIM) Study, a Substudy of ACTFAST**

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Martineau, Gonzalo Hernández, Jesús Egido and on behalf of the ACTFAST
investigators

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Increased Soluble Fas Plasma Levels in Subjects at High Cardiovascular Risk

Atorvastatin on Inflammatory Markers (AIM) Study, a Substudy of ACTFAST

Luis M. Blanco-Colio, Jose L. Martín-Ventura, Eduardo de Teresa, Csaba Farsang, Allan Gaw, GianFranco Gensini, Lawrence A. Leiter, Anatoly Langer, Pierre Martineau, Gonzalo Hernández, Jesús Egido, on behalf of the ACTFAST investigators

Objectives—Increasing evidence indicates that the Fas/Fas ligand interaction is involved in atherogenesis. We sought to analyze soluble Fas (sFas) and soluble Fas ligand (sFasL) concentrations in subjects at high cardiovascular risk and their modulation by atorvastatin treatment.

Methods and Results—ACTFAST was a 12-week, prospective, multicenter, open-label trial which enrolled subjects (statin-free or statin-treated at baseline) with coronary heart disease (CHD), CHD-equivalent, or 10-year CHD risk >20%. Subjects with LDL-C between 100 to 220 mg/dL (2.6 to 5.7 mmol/L) and triglycerides ≤600 mg/dL (6.8 mmol/L) were assigned to a starting dose of atorvastatin (10 to 80 mg/d) based on LDL-C at screening. Of the 2117 subjects enrolled in ACTFAST, AIM sub-study included the 1078 statin-free patients. At study end, 85% of these subjects reached LDL-C target. Mean sFas levels were increased and sFasL were reduced in subjects at high cardiovascular risk compared with healthy subjects. Atorvastatin reduced sFas in the whole population as well as in patients with metabolic syndrome or diabetes. Minimal changes were observed in sFasL.

Conclusions—sFas concentrations are increased and sFasL are decreased in subjects at high cardiovascular risk, suggesting that these proteins may be novel markers of vascular injury. Atorvastatin reduces sFas, indicating that short-term treatment with atorvastatin exhibits antiinflammatory effects in these subjects. (*Arterioscler Thromb Vasc Biol.* 2007;27:168-174.)

Key Words: inflammation ■ atorvastatin ■ soluble Fas ■ C-reactive protein ■ statins

Cardiovascular events arise from the disruption of atherosclerotic plaques that contain numerous inflammatory cells.¹ Inflammatory and resident cells (endothelial and vascular smooth muscle cells) release different proteins that can generate a chronic inflammatory response in the injured artery. Measurement of circulating markers of inflammation may provide some insight into this process. Proteins secreted by cells implicated in atherosclerotic lesions, including soluble Fas (sFas) and soluble Fas ligand (sFasL), circulate in small, but detectable, amounts. Soluble Fas is generated by alternative messenger RNA splicing capable of encoding a soluble Fas molecule lacking the transmembrane domain,² whereas sFasL is released in serum from membrane-bound FasL processed by a metalloproteinase.³ It has been demonstrated that Fas and FasL are expressed in atherosclerotic

lesions and the Fas/Fas ligand system is related to the apoptotic and inflammatory responses present in atherosclerotic plaques.⁴⁻⁵ Fas and its ligand are typical members of the tumor necrosis factor (TNF) receptor superfamily. Similar to other members of this family, FasL induces apoptosis or programmed cell death when bound to its receptor Fas.⁶ However, depending on the conditions, Fas/FasL interactions may be related to augmentation of proliferation and inflammatory response.⁷ In this sense, signals initiated by regulated Fas-associated death domain protein overexpression in the carotid artery induce expression of monocyte-chemoattractant protein-1 and interleukin (IL)-8, and cause massive migration of macrophages in vivo,⁸ indicating that Fas and Fas ligand act also as proinflammatory proteins.

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From the Vascular Research Lab (L.M.B.-C., J.L.M.-V.), Fundación Jiménez Díaz, Autonoma University, Madrid, Spain; University of Malaga & V. de la Victoria Hospital (E.d.T.), Malaga, Spain; 1st Department Med. Semmelweis University (C.F.), Budapest, Hungary; University of Glasgow (A.G.), Glasgow, UK; University of Florence, Careggi Hospital (G.G.), Firenze, Italy; University of Toronto & St-Michael's Hospital (L.A.L., A.L.), Toronto, ON, Canada; Canadian Heart Research Centre (A.L.), Toronto, ON, Canada; Medical Division, Pfizer Canada (P.M.), Kirkland, QC, Canada; and R&D Department, Medical Division, Pfizer Spain (G.H.), Madrid, Spain.

Correspondence to Professor Jesús Egido, MD, PhD, Vascular Research Laboratory, Fundación Jiménez Díaz, Avenida Reyes Católicos 2, 28040, Madrid, Spain. E-mail jegido@fjd.es

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Several trials have demonstrated that 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitor (statin) therapy lowers the risk of cardiovascular events by reducing plasma cholesterol levels,⁹ and practice guidelines for high cardiovascular risk patients describe the importance of reaching low-density lipoproteins cholesterol (LDL-C) targets.¹⁰ The Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration (ACTFAST) study was designed to determine whether using atorvastatin at starting doses appropriate for the degree of LDL-C reduction required would achieve LDL-C targets quickly with either no titration or just one titration step.

This study included patients with coronary heart disease (CHD), CHD-equivalent (defined as diabetes, peripheral vascular disease, or cerebrovascular disease), or a 10-year CHD risk >20%. In the Atorvastatin on Inflammatory Markers (AIM) substudy of ACTFAST, we have analyzed sFas, sFasL, and C-reactive protein (CRP) concentrations in patients that were statin-free at baseline. Specifically, as a post-hoc analysis, we have analyzed whether subjects at high cardiovascular risk have modified sFas and sFasL levels with respect to healthy subjects. We have also evaluated whether atorvastatin treatment changed the levels of these markers comparing simultaneously, for the first time, all available doses of atorvastatin.

Materials and Methods

Study Design

The ACTFAST study is a 12-week, prospective, multicenter, open-label trial which enrolled subjects (either statin-free or statin-treated at baseline) with CHD, a CHD-equivalent (defined as diabetes, peripheral vascular disease, or cerebrovascular disease) or a 10-year CHD risk >20%.¹¹ In addition, subjects had to present with a LDL-C >2.6 mmol/L and \leq 5.7 mmol/L, as well as triglycerides \leq 6.8 mmol/L and had to be willing to follow the NCEP III multifaceted lifestyle approach (or local equivalent). The substudy only included patients who were statin-free (either never previously prescribed a statin or who have been statin-free for a minimum of two months before recruitment) at baseline.

Exclusion criteria included acute liver disease or hepatic dysfunction (AST or ALT \geq two times the upper limit of normal), elevated serum creatinine (\geq 181 μ mol/L), CPK >3 times the upper limit of normal, uncontrolled diabetes (HbA1c >10%), evidence of gastrointestinal disease that could decrease absorption, uncontrolled hypertension (sitting blood pressure >160/100 mm Hg), uncontrolled primary hypothyroidism (thyroid-stimulating hormone (TSH) \geq 1.5 times the upper limit of normal), known intolerance or hypersensitivity to HMG-CoA reductase inhibitors. In addition, patients using prohibited medication were also excluded. These drugs included strong CYP3A4 inhibitors (eg, erythromycin or azole antifungals), other lipid-regulating drugs such as niacin, probucol, fibrates, and derivatives, bile acid sequestering resins, ezetimibe, fish oils, and other HMG-CoA reductase inhibitors. Antiinflammatory drugs in the previous year, with the exception of low doses of acetylsalicylic acid (<325 mg/d), or immunosuppressive drugs were also excluded in the per protocol analysis of inflammatory markers. Subjects were also ineligible for the study if they had any severe disease or had undergone a surgical procedure within 3 months before screening, or women who were pregnant or lactating or of childbearing potential not using an acceptable method of contraception.

The institutional review board of all participating centers approved the ACTFAST study protocol and all participants provided written informed consent. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki.¹²

For comparison, age and gender-matched blood donors (N=130) were used as controls. All of them have blood pressure and lipids concentrations in the normal range. None had cardiovascular disease.

Dose Assignment

Subjects were assigned to a starting dose of atorvastatin (10 to 80 mg/d) based on LDL-C at screening (100 to 149, 150 to 159, 160 to 169, and 170 to 220 mg/dL were assigned to 10, 20, 40, or 80 mg/d, respectively). After 6 weeks, if not already at maximum dose, subjects not reaching LDL-C target (< 100 mg/dL) had their dose doubled. Subjects initially allocated to atorvastatin 80 mg/d who did not reach LDL-C targets, were continued on that dose and a more intense therapeutic lifestyle intervention (NECP step II diet or equivalent) was recommended.

Laboratory Determinations

As part of the main protocol, fasting venous blood samples were collected into tubes with EDTA anticoagulant at baseline and at 12 weeks. Plasma was isolated by low speed centrifugation and shipped to a core laboratory for storage at -70°C . The paired baseline and 12-week samples were then shipped to the laboratory (Madrid, Spain) and measured in batches. sFas and sFasL were measured with commercially available enzyme-linked immuno-sorbent assay (ELISA) kits (R&D Systems). The minimum detectable concentrations of sFas and sFasL were 20 pg/mL and 2.6 pg/mL, respectively. The reproducibility of the assays over the study was excellent. Intraassay and interassay coefficients of variation were 2.9% to 4.1% (sFas) and 3.9% to 6.4% (sFasL), respectively.

Statistical Analysis

This report focuses on the 1078 subjects in the AIM substudy with baseline and 12-week samples available for measurement of inflammatory markers. As expected, the distributions of the inflammatory markers were skewed. To meet the distributional assumptions of the statistical models, the markers were log-transformed for the statistical models and antilog-transformed for descriptive purposes, yielding geometric means and 95% confidence intervals (CI) for baseline, week 12 concentrations, and the change in concentrations over the study period. The prespecified primary end point was the effect of atorvastatin 10, 20, 40, and 80 mg on decreasing sFas and in increasing sFasL levels over the 12-week study period. The primary end point was assessed by ANCOVA, adjusted for the initial level of the marker. Secondary endpoints included the effect of atorvastatin on the changes of the sFas or sFasL levels over 12 weeks, according to the presence of diabetes or metabolic syndrome [definition of metabolic syndrome according to NCEP-III: When 3 or more of the following are present: waist circumference >102 cm in men or >88 cm in women; triglycerides \geq 150 mg/dL (1.7 mmol/L); HDL-C <40 mg/dL (1.0 mmol/L) in men, < 50 mg/dL (1.3 mmol/L) in women; blood pressure \geq 130/ \geq 85 mm Hg, and FPG \geq 110 mg/dL (6.1 mmol/L)].¹⁰ Post-hoc analyses were designed to analyze the differences between sFas and sFasL concentrations in subjects at high cardiovascular risk and healthy subjects matched for age and sex. In addition, changes on sFas and sFasL over 12 weeks, according to the presence of hypertension were analyzed. The association between sFas and sFasL (both log-transformed) versus continuous variables were explored using Pearson correlation coefficients, without adjustment for doses used. Statistical significance was defined as a value of $P<0.05$.

Results

Soluble Fas Concentrations Are Increased and Soluble Fas Ligand Levels Are Decreased in Subjects at High Cardiovascular Risk

The baseline characteristics of the studied population are summarized in Table 1. Of the 2117 subjects enrolled in ACTFAST, 1078 were included in the AIM substudy. We measured sFas and sFasL in plasma samples of high-risk

TABLE 1. Baseline Characteristics of the 1078 Subjects Included in the AIM Substudy

Variable Mean (SD) or n (%)	Atorvastatin 10 mg (n=560)	Atorvastatin 20 mg (n=149)	Atorvastatin 40 mg (n=131)	Atorvastatin 80 mg (n=238)	Overall (n=1078)
Age, y	64.2 (10.6)	64 (11.3)	62.1 (10.8)	62 (11)	63.4 (10.8)
Weight, kg	82.8 (17.6)	79.7 (15.5)	81.9 (15.6)	81.1 (16.5)	81.9 (16.9)
Body mass index*	29.4 (5.5)	28.5 (4.9)	29.2 (5.3)	29.2 (5.2)	29.2 (5.3)
Men	384 (68.6)	93 (62.4)	81 (61.8)	141 (59.2)	699 (64.8)
White	518 (92.5)	140 (94)	124 (94.7)	229 (96.2)	1011 (93.8)
Smoking Status					
Current Smoker	86 (15.4)	33 (22.1)	35 (26.7)	73 (30.7)	227 (21.1)
Past or nonsmoker	474 (84.6)	116 (77.9)	96 (73.3)	165 (69.3)	851 (78.9)
Alcohol Consumption	261 (46.6)	72 (48.3)	70 (53.4)	122 (51.3)	525 (48.7)
History of Hypertension	383 (68.4)	105 (70.5)	94 (71.8)	166 (69.8)	748 (69.4)
History of Diabetes Mellitus	271 (48.4)	64 (43)	63 (48.1)	96 (40.3)	494 (45.8)
Metabolic Syndrome	274 (48.9)	61 (40.9)	73 (55.7)	113 (47.5)	521 (48.3)
History of CVD	49 (8.8)	19 (12.8)	10 (7.6)	23 (9.7)	101 (9.4)
History of PVD	52 (9.3)	8 (5.4)	12 (9.2)	16 (6.7)	88 (8.2)
History of CHD	308 (55)	70 (47)	54 (41.2)	112 (47.1)	544 (50.5)

* Calculated as weight in kilograms divided by the square of the height in meters.

CVD indicates cerebrovascular disease; PVD, peripheral vascular disease; CHD, coronary heart disease.

patients enrolled in the AIM substudy and compared them with those of 130 healthy subjects matched for age and sex. Post-hoc analyses of circulating sFas concentrations showed that sFas were higher in subjects at high cardiovascular risk compared with healthy subjects [7392 pg/mL (7249 to 7537) versus 4640 (4480 to 4798); Geometric mean (95% CI), $P < 0.0001$]. In addition, sFasL concentrations were lower in subjects at high cardiovascular risk [58.8 pg/mL (57.4 to 60.3) versus 70.2 (66.6 to 74.1); $P < 0.0001$]. When sFas or sFasL were considered as a continuous variable, we observed that sFas levels correlated with systolic blood pressure ($r = 0.12$; $P = 0.0002$) and sFasL correlated positively with HDL-C concentrations ($r = 0.11$; $P = 0.0006$) and inversely with C-reactive protein (CRP) concentrations ($r = -0.1$; $P = 0.003$). However, no evidence of association was observed between sFas or sFasL and total cholesterol, LDL-C, or triglycerides. The baseline levels of sFas were not higher in subjects with LDL-C below the median value (148 mg/dL) compared with those with LDL-C above the median (7357 versus 7427 pg/mL, respectively; $P = 0.72$). Furthermore, patients with high LDL-C levels have lower sFasL levels, but this change was not significant (58.5 versus 59.2 pg/mL; $P = 0.5$).

Of interest was the analysis of subgroups in subjects at high cardiovascular risk studied according to the following categories defined by presence of diabetes, the metabolic syndrome (prespecified subgroups), or hypertension (post-hoc analyses). Univariate analyses demonstrated that circulating sFas levels are increased in patients with hypertension, diabetes, or the metabolic syndrome compared with patients without these pathologies (Table 2). Furthermore, sFasL concentrations were also lower in patients with hypertension or the metabolic syndrome.

Effects of Atorvastatin Treatment on sFas and sFasL Levels

At baseline, 52%, 14%, 12%, and 22% of subjects were assigned to 10, 20, 40, and 80 mg, respectively. Figure 1 shows the changes in lipid parameters in subjects treated with atorvastatin during 12 weeks. At this time, atorvastatin treatment significantly decreased total cholesterol [223 (220.9 to 224.7) to 150 mg/dL (146.4 to 154.7); $P < 0.0001$], LDL-C [147 (146.1 to 149.1) to 80 mg/dL (79 to 81.2); $P < 0.0001$], triglycerides [151 (146.4 to 154.7) to 114 mg/dL (111.2 to 117.3); $P < 0.0001$], and ApoB [1.11 (1.1 to 1.12) to 0.66 g/L (0.65 to 0.67); $P < 0.0001$], and increased HDL-C [48 (47.6 to

TABLE 2. Soluble Fas and sFasL Markers According to the Presence of Hypertension, Diabetes or the Metabolic Syndrome at Baseline

Marker	Soluble Fas (pg/mL)		Soluble FasL (pg/mL)	
	Mean (95% CI)	<i>P</i>	Mean (95% CI)	<i>P</i>
Diabetes (n=444)	7587.3 (7373.2–7807.6)	0.007	57.9 (55.8–60.1)	0.26
No diabetes (n=534)	7232.7 (7042.9–7427.6)		59.7 (57.7–61.6)	
Metabolic syndrome (n=506)	7626.2 (7418.4–7839.8)	0.0007	57.3 (55.2–59.4)	0.03
No metabolic syndrome (n=469)	7175.3 (6980.8–7375.3)		60.3 (58.3–62.3)	
Hypertension (n=676)	7495.5 (7318.3–7676.9)	0.02	57.9 (56.2–59.5)	0.04
No hypertension (n=294)	7159.9 (6925.1–7402.7)		61.2 (58.3–64.2)	

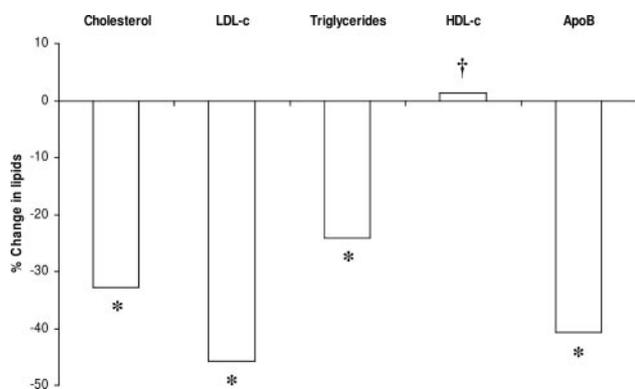


Figure 1. Effect of atorvastatin treatment on total cholesterol, LDL-C, triglycerides, HDL-C, and ApoB (mean change from baseline) in subjects at high cardiovascular risk. Probability values from paired *t* test. **P*<0.0001; †*P*=0.001.

48.9) to 49 mg/dL (48.2 to 49.6); *P*=0.001]. Percentages of patients reaching LDL-C <100 mg/dL were 87%, 84%, 91%, and 78% (atorvastatin 10, 20, 40, and 80 mg/d), respectively. In the whole population, 85% of subjects reached LDL-C target. The effect of atorvastatin on sFas, sFasL, and CRP concentrations is shown in Table 3. In the whole group, atorvastatin lowered circulating sFas in subjects at high cardiovascular risk. However, sFasL concentrations were minimally modified by atorvastatin treatment. As expected, all doses of atorvastatin diminished CRP concentrations.

Additional post-hoc analyses were designed to assess whether the observed atorvastatin-induced changes in sFas were related to atorvastatin-induced changes in lipid parameters. We found no evidence of association between percentage change in sFas and percentage change observed in total cholesterol, LDL-C, triglycerides, or HDL-C (*r*=0.004, *P*=0.87; *r*=0.01, *P*=0.72; *r*=0.05, *P*=0.1, *r*=-0.04, *P*=0.12; respectively). Furthermore, the reduction of sFas concentrations was significantly greater with atorvastatin in both the high and the low LDL-C subgroups (-3.1%, LDL-C <148 mg/dL, *P*=0.009; -3.8%, LDL-C ≥148 mg/dL, *P*=0.001). When we analyzed the effect of different atorvastatin doses used, we observed that all doses decreased sFas levels, and this was statistically significant for atorvastatin 10 and 40 mg/d (Table 3). Interestingly, we observed an association between percentage change in sFas and percentage change observed in CRP (*r*=0.1; *P*=0.002). Furthermore, in the highest quartile, all doses of atorvastatin diminished sFas concentrations (*P*<0.0001 for all doses) though the greatest effect was noted with 40 mg/d atorvastatin (Figure 2A).

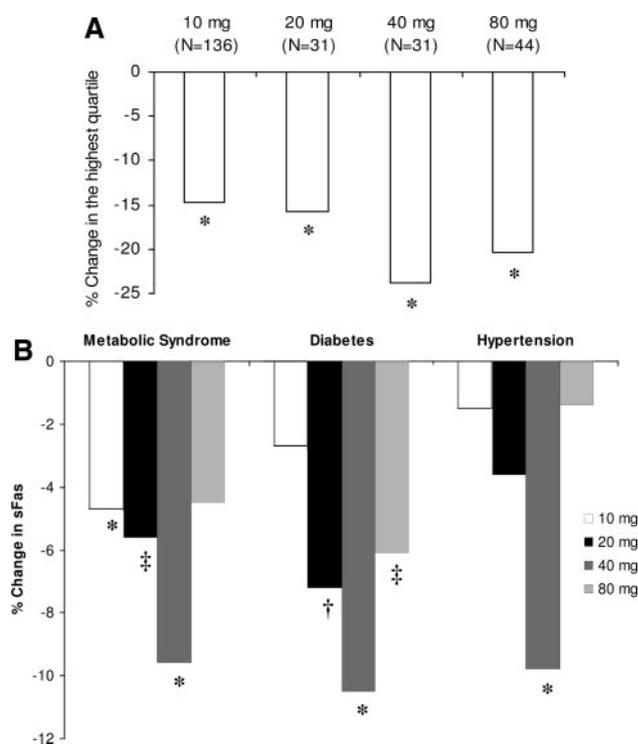


Figure 2. A, Effect of all doses of atorvastatin on soluble Fas in the highest quartile. Probability values from *t* test * *P*<0.0001. B, Effect of all atorvastatin doses on soluble Fas (mean change from baseline) according to the presence of hypertension, diabetes, or the metabolic syndrome. Probability values from paired *t*-test. **P*<0.005; †*P*=0.01; ‡*P*<0.05.

Treatment with atorvastatin diminished circulating sFas (geometric mean [95% CI] before and after treatment) in patients with diabetes [7587 (7373 to 7807) versus 7209 pg/mL (7010 to 7415), *P*<0.0001], the metabolic syndrome [7626 (7418–7839) versus 7208 pg/mL (7019 to 7402); *P*<0.0001] or hypertension [7495 (7318 to 7676) versus 7279 pg/mL (7117 to 7444), *P*=0.004] (Table 4). In these subgroups, atorvastatin 40 mg/d had the greatest effect in sFas reduction (Figure 2B).

Atorvastatin also decreased sFas in normotensive patients, but was less effective in lowering sFas in subjects without the metabolic syndrome or diabetes. Interestingly, the percentage reduction in subjects with the metabolic syndrome was significantly higher than in those without metabolic syndrome (*P*=0.018). However, atorvastatin treatment did not change sFasL levels in patients with diabetes, hypertension, or metabolic syndrome (data not shown).

TABLE 3. Changes in sFas, sFasL, and hs-CRP Values From Baseline in all Atorvastatin Dose Studied

Atorvastatin Dose	Soluble Fas Change (95% CI)	<i>P</i>	Soluble Fas Ligand Change (95% CI)	<i>P</i>	C-Reactive Protein Change (95% CI)	<i>P</i>
10 mg	-3.1% (-5.3%, -0.9%)	0.007	-0.5% (-3.5%, 2.6%)	0.74	-23.4 (-29.1, -17.2)	<0.0001
20 mg	-3.3% (-6.8%, 0.3%)	0.07	-6.1% (-10.2%, -1.9%)	0.005	-27.4 (-36.4, -17.1)	<0.0001
40 mg	-7.4% (-11.8%, -2.9%)	0.001	-2.8% (-8%, 2.6%)	0.29	-21.7 (-32.1, -9.7)	0.0009
80 mg	-2% (-5.6%, 1.8%)	0.29	-3.3% (-7.7%, 1.3%)	0.15	-38.3 (-45.3, -30.4)	<0.0001
Overall	-3.5% (-5%, -1.8%)	<0.0001	-2.2% (-4.2%, -0.1%)	0.04	-27.3 (-31.1, -23.2)	<0.0001

P values from paired *t* test.

TABLE 4. Effect of Atorvastatin on Soluble Fas at Follow-Up According to Presence of Hypertension, Diabetes, or the Metabolic Syndrome

	Metabolic Syndrome Change (95% CI)	<i>P</i>	Diabetes Change (95% CI)	<i>P</i>	Hypertension Change (95% CI)	<i>P</i>
Overall	-5.5 (-7.6%, -3.3%)	<0.0001	-5% (-7.2%, -2.7%)	<0.0001	-2.9% (-4.8%, -0.9%)	0.004
	No Metabolic Syndrome Change (95% CI)		No Diabetes Change (95% CI)		No Hypertension Change (95% CI)	
Overall	-1.7% (-4%, 0.7%)	0.17	-2.1% (-4.4%, 0.1%)	0.06	-4.7% (-7.5%, -1.8)	0.001

P values from paired *t* test.

Discussion

Our data indicate that sFas levels are increased and sFasL levels are decreased in subjects at high cardiovascular risk compared with healthy subjects. Our results also demonstrate that sFas and sFasL concentrations are related to different cardiovascular risk factors such as diabetes, metabolic syndrome, or hypertension. This is the first time that circulating sFas and sFasL have been measured in a large, multi-center study.

These data may have clinical relevance for several reasons. Our findings could be of pathophysiological importance, because they provide evidence that diminishing inflammation in general, and perhaps the levels of sFas in particular, may well have a role to play in altering the atherosclerotic process. Several single-center studies have demonstrated that sFas was elevated in patients with myocarditis, chronic congestive heart failure, and coronary artery disease, suggesting that the Fas/FasL system may contribute to the pathogenesis of cardiovascular disease.^{13–15} Moreover, Troyanov et al have suggested that sFas could be a predictor of atherosclerosis in dialysis patients.¹⁶ Now, we have observed in a large-population study that sFas concentrations are elevated in patients with different cardiovascular risk factors, suggesting that sFas may be a novel marker of vascular damage. Furthermore, we have observed that short-term treatment with atorvastatin lowers circulating sFas concentrations in these patients. This effect may be related to the known antiinflammatory properties of statins.¹⁷ In experimental studies, Fas and FasL interaction induce the expression and release of proinflammatory cytokines.¹⁸ Overexpression of Fas-associated death domain protein in the carotid artery induces expression of proinflammatory chemokines such as monocyte chemoattractant protein 1 and IL-8, and cause massive migration of macrophages *in vivo*.⁸

The signaling pathways involved in Fas-induced chemokine production are ill-defined. Although Fas-induced chemokine production has been associated with apoptosis, this can also occur independently of apoptosis. In this way, overexpression of FasL in arteries of hypercholesterolemic rabbits accelerates atherosclerotic lesions formation. This effect is related to an augmentation of inflammatory cell recruitment without changes in the number of apoptotic cells, indicating that Fas/FasL interaction may be related to the inflammatory response and can contribute to the progression of atherosclerosis.¹⁹

On the other hand, FasL expression by endothelial cells is related with negative regulation of inflammation.²⁰ Consis-

tent with its role in preventing leukocyte extravasation, FasL expression is markedly downregulated when endothelial activation is induced by proinflammatory cytokines.²¹ The FasL expression by endothelial cells may contribute to the normal concentrations of circulating sFasL. We have observed that subjects at high cardiovascular risk have markedly lower sFasL concentrations than healthy subjects. It is possible that endothelial dysfunction occurring in subjects at high cardiovascular risk may be responsible for this finding, probably because of reduced synthesis and/or release into the blood. This result confirms in a large-population study that sFasL may be a novel marker of vascular injury possibly related to endothelial function. Soluble FasL concentrations were only minimally affected by atorvastatin treatment in the AIM substudy. However, in a previous report, we demonstrated that atorvastatin treatment can normalize sFasL levels in patients with familial combined hyperlipidemia after one year of treatment,²² suggesting that the short-term treatment with atorvastatin in the AIM substudy may be responsible for the minimal effect observed.

Secondly, change in sFas concentration is largely unrelated to the observed change in LDL-C concentration. In our study, circulating sFas concentrations did not correlate with any lipid parameter analyzed at baseline and the reduction observed in lipid values after atorvastatin treatment was not related with the change in sFas concentrations. In addition, the reduction observed in patients with LDL-C above the median value was not statistically different from the one observed in patients with LDL-C below the median. Statins have been shown to provide clinical benefit in reducing cardiovascular events, even in patients without elevated LDL-C, raising the possibility that the benefit may be attributable to effects beyond changes in plasma lipoproteins.²³ However, it cannot be excluded that the effect observed with atorvastatin in our study could be also related to the lipid lowering properties of statins.

Thirdly, different studies have compared the effect of intensive (80 mg/d) versus moderate (40 mg/d) treatment but using different statins.^{24–25} Recently, the NASDAC study evaluated the safety and lipid-lowering efficacy of atorvastatin at starting doses of 10, 20, 40, and 80 mg.²⁶ However, the present report is the first study comparing the effect of atorvastatin on soluble plasma markers simultaneously with all available doses.

Atorvastatin, at doses between 10 to 40 mg/d, diminished sFas in a dose-dependent manner. However, atorvastatin 80 mg/d was less effective in sFas reduction. Furthermore, when we analyzed the effect of atorvastatin in different subgroups

(diabetes, hypertension, or the metabolic syndrome), we also observed that 40 mg/d atorvastatin reduced sFas concentrations by approximately 10%, and this effect was more than that observed with the other doses used. These results could indicate that intensive treatment with atorvastatin (80 mg/d) did not provide additional beneficial antiinflammatory effects compared with those obtained with intermediate doses, at least with regards to sFas concentrations. However, when we analyzed the highest quartile, all doses of atorvastatin diminished sFas levels. Furthermore, it is important to keep in mind that the 40 mg group was the smallest one in size and this may have influenced the results. More studies comparing atorvastatin 40 mg versus 80 mg are needed to confirm the lack of dose-response observed in our study for sFas.

Finally, we observed that atorvastatin treatment lowered sFas mainly in patients with diabetes or the metabolic syndrome, and in particular that the percentage reduction in subjects with the metabolic syndrome was significantly higher than in those without it. However, although sFas concentrations were associated with systolic blood pressure at baseline, the magnitude of this association was small in absolute terms and atorvastatin diminished sFas concentrations in both subjects with or without hypertension.

The specific pathways by which atorvastatin decreases sFas blood concentrations remain uncertain. Intensive research efforts have related statin treatment with diminution of different components of the inflammatory response.²⁷ In this sense, we have observed that all doses of atorvastatin diminished CRP, in agreement with previous data from other clinical trials.^{24,25,28} Recent studies suggest that statins may have antiinflammatory effects in the vascular wall. These data are of particular interest given the recent demonstration that Fas/FasL interaction can induce the expression of proinflammatory cytokines implicated in the development of atherosclerosis.^{7–8,18} However, more studies are needed to determine the precise mechanism(s) by which atorvastatin diminishes sFas concentrations.

In summary, this large-population study provides, for the first time, a direct confirmation that sFas concentrations are increased and sFasL levels are decreased in subjects at high cardiovascular risk, suggesting that sFas and sFasL may be novel markers of vascular injury. Unfortunately, this study was not designed to analyze cardiovascular events because of the short period of follow-up. Therefore, the potential predictive value of sFas and sFasL in subjects at high cardiovascular risk need to be evaluated in future studies. The AIM substudy provides evidence that short-term treatment with atorvastatin reduces sFas concentrations, more markedly so in patients with the metabolic syndrome and diabetes. Our results indicate that short-term treatment with atorvastatin exhibits antiinflammatory and, probably, antiapoptotic effects in subjects at high cardiovascular risk.

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Disclosures

Drs Martineau and Hernandez are employees of Pfizer, Inc. Drs de Teresa, Farsang, Gaw, Gensini, Leiter, Langer, and Egido have served as consultants to manufacturers of lipid-lowering pharmaceuticals.

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