Statins Suppress Apolipoprotein CIII-Induced Vascular Endothelial Cell Activation and Monocyte Adhesion

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Aims	Activation of vascular endothelial cells (ECs) contributes importantly to inflammation and atherogenesis. We previously reported that apolipoprotein CIII (apoCIII), found abundantly on circulating triglyceride-rich lipoproteins, enhances adhesion of human monocytes to ECs *in vitro*. Statins may exert lipid-independent anti-inflammatory effects. The present study examined whether statins suppress apoCIII-induced EC activation *in vitro* and *in vivo*.
Methods and results	Physiologically relevant concentrations of purified human apoCIII enhanced attachment of the monocyte-like cell line THP-1 to human saphenous vein ECs (HSVECs) or human coronary artery ECs (HCAECs) under both static and laminar shear stress conditions. This process mainly depends on vascular cell adhesion molecule-1 (VCAM-1), as a blocking VCAM-1 antibody abolished apoCIII-induced monocyte adhesion. ApoCIII significantly increased VCAM-1 expression in HSVECs and HCAECs. Pre-treatment with statins suppressed apoCIII-induced VCAM-1 expression and monocyte adhesion, with two lipophilic statins (pitavastatin and atorvastatin) exhibiting inhibitory effects at lower concentration than those of hydrophilic pravastatin. Nuclear factor κB (NF-κB) mediated apoCIII-induced VCAM-1 expression, as demonstrated via loss-of-function experiments, and pitavastatin treatment suppressed NF-κB activation. Furthermore, in the aorta of hypercholesterolaemic *Ldlr*$^{-/-}$ mice, pitavastatin administration *in vivo* suppressed VCAM-1 mRNA and protein, induced by apoCIII bolus injection. Similarly, in a subcutaneous dorsal air pouch mouse model of leucocyte recruitment, apoCIII injection induced F4/80+ monocyte and macrophage accumulation, whereas pitavastatin administration reduced this effect.
Conclusions	These findings further establish the direct role of apoCIII in atherogenesis and suggest that anti-inflammatory effects of statins could improve vascular disease in the population with elevated plasma apoCIII.
Keywords	Apolipoprotein CIII • Vascular endothelial cells • Monocytes • HMG-CoA reductase inhibitors • Atherosclerosis

Introduction

Atherosclerosis is a chronic inflammatory disease.[1,2] Expression of adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), by activated endothelial cells (ECs) plays an important role in the initiation of arterial inflammation by mediating firm adhesion, diapedesis, and retention of mononuclear leucocytes to the intima of the blood vessel wall.[3,4] The recruitment of inflammatory cells into the vascular intima also contributes to the progression and instability of atherosclerotic plaques.[2,5]

Much effort has explored the inciting factors for the activation of vascular EC and the pathogenesis of atherosclerosis. Increasing evidence demonstrates that apolipoprotein CIII (apoCIII), a surface protein component that abundantly presents on circulating triglyceride-rich apoB lipoproteins and HDL, is a strong contributor to atherosclerosis. Plasma levels of apoCIII and the lipoproteins that carry apoCIII independently predict increased risk for coronary heart disease in prospective human cohorts after adjusting for blood lipids.[6–8] Overexpression of apoCIII causes hyperlipidaemia and promotes atherosclerotic lesion development in mouse models,[9] while apoCIII deficiency protects against dyslipidaemia and atherogenesis.[10] ApoCIII also promotes pancreatic β-cell death,[11,12] while antisense treatment against apoCIII delays the onset of type 1 diabetes in rats.[13] We recently reported that apoCIII promotes hyperlipidaemia in humans by inhibiting clearance of plasma triglyceride-rich lipoproteins and channeling them to conversion to small, dense LDL.[14] In addition to its adverse effects on blood lipids, we and others also have reported that apoCIII alone, or as a component of VLDL or LDL, induces monocyte activation and adhesion to ECs.[15,16] These findings suggest that apoCIII plays a causal role in atherosclerotic lesion development independent of its deleterious effects on lipid metabolism.

Potent cholesterol-lowering drugs 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce the progression of atherosclerotic lesions and the onset of acute thrombotic complications of coronary atherosclerosis.[17] In addition to lipid lowering, statins may exert pleiotropic effects independent of their regulation of cholesterol metabolism.[18] In the present study, we tested the hypothesis in vitro and in vivo that statins suppress apoCIII-induced EC activation and monocyte adhesion, key events in early atherogenesis.

Methods

A detailed Methods section is available as Supplementary material online.

Results

Apolipoprotein CIII induces vascular cell adhesion molecule-1 expression in human arterial and venous endothelial cells *in vitro*

For reference, plasma apoCIII concentration ranges from 50 to 100 μg/mL in normolipidaemic humans, while hyperlipidaemic patients have higher levels.[7] When we applied clinically relevant concentrations of purified human apoCIII protein, we found that apoCIII treatment significantly affected VCAM-1 expression in cultured human saphenous vein ECs (HSVECs) (ANOVA $P = 0.012$), and furthermore apoCIII induced VCAM-1 expression in a dose-dependent manner (Figure 1A, $P < 0.05$ for linear regression analysis using apoCIII concentration as independent variable). The expression of ICAM-1, another major adhesion molecule, was not significantly affected. Similarly, physiological concentrations of apoB lipoproteins (VLDL and LDL) containing apoCIII, isolated from fresh human plasma, significantly increased the
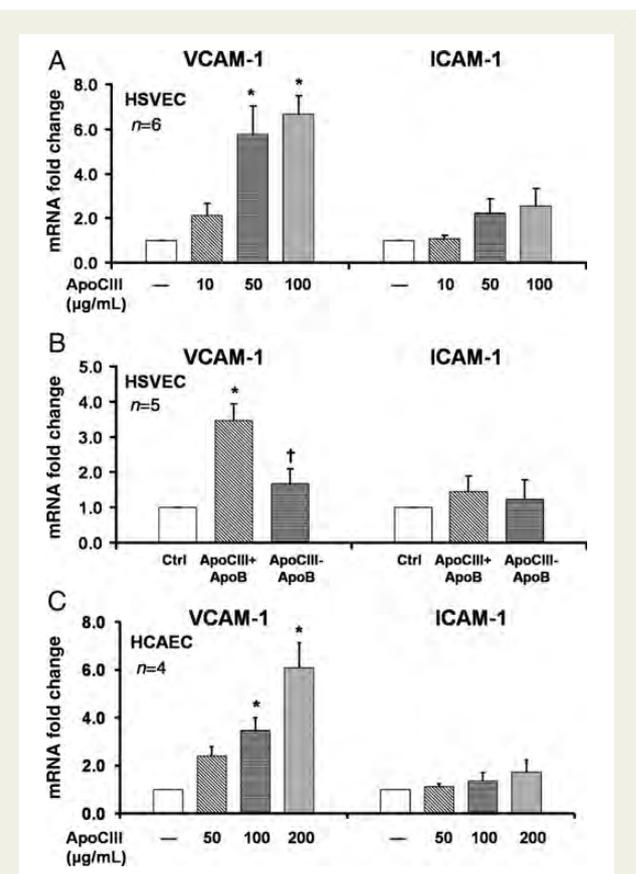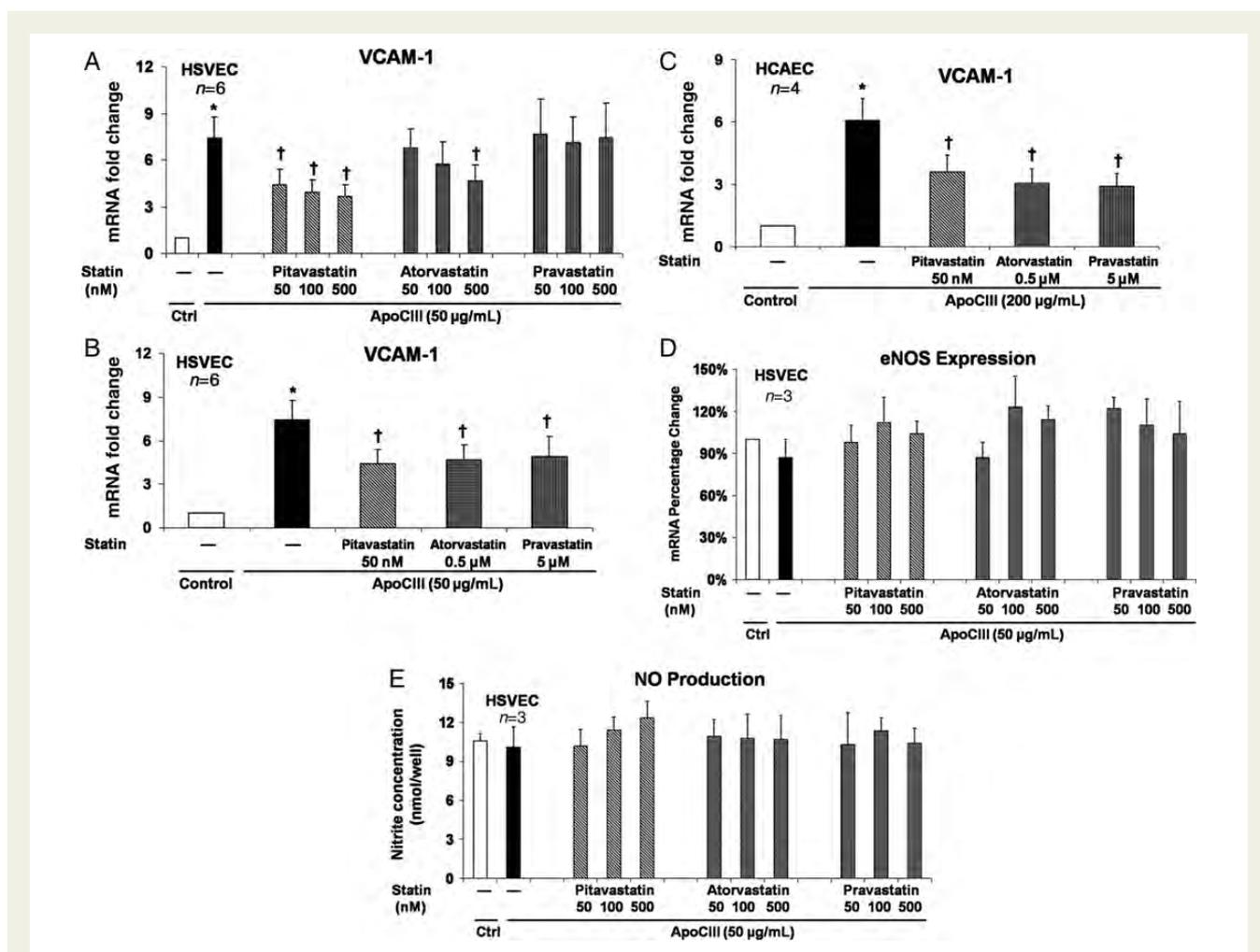

Figure 1 ApoCIII induces expression of VCAM-1 in cultured human venous and arterial vascular ECs. Confluent HSVECs were incubated with purified human apoCIII at indicated concentrations (A) or apoB lipoproteins with apoCIII (apoCIII+) or without apoCIII (apoCIII-) isolated from fresh human plasma by immunoaffinity chromatography (apoB concentration 100 μg/mL) (B). Concentration of apoCIII in apoB lipoproteins is between 15 and 30 μg/mL. HSVECs were lysed 3 h after treatment, and we performed real-time qPCR to measure VCAM-1 and ICAM-1 expression. In other experiments, confluent HCAECs were incubated with purified human apoCIII (C). Data represent mean ± SEM from multiple experiments with HSVECs or HCAECs from different donors. *$P < 0.05$ between treatment and control [Dunnett's test for (A and C); unpaired t-test for (B)] and †$P < 0.05$ between apoB lipoproteins with and without apoCIII (unpaired t-test).

expression of VCAM-1, but not ICAM-1, compared with the same concentrations of apoB lipoproteins without apoCIII, indicating that VCAM-1-inducing effect was due to apoCIII (Figure 1B). ApoCIII concentration in apoCIII-containing apoB lipoproteins was ~15–30 μg/mL. In addition to HSVECs, purified apoCIII also induced expression of VCAM-1 in a dose-dependent manner in cultured human coronary artery ECs (HCAECs) (Figure 1C, $P < 0.05$ for linear regression analysis using apoCIII concentration as independent variable). VCAM-1 activation in HCAECs requires higher concentrations of apoCIII compared with HSVECs but still in the range found in mild–to-moderate hyperlipidaemia.

Statin treatment reduces apolipoprotein CIII-induced vascular cell adhesion molecule-1 expression in human arterial and venous endothelial cells

We pre-treated HSVECs and HCAECs with statins before administration of purified human apoCIII and examined the effects of statins on apoCIII-induced EC activation. We applied two lipophilic statins (pitavastatin and atorvastatin) and one hydrophilic statin (pravastatin). Our results showed that statins overall affected apoCIII-induced VCAM-1 expression (*Figure 2A–C*, ANOVA $P < 0.05$). Pitavastatin and atorvastatin attenuated apoCIII-induced VCAM-1 expression in HSVECs (*Figure 2A*, $P < 0.05$ Dunnett's test), but pravastatin did not exert this effect

at the concentrations used (50–500 nM). To achieve a similar magnitude of reduction in apoCIII-induced VCAM-1 expression by statins in HSVECs, the minimum concentrations required were different among the three statins (pitavastatin < atorvastatin < pravastatin, *Figure 2B*). Notably, 50 nM of pitavastatin is clinically achievable. Similar to what was observed in HSVECs, statin pre-treatment also attenuated apoCIII-induced VCAM-1 expression in HCAECs (*Figure 2C*). These concentrations of statins did not affect cell viability of HSVECs substantially (MTS assay, Supplementary material online, *Figure S1*, ANOVA $P = 0.56$). Unlike VCAM-1 induction, apoCIII or statins did not affect eNOS mRNA expression or NO production, as measured by nitrite concentration in the cell culture supernatant, in HSVECs (*Figure 2D* and *E*, ANOVA $P = NS$).

Figure 2 Statin treatment attenuates apoCIII-induced VCAM-1 expression in cultured human vascular ECs. Confluent HSVECs or HCAECs were incubated with pitavastatin, atorvastatin, and pravastatin at indicated concentrations for 12 h before treatment with purified human apoCIII for additional 4 h. Expression of VCAM-1 (*A–C*) or eNOS (*D*) was determined by qPCR. (*A* and *D*) HSVECs were treated with 50, 100, and 500 nM of statins plus 50 μg/mL apoCIII. (*B*) HSVECs from the same donors were treated with statins at specified concentrations. (*C*) HCAECs were treated with statins plus 200 μg/mL apoCIII ($P = NS$ for ANOVA). (*E*) In some other experiments, nitrite concentration in the supernatant of HSVECs cultured on 96-well plates was measured by a colorimetric assay kit 8 h after apoCIII treatment ($P = NS$ for ANOVA). Data represent mean ± SEM from multiple experiments with HSVECs or HCAECs from different donors. *$P < 0.05$ between apoCIII treatment (unpaired *t*-test) and control and [†]$P < 0.05$ between statin treatment and apoCIII alone (Dunnett's test).

Statin treatment reduces apolipoprotein CIII-induced monocyte adhesion to endothelial cells under static conditions

To examine whether VCAM-1 induction increases monocyte adhesion, we performed monocyte−EC adhesion assay under static conditions. We cultured HSVECs in 96-well plates with statins prior to addition of 50 μg/mL of purified human apoCIII, and measured the adherence of fluorescence-labelled THP-1 cells. ApoCIII treatment significantly altered the adhesion of THP-1 to HSVECs after apoCIII treatment (*Figure 3A−C*, ANOVA $P < 0.05$). VCAM-1 appeared to mediate this increased adhesion because the addition of VCAM-1-neutralizing antibody abolished apoCIII-induced monocyte adhesion, whereas control antibody had no effect (*Figure 3A*). Treatment of cultured HSVECs with lipophilic pitavastatin and atorvastatin significantly reduced monocyte adhesion at lower concentrations than those of pravastatin (*Figure 3B*). In addition to THP-1, we further examined the effects of statins and apoCIII on the adhesion of human primary monocytes to HCAECs. ApoCIII induced adhesion of peripheral blood-derived CD14+ cells to HCAECs, which was significantly reduced by statin pre-treatment (*Figure 3C*).

Statin treatment reduces apolipoprotein CIII-induced monocyte adhesion to endothelial cells under laminar flow

We then performed monocyte adhesion assays under laminar shear flow conditions. We pre-treated HSVECs with statins followed by incubation with apoCIII, and recorded adhesion of THP-1 cells. ANOVA analysis showed that statin pre-treatment significantly affected apoCIII-induced adhesion of THP-1 cells to HSVECs (*Figure 4*, ANOVA $P < 0.05$ for all panels). Analysis of video recordings showed that pre-treatment with lipophilic statins significantly reduced apoCIII-induced monocyte adhesion to the HSVEC monolayer (representative still images, *Figure 4A*). Pitavastatin and atorvastatin produced statistically significant reductions in apoCIII-induced THP-1 adhesion under laminar flow (*Figure 4B* and *C*). At shear stress level of 0.75 dyne/cm^2, statin treatment reduced apoCIII-induced THP-1 adhesion (*Figure 4B*). When the shear stress decreased to 0.50 dyne/cm^2, this pattern persisted and we observed more monocyte adhesion overall (*Figure 4C*). At higher concentration, pravastatin also reduced apoCIII-induced monocyte adhesion (*Figure 4D−F*). The addition of the VCAM-1 monoclonal blocking monoclonal antibody (clone E1/6) almost completely abolished apoCIII-induced monocyte adhesion to HSVECs but not the control monoclonal antibody that recognizes VE-cadherin (clone HEC 1.2), indicating that THP-1 adhesion depends on VCAM-1 (*Figure 4D−F*). Similar to what was observed for HSVECs, statin pre-treatment also significantly reduced apoCIII-induced THP-1 adhesion to HCAECs under laminar flow conditions (*Figure 4G−I*).

Statin administration reduces apolipoprotein CIII-induced vascular endothelial cell activation *in vivo*

To investigate the inhibitory effects of statins on apoCIII-triggered vascular EC activation *in vivo*, we administered purified human
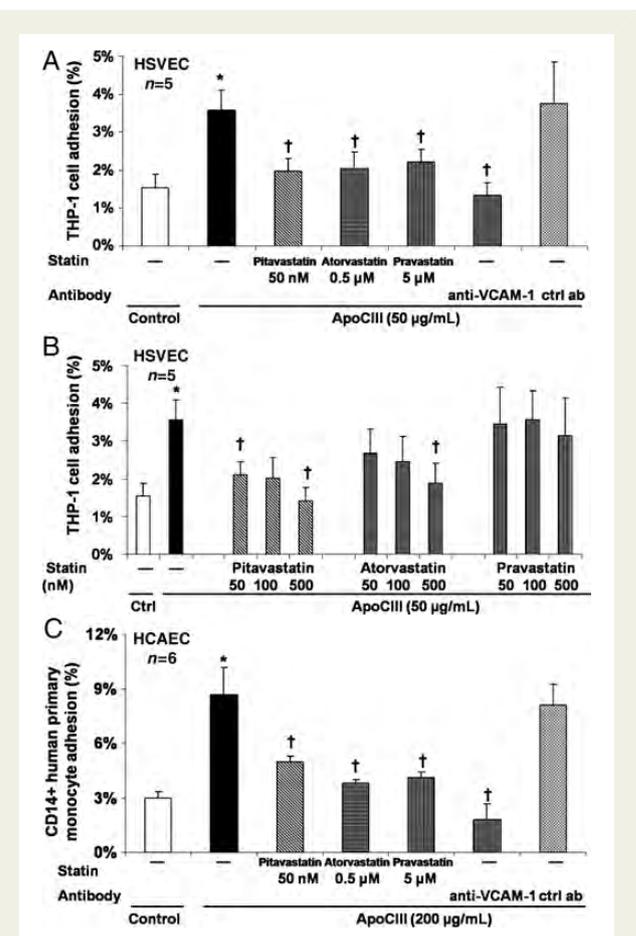
Figure 3 Statin treatment attenuates apoCIII-induced monocyte adhesion to cultured human primary ECs under static condition. Confluent HSVECs or HCAECs were cultured on 96-well plates with pitavastatin, atorvastatin, and pravastatin at specified concentrations for 12 h before the addition of purified human apoCIII (final concentration 50 μg/mL). After another 16 h, THP-1 cells, a human monocytic cell line (*A* and *B*), or human primary CD14+ monocytes (*C*) (cell density: 1×10^5/mL) labelled with BCECF-A were added into each well, and allowed to adhere for 10 min. Non-adherent cells were removed by washing, the fluorescent intensity of adhered and total cells applied to the well was measured by a fluorescence plate reader, and the ratio of adherent to total cells was calculated. (*A*) Quantified results of the percentage of THP-1 cells attached to HSVEC-covered 96-well plates. HSVECs from the same donors were treated with statins at specified concentrations. (*B*) THP-1 cells were applied to HSVECs treated with 50, 100, and 500 nM of statins. (*C*) Human CD14+ monocytes were applied to HCAECs treated with statins. Data represent mean ± SEM from multiple experiments with HSVECs from different donors. *$P < 0.05$ between apoCIII treatment and control (unpaired *t*-test) and †$P < 0.05$ between statin treatment and apoCIII alone (Dunnett's test).

apoCIII via tail vein injection (200 μg apoCIII) to hypercholesterolaemic *Ldlr*$^{-/-}$ mice on high-fat diet for 12 weeks that were treated with pitavastatin. We administered pitavastatin by oral gavage for 5 days at the physiological dosage of 60 mg/day/kg body weight.
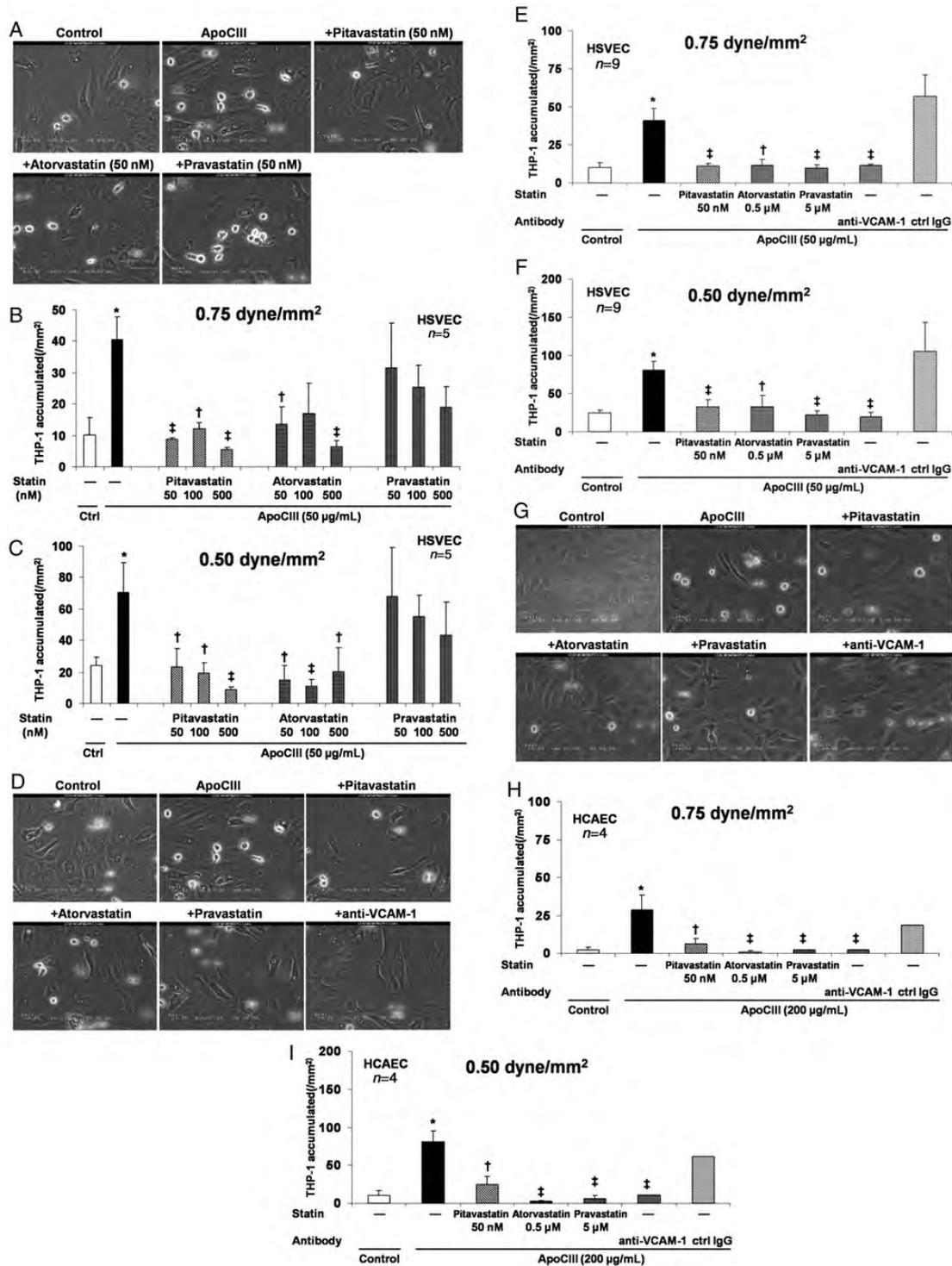
Figure 4 Statin treatment attenuates apoCIII-induced THP-1 cell adhesion to cultured human primary ECs under unidirectional laminar flow. Confluent HSVECs or HCAECs cultured on fibronectin-coated 25 mm glass coverslips were incubated with pitavastatin, atorvastatin, and pravastatin at specified concentrations for 12 h before the addition of purified human apoCIII (50 μg/mL). After 16 h, these coverslips were inserted into an *in vitro* flow chamber and 5×10^5/mL THP-1 cells in DPBS containing 0.1% HSA were drawn across the EC monolayer first at 0.75 dyne/mm^2 and then at 0.50 dyne/mm^2. THP-1 adhesion was recorded by digital video microscopy and the adhesion of THP-1 cells was assessed as previously described.[15] (*A*, *D*, and *G*) Representative still images of THP-1 cells attachment to HSVECs or HCAECs at indicated conditions. (*B*, *C*, *E*, and *F*) Quantified results of the number of THP-1 cells attached to HSVECs after treatment with apoCIII and statins at indicated concentrations. (*H* and *I*) Quantified results of the number of THP-1 cells attached to HCAECs after treatment with apoCIII and statins at indicated concentrations. Data represent mean $\pm$ SEM from multiple experiments from different donors of HSVECs or HCAECs. *$P < 0.05$ between apoCIII treatment and control (unpaired *t*-test); †$P < 0.05$ and ‡$P < 0.01$ between statin treatment and apoCIII alone (Dunnett's test).

Bolus injection of this physiologically relevant amount of apoCIII (200 μg apoCIII) induced accumulation of VCAM-1 at the aortic arch (*Figure 5A*). Our image analysis colocalized immunoreactive VCAM-1 with CD31, a well-characterized EC marker. Pitavastatin administration reduced apoCIII-induced VCAM-1 expression to the levels similar to control mice. Quantitative analysis showed that apoCIII injection increased the VCAM-1 immunopositive area in aortic endothelium by 2.6-fold and pitavastatin administration significantly reduced this increase (*Figure 5B*). Similarly, pitavastatin abolished elevation of VCAM-1 mRNA levels in the lysates of aorta following apoCIII injection (*Figure 5C*).

Statin administration reduces apolipoprotein CIII-induced monocyte transmigration *in vivo*

Using a mouse air pouch model of leucocyte recruitment, we furthermore explored another line of *in vivo* evidence for the effects of pitavstatin on apoCIII-mediated inflammation and monocyte/macrophage infiltration. After pre-treating wild-type C57BL/6 mice with pitavastatin via gavage (60 mg/day/kg body weight), we injected apoCIII (200 μg) to the air pouch to induce leucocyte infiltration. We analysed the cell contents of the air pouch cavity 24 h after apoCIII injection through fluorescence-activated cell sorting (FACS), and found that apoCIII injection significantly induced the percentage as well as the total cell number of monocytes/macrophages (F4/80+) (*Figure 5D−F*). Oral administration of pitavastatin almost completely abrogated these apoCIII's effects on infiltration of cells, including monocytes/macrophages into the air pouch.

Nuclear factor κB mediates apoCIII-induced endothelial cell activation

Transcription factor nuclear factor κB (NF-κB) is a key regulator of vascular inflammatory reactions and drives expression of various atherogenic molecules, including VCAM-1. ApoCIII induced NF-κB activation in HSVECs as evidenced by the nuclear location of its p65 subunit (*Figure 6A*). In addition, an inhibitor peptide to NF-κB, SN50, significantly reduced apoCIII-induced VCAM-1 expression in these cells (*Figure 6B*), indicating that apoCIII-induced EC activation is dependent on NF-κB. In addition, pitavastatin treatment at a clinically achievable concentration (50 nM) significantly attenuated apoCIII-induced NF-κB activation (*Figure 6A*). Therefore, inhibition of the NF-κB pathway may be the major mechanism by which statins reduce apoCIII-triggered EC activation. On the other hand, in contrast to apoCIII-induced activation of mitogen-activated protein (MAP) kinase components p38, ERK1/2, and JNK observed in pancreatic β-cells,[11,12] pre-treatment of HSVECs with inhibitors of p38, ERK1/2, and JNK did not affect apoCIII-induced VCAM-1 activation (*Figure 6C*, ANOVA $P = $ NS for apoCIII-treated cells with or without inhibitors).

Discussion

Recent research by our group and others has established that apoCIII, a well-characterized regulator of lipid metabolism, can also act independently of its effects on lipoproteins to promote inflammation in vascular cells[15,16] and pathological changes in other cell types.[11−13] Owing to its lipid-independent and lipid-dependent effects in atherogenesis, apoCIII appears to be an attractive target for pharmacological intervention in patients with hyperlipidaemia. In this study, we demonstrated that statin treatment reduced apoCIII-induced pro-inflammatory and pro-atherogenic reactions in venous and arterial vascular ECs *in vivo* and *in vitro*. Statins directly inhibited apoCIII-induced EC activation as assessed by expression of adhesion molecules, monocyte adhesion and activation of the pro-inflammatory NF-κB pathway, independent from their cholesterol-lowering effects. To our knowledge, this study is the first *in vivo* demonstration of therapeutic suppression of apoCIII-induced vascular inflammation.

Increasing evidence suggests that apoCIII is a novel and potent target for the prevention and treatment of CVD. Epidemiological studies have demonstrated that apoCIII is a strong, independent predictor of CVD after adjusting for the classical blood lipid risk factors.[6−8] To elucidate the molecular mechanism behind oversized atherogenic risk associated with apoCIII, a series of our published studies documented that apoCIII alone, or as a component of apoB containing lipoproteins, activated monocytes and EC and enhances their adhesive interaction *in vitro*.[15,16] To extend these findings *in vivo*, we administered apoCIII to hypercholesterolaemic mice and observed that apoCIII induced the expression and accumulation of adhesion molecule VCAM-1 at the aortic endothelium. Together with our earlier findings, this line of evidence suggests that apoCIII-induced EC activation and monocyte adhesion is one of the major mechanisms through which apoCIII induces atherosclerosis.

EC dysfunction is a hallmark of atherosclerotic lesion initiation and progression. As cells lining the entire inner surface of blood vessels, vascular EC are perpetually exposed to a wide variety of blood borne plasma stimulants. Recruitment of monocytes by adhesion to the vascular wall, the ensuing transendothelial migration and their retention in a complex milieu of pro-atherosclerotic stimuli drive monocyte transformation into macrophages and macrophage-derived foam cells.[4,5] In this study, physiological levels of apoCIII induced the expression of VCAM-1 *in vitro* and *in vivo* and enhanced monocyte adhesion under flow conditions. Given the importance of EC dysfunction in atherogenesis and its clinical manifestation (i.e. acute myocardial infarction), one could theorize that patients with elevated plasma apoCIII are at higher risk for CVD because of the constant exposure of the vascular wall to circulating apoCIII-containing lipoproteins. To counter apoCIII's direct atherogenic effects, we investigated statin treatment on apoCIII-induced EC dysfunction. In addition to lipid lowering, statins may exert anti-inflammatory effects in vascular cells. However, the effects of statins on adhesion molecule expression in ECs remain controversial. Whereas some studies demonstrated that statins reduced the VCAM-1 induction in ECs after exposure to TNF-α,[19,20] others reported that statins enhanced expression of these adhesion molecules[21,22] or had no effect.[23] From the wide range of reported effects, it appears that adhesion molecule expression by statins is greatly influenced by the experimental system, including leucocytes and EC types examined, type of statin experimented, conditions of shear stress, and other factors. In the present study, by using cultured human primary
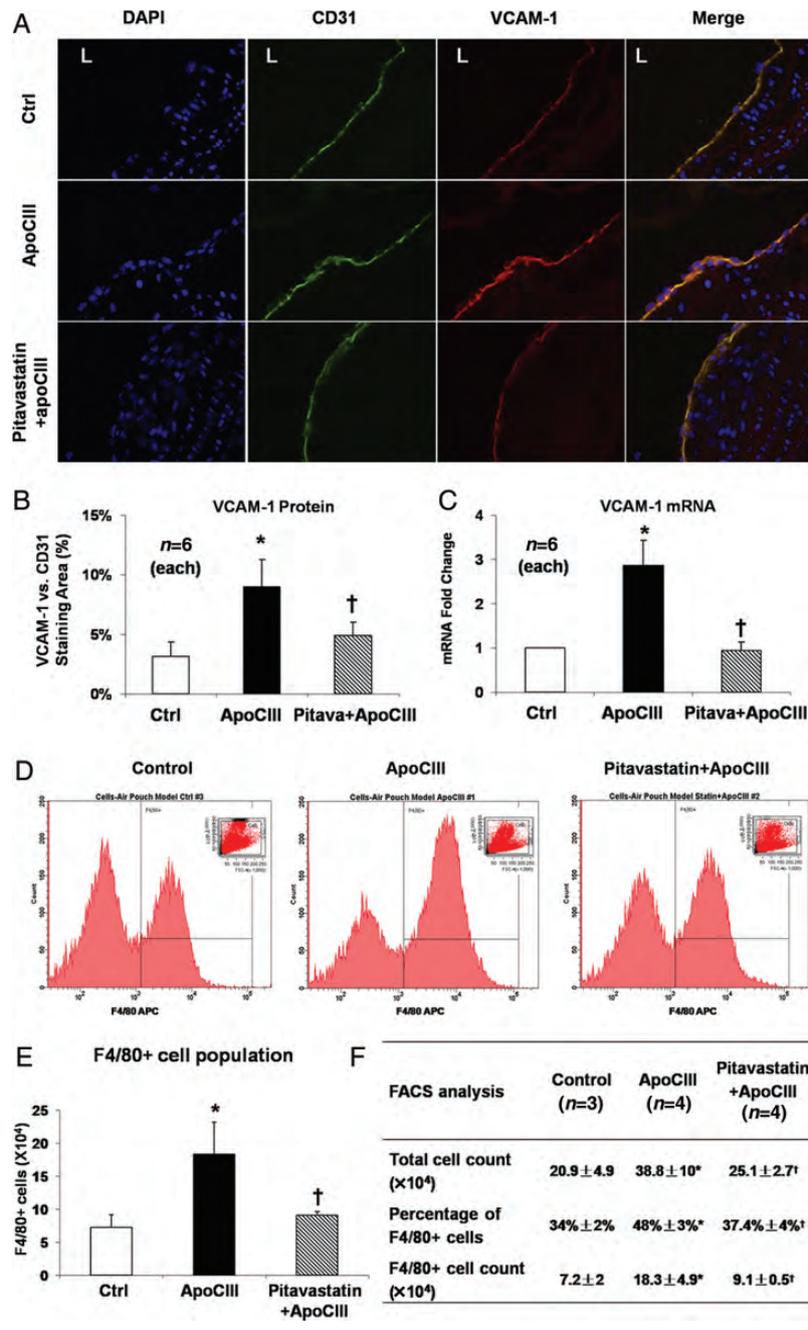
Figure 5 Pitavastatin administration significantly attenuates apoCIII-induced VCAM-1 activation and monocyte infiltration *in vivo*. (A–C) Pitavastatin was administered by gavage for 5 days to $Ldlr^{-/-}$ mice on a high-fat diet for 3 months at the dosage of 60 mg/day/kg body weight. At the end of pitavastatin administration, we injected 200 μg of purified human apoCIII to these mice through the tail vein. After 16 h, we collected aorta from these mice and examined VCAM-1 protein accumulation at the aortic arch by immunohistochemistry analysis (A and B) and VCAM-1 mRNA levels in the whole aorta by qPCR (C). 'L' indicates lumen. Each group had six mice. (D–F) In some other experiments, pitavastatin was administered by gavage for 5 days to wild-type C57BL/6 mice at the dosage of 60 mg/day/kg body weight. These mice were about 5 months old and fed on regular chow diet. Dorsal air pouch was created by injection of 3 mL of sterile air subcutaneously, repeated twice every 3 days. ApoCIII (200 μg in 200 μL PBS) was injected with 3 mL of air. After 24 h, we collected cells from the air pouch by lavage. Infiltrated monocytes and macrophages were stained with the F4/80 antibody, and F4/80+ cell population was determined by FACS analysis. Total cell populations were gated by side/forward scatter (D inserts) and the F4/80+ population was determined. Representative images (D) and quantified results (E and F) from FACS analysis. Data represent mean ± SEM. *$P < 0.05$ between apoCIII treatment and control; $^{\dagger}P < 0.05$ between mice treated with or without pitavastatin (Mann–Whitney test).
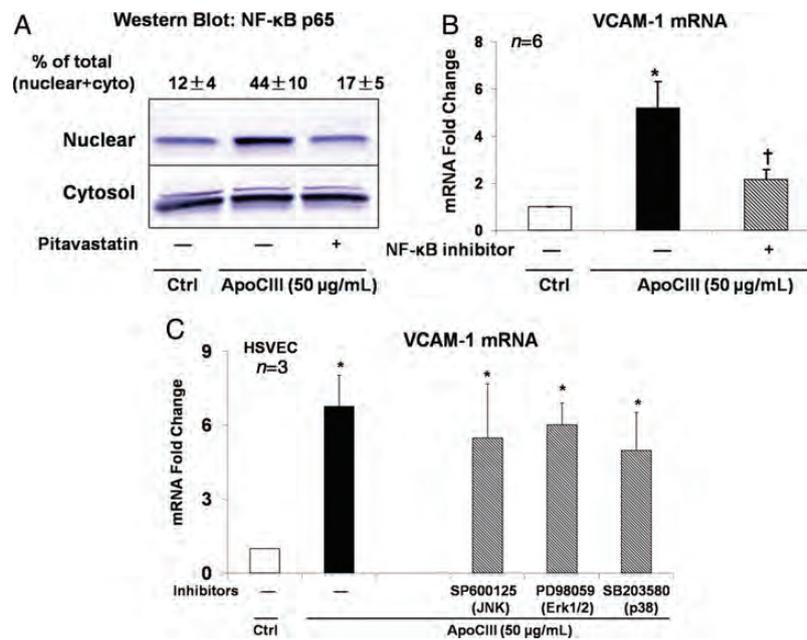
Figure 6 Pitavastatin suppresses apoCIII-induced NF-κB activation. (*A*) Confluent HSVECs were incubated with pitavastatin (50 nM) for 12 h before treatment with purified human apoCIII (50 μg/mL) for 4 h. After cells were lysed, nuclear and cytoplasmic fractions were isolated. Western blotting was performed using a monoclonal antibody against the p65 subunit of NF-κB. The density of the bands was scanned and quantified. In some other experiments, confluent HSVECs were incubated with (*A*) an inhibitor for NF-κB for 30 min or (*B*) inhibitors for JNK (SP600125, 50 μM), ERK1/2 (PD98059, 20 μM), and p38 (SB203580, 10 μM) for 2 h before treatment with purified human apoCIII (50 μg/mL) for 4 h. VCAM-1 expression was determined by qPCR. Data represent mean ± SEM. *P < 0.05 between apoCIII treatment and control [unpaired *t*-test for (*B*), Dunnett's test for (*C*)]; †P < 0.05 between statin treatment and apoCIII alone (unpaired *t*-test).

ECs from saphenous veins and coronary arteries and applying physiologically relevant levels of laminar shear stress, we demonstrated that clinically achievable concentrations of statins suppressed apoCIII-induced VCAM-1 expression and monocyte adhesion without affecting the viability of these cells. Furthermore, *in vivo* administration of pitavastatin at clinically relevant dosages significantly reduced VCAM-1 expression in the aorta of hypercholesterolaemic mice after apoCIII injection and also attenuated the infiltration of monocytes and macrophages in a subcutaneous air pouch model. Collectively, these results indicate that statins may suppress direct atherogenic effects on EC activation by apoCIII in patients.

In addition to inhibiting apoCIII-induced EC activation, statins modestly lower plasma concentrations[24] and hepatic cellular mRNA levels[25] of apoCIII, independent of their effects on LDL-receptor activity. Although the precise mechanism for the effects of statins on apoCIII lowering is not fully understood, it may relate to the activation of the hepatic PPARα pathway.[26] PPARα agonists have been shown to consistently reduce apoCIII expression and plasma levels.[27]

We and others established that lipid lowering is an anti-inflammatory therapy.[5,17] Diet-induced lipid lowering in hypercholesterolaemic rabbits reduced oxidative stress and EC expression of VCAM-1.[3] While evidence clearly suggests that the effects of statin treatment on prevention of coronary events in patients closely associate with the magnitude of cholesterol reduction,[28] clinical significance of statins' pleiotropic effects remains obscure.

Our results suggested that lipophilic statins exerted more pronounced effects in inhibiting apoCIII-induced VCAM-1 expression in vascular EC and monocyte adhesion than a hydrophilic statin, probably via their permeability to peripheral tissues. These data agree with our earlier publications that reported strong lipid-independent effects of lipophilic statins.[29,30]

Our results demonstrated that apoCIII induced the expression of VCAM-1 without affecting ICAM-1. The pathophysiological outcomes of apoCIII differentially mediating the expression of VCAM-1 and ICAM-1 by vascular ECs remain to be further studied. Both VCAM-1 and ICAM-1 are expressed in vascular ECs and play important but different roles in leucocyte recruitment in that VCAM-1 is more important in leucocyte arrest and firm adhesion while ICAM-1 in slow rolling and migration.[31,32] Nonetheless, VCAM-1 is a major adhesion molecule that is expressed by ECs in atherosclerotic plaques and plays an important role in the initiation of atherosclerosis in animal models.[33]

Our study also showed that NF-κB may be a central regulator of apoCIII-induced EC activation and NF-κB inhibition could be the major mechanism by which statins reduce apoCIII-induced EC activation. The key step in NF-κB regulation is the translocation of activated NF-κB from the cytoplasm to the nucleus. Our results demonstrated that statin pre-treatment abolishes apoCIII-induced nuclear translocation of the NF-κB p65 subunit. Consistent with our observation, Størling *et al.*[34] examined apoCIII-induced pancreatic β-cell apoptosis, and reported that apoCIII induced IκBα degradation and NF-κB reporter activity. Previous reports

showed that statins inhibited the expression of pro-inflammatory cytokines through NF-κB inhibition.[35] Since the NF-κB pathway plays an important role in apoCIII-induced pro-inflammatory and pro-atherogenic effects in other vascular cells such as monocytes[15] and macrophages (Zheng et al., unpublished observations), statins may also help to reduce apoCIII's adverse effects in these cells. Interestingly, we did not observe evidence for the involvement of MAP kinases (JNK, Erk1/2, or p38) in apoCIII-induced EC activation. MAP kinases were previously implied in apoCIII-induced apoptosis in a rat insulinaemia cell line,[12] but not in primary rat pancreatic islets.[34] The role of MAPK in apoCIII-induced EC dysfunction remains to be studied.

In conclusion, this study has extended our previous *in vitro* observations to demonstrate that apoCIII, a major surface component of circulating blood lipoproteins, induces the expression of the adhesion molecule VCAM-1 in cultured human arterial and venous ECs. Furthermore, apoCIII also induces aortic EC activation and monocyte/macrophage infiltration *in vivo*, both of which were significantly reduced by statin administration. Our observations provide novel insights into the role for apoCIII as a distinct contributor to atherosclerotic lesion initiation and progression. Statin treatment, especially with lipophilic statins, effectively reduces apoCIII-induced EC activation both *in vitro* and *in vivo*. Although previous studies have shown that statins only modestly lower plasma apoCIII, our results demonstrate that statins directly reduce apoCIII-induced endothelial adhesiveness and suggest a novel vascular protective effect of statins in patients with elevated plasma levels of apoCIII.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Corrigendum

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The surname of one of the corresponding authors was incorrect. Francesca Romana Pluchinotta's surname is Pluchinotta, not Romana Pluchinotta. This has been incorrectly tagged in the journal website and on other databases. The authors apologize for this error.