Acute activation and phosphorylation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors

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The first study to demonstrate that acute statin treatment of endothelial cells results in rapid NO release was conducted by measuring NO release with a NO electrode and by a methemoglobin technique (13). NO release in bovine aortic endothelial cells (BAECs) stimulated with 0.1, 1, and 10 μM pravastatin (PRA) or simvastatin (SIM) was detected as early as 30 s and was blocked by pretreatment with N-nitro-L-arginine methyl ester (L-NAME). In addition to these in vitro experiments, this study demonstrated that both PRA and SIM (1 nM to 10 μM) increased vasorelaxation responses in rat aortic rings. Maximal vasorelaxation of rat aortic rings occurred ~8 min after treatment with a 10 μM concentration of the statins and was completely abolished in endothelium denuded rings. The results of this study demonstrated that statins rapidly stimulate endothelial NO release through a NOS-dependent mechanism.

More recently, another group published several papers that demonstrated the same acute effect of statin treatment on endothelial cells (6, 14). NO and superoxide were measured with microsensors in fetal BAECs in response to treatment with lovastatin (LOV), SIM, PRA, and atorvastatin (ATV) (6) and in human umbilical vein endothelial cells (HUVECs) in response to cerivastatin (14). A maximal increase in NO release was reported with 1 μM SIM and LOV at 9 and 32 s, respectively (6). In a follow-up study, these authors reported pulses of NO release in HUVECs in the first 3 min after treatment with 1 μM cerivastatin that were followed by a sustained but ~63% lower NO release (14). In addition, both of these studies, the authors also observed a smaller increase in superoxide generation that lagged ~2 s behind the observed NO release. The initial increase in NO release and superoxide generation was ~32 and ~48% of that produced by treatment with a calcium ionophore (1 μM A-23187), depending on the statin used. These studies, in addition to the first study (13), clearly indicate by several methods that statins acutely (within s to min) increase endothelial NO release, which appears to be due to the activation of eNOS. The mechanism of eNOS activation, however, is not known.

Previously, it has been reported that activation of eNOS by statins in a time frame of hours to days may occur via posttranslational mechanisms such as altered phosphorylation (3, 15, 21). Several different laboratories have reported that eNOS is phosphorylated in endothelial cells at Ser-1179 (bovine sequence) by Akt protein kinase, resulting in a twofold

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3-HYDROXY-3-METHYLGLUTARYL-COA (HMG-CoA) reductase inhibitors, statins, are among the most widely used prescription drugs and provide beneficial effects independent of lipid lowering (19). Beneficial effects independent of lipid lowering include improvements in endothelial function and angiogenesis that may be due to altered endothelial nitric oxide (NO) synthase (eNOS) expression and activity (19). Most of these studies have examined the effects of statin treatment in a time frame of hours to days and are dependent on HMG-CoA reductase inhibition, which results in increased eNOS expression by posttranscriptional mechanisms (7). However, several recent studies have reported that statins can stimulate endothelial NO release in an acute time frame of seconds to minutes (6, 13, 14).
increase in eNOS catalytic activity and a decrease in the Ca^{2+}-calmodulin (CaM) dependency (5, 9, 17). One study (15) demonstrated that SIM (1 μM) treatment of HUVECs for 30 min resulted in an Akt-dependent increase in eNOS phosphorylation measured by [32P]orthophosphate incorporation. Furthermore, treatment of BAECs for 1 h with 1 μM SIM resulted in a 1.7-fold increase in NO release. These results led the authors to conclude that like other eNOS agonists, statin-induced endothelial NO release was due, in part, to Akt-dependent phosphorylation of eNOS at Ser-1179. Another study demonstrated that ATV not only increased phosphorylation of Ser-1177 (human sequence corresponding to Ser-1179 in the bovine sequence) but also altered the interaction of eNOS with caveolin, heat shock protein 90, and Akt (3). Increased eNOS phosphorylation at Ser-1179 was only reported at 30 min after short-term treatment of HUVECs (0, 5, and 30 min) with ATV (1 μM). Additional experiments demonstrating increased eNOS phosphorylation after statin treatment were conducted in this study using endothelial cells from a variety of species, demonstrating that these regulatory pathways function in a variety of models.

As mentioned previously, the reported time course of statin-induced Ser-1179 phosphorylation in these studies (3, 15, 21) occurred well after the transient increase in NO release described earlier (6, 13, 14). Previous studies in our laboratory have shown that bradykinin (BK) results in rapid (within minutes) and transient NO release from BAECs (22). Furthermore, we showed that BK-stimulated NO release is associated with an increase in phosphorylation at Ser-1179, Ser-635, and Ser-617 (12, 18). In addition, we and others (8, 12) have reported that agonist-induced activation of eNOS is also due, in part, to rapid dephosphorylation of Thr-497 (bovine sequence). Therefore, it is possible that like BK, the acute increase in NO release observed after treatment of endothelial cells with statins may be due to changes in eNOS phosphorylation. Recently, it has been shown that administration of AT II in the range of 25–100 μM during reperfusion significantly reduced infarct size after ischemia in isolated perfused mouse hearts, which was blocked by the phosphatidylinositol 3-kinase (PI3-kinase) inhibitor wortmannin (1). Furthermore, these authors reported a significant increase in Akt and eNOS phosphorylation (Ser-1179) as early as 5 min after reperfusion with 25 μM AT II, suggesting that the improved myocardial protection was due to acute activation and phosphorylation of eNOS. However, NO production was not measured directly, and changes in the other known eNOS phosphorylation sites were not determined. Furthermore, because of the short time course of acute statin-stimulated NO release, it is likely that this effect is not due to HMG-CoA reductase inhibition. However, previous studies reporting acute (within seconds to minutes) statin-stimulated NO release did not explore this possibility.

Therefore, the purpose of the present study was to examine the effects of acute (0–30 min) statin treatment of endothelial cells on the phosphorylation of the five currently known sites on eNOS: Ser-1179, Ser-635, Ser-617, Thr-497, and Ser-116. In addition, we confirmed that statins could acutely stimulate NO release from endothelial cells using an alternative technique and examined the potential role of statin-stimulated eNOS phosphorylation on this response. Finally, we determined whether these effects were dependent or independent of HMG-CoA reductase inhibition.

**METHODS**

**Cell culture.** BAECs were passaged from primary cultures and used for experiments during passages 2–6. Cultures were maintained in water-jacketed incubators at 37°C and 5% CO2 and in M199 medium supplemented with 10% fetal bovine serum, 3% iron-supplemented calf serum, 20 μg/ml l-glutamine, 1× MEM, 0.6 μg/ml thymidine, 500 IU/ml penicillin, and 500 μg/ml streptomycin. Rat aortic smooth muscle cells were also passaged from primary cultures and used in experiments during passages 2–5. Cultures were maintained in DMEM containing 10% fetal bovine serum, 500 IU/ml penicillin, and 500 μg/ml streptomycin.

**Immunoblotting.** Immunoblotting of BAECs was carried out as previously described (10). Before treatment, BAEC cultures were placed in serum-free medium overnight. After treatment, BAECs were washed twice with ice-cold phosphate-buffered saline containing 1 mM Na3VO4. Cells were then lysed in ice-cold buffer containing 20 mM Tris-HCl (pH 7.4), 2.5 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM NaF, 10 mM Na2HPO4, 1 mM Na3VO4, and 1% phenylmethylsulfonyl fluoride (PMSF). Cell lysates were centrifuged at 10,000 g for 20 min to remove insoluble material. For detection of phospho-eNOS by immunoblotting, eNOS was partially purified from cell lysates by affinity binding, by adding 80 μl of a 50% 2’,5’-ADP-Sepharose slurry to 1 ml of the cleared lysate, and then incubated at 4°C overnight. Purified samples were then loaded and run on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane, which was subsequently immunoblotted with one of the following phospho-eNOS antibodies: 1) Ser-1179 (Upstate; no. 07-428), 2) Ser-635 (gift from Kemp), 3) Ser-617 (gift from Kemp), 4) Thr-497 (Upstate; no. 07-185), or 5) Ser-116 (Upstate; no. 07-011). Blots were stripped and reprobed with anti-eNOS antibody (N30020; Transduction Laboratories) to demonstrate equal loading.

**Endothelial NO release.** NO release from BAECs was measured as previously described (11). Briefly, BAEC cultures were switched to serum-free medium overnight and then equilibrated in Locke’s buffer [154.0 mM NaCl, 5.6 mM KCl, 2.0 mM CaCl2, 1.0 mM MgCl2, 3.6 mM NaHCO3, 5.6 mM glucose, 10.0 mM HEPES (pH 7.4), 20 U/ml superoxide dismutase, and 0.3 mM isobutylmethyl xanthine]. Cells were treated with either 10 μM LOV, 10 μM PRA, or 1 μM BK for 0, 1, 2.5, 5, 10, or 30 min. In separate series of experiments, BAECs were preincubated with either t-NAME (1 mM, 1 h), wortmannin (100 nM, 30 min), KT-5720 (500 nM, 30 min), or mevalonic acid (500 μM, 30 min), prepared as described previously (23), before treatment with either 10 μM LOV or PRA for 2.5 min. After treatment with LOV or PRA, the bathing medium was transferred to confluent rat aortic smooth muscle cells (RASMCs) in six-well plates. After the transfer, the RASMCs were incubated for 3 min and then lysed in ice-cold 20 mM sodium acetate, pH 4.0. Lysates were frozen at −20°C until assay for cGMP concentrations using an enzyme immunoassay kit (Cayman). cGMP concentrations in the lysates were quantified and reflect NO released by the endothelial cells.

**Statistics.** Descriptive data (means ± SD and SE) were calculated for each dependent variable. Overall differences between groups were analyzed using a two-way ANOVA with Student-Newman-Keuls post hoc analysis for determining differences between the means when more than two groups were compared. An independent t-test was used when only two groups were compared. In all tests, a probability level of P < 0.05 was used as the decision rule for significance testing.

**RESULTS**

**eNOS phosphorylation.** As illustrated (see Figs. 1–3), Western blot analysis revealed that treatment of BAECs with 10 μM LOV for 0, 2.5, 5, 10, 15, and 30 min (Fig. 1) or 0, 1, 2.5, 5,
10, and 30 min (Figs. 2 and 3) resulted in an increase in eNOS phosphorylation at Ser-1179, Ser-635, and Ser-617, respectively. Figures 1 and 3 also show that Ser-1179 and Ser-617 phosphorylation is attenuated by pretreatment with the PI3-kinase/Akt inhibitor wortmannin, and similar results were obtained with another PI3-kinase/Akt inhibitor, LY-294002 (20 μM for 30 min). In addition, Fig. 2 shows that Ser-635 phosphorylation is attenuated by pretreatment with the protein kinase A (PKA) inhibitor KT-5720 (500 nM for 30 min). No change in eNOS phosphorylation at Thr-497 and Ser-116 was observed at 0, 1, 2.5, 5, 10, and 30 min. Similar results to those shown in Figs. 1–3 were obtained after treatment of BAECs with 10 μM PRA.

**Endothelial NO release.** Figure 4 shows the time course of LOV- and PRA-stimulated NO release compared with BK, measured by the accumulation of cGMP in smooth muscle reporter cells. LOV (10 μM) and PRA (10 μM) treatment of BAECs resulted in a significant (*P < 0.05*) 4-fold and 3.5-fold increase in NO release, respectively, at 2.5 min compared with the 10-fold increase at 5 min induced by BK (1 μM).

Figure 5 shows the effects of various inhibitors on the peak LOV- and PRA-induced NO release at 2.5 min. Values are expressed relative to the basal NO release set at 100%. Pretreatment with mevalonic acid (500 μM, 30 min) had no significant effect (*P > 0.05*) on LOV-stimulated NO release, which was almost completely blocked by pretreatment with l-NAME (1 mM, 1 h; *P < 0.05*). In addition, pretreatment with KT-5720 (500 nM, 30 min) partially blocked LOV-stimulated NO release (*P < 0.05*), and pretreatment with wortmannin

Fig. 1. Lovastatin (LOV)-stimulated endothelial nitric oxide (NO) synthase (eNOS) phosphorylation at Ser-1179 in bovine aortic endothelial cells (BAECs). BAECs were treated with LOV (10 μM) for the indicated times, and cells were lysed. eNOS was partially purified by affinity binding to 2',5'-ADP-Sepharose and immunoblotted (IB) with anti-phospho-Ser-1179 antibody (A and B) and nonphosphospecific anti-eNOS antibody (C). Cells in B were pretreated for 30 min with 100 nM wortmannin (WT). D: histogram summarizing changes in eNOS phosphorylation at Ser-1179 (relative to LOV time 0) from 3 independent experiments. Values are means ± SE; n = 3. *P < 0.05. Similar results were obtained with 10 μM pravastatin (PRA).

Fig. 2. LOV-stimulated eNOS phosphorylation at Ser-635 in BAECs. BAECs were treated with LOV (10 μM) for the indicated times, and cells were lysed. eNOS was partially purified by affinity binding to 2',5'-ADP-Sepharose and immunoblotted with anti-phospho-Ser-635 antibody (A and B) and nonphosphospecific anti-eNOS antibody (C). Cells in B were pretreated for 30 min with 100 nM KT-5720 (500 nM for 30 min). No change in eNOS phosphorylation at Thr-497 and Ser-116 was observed at 0, 1, 2.5, 5, 10, and 30 min. Similar results to those shown in Figs. 1–3 were obtained after treatment of BAECs with 10 μM PRA.
(100 nM, 10 min) significantly \( P < 0.05 \) blocked LOV- and PRA-stimulated NO release. Similar results were obtained with another Akt inhibitor, LY-294002 (20 \( \mu \)M for 30 min).

**DISCUSSION**

Previous studies have demonstrated that statins can stimulate NO release from endothelial cells in a time frame of seconds to minutes; however, the mechanism of this action is unknown (6, 13, 14). The results of this study demonstrate that two different statins, LOV and PRA, stimulate increased eNOS phosphorylation at Ser-1179 and Ser-617 as well as Ser-635 through PI3-kinase/Akt and PKA pathways, respectively. In addition, blocking of the phosphorylation of eNOS with inhibitors of these pathways attenuates acute statin-stimulated NO release, suggesting that eNOS phosphorylation is one potential mechanism of acute statin-stimulated NO release. Furthermore, this study demonstrates that acute statin-stimulated NO release is dependent on eNOS activation and is the first to demonstrate that this is independent of HMG-CoA reductase inhibition.

In the first series of experiments, we evaluated changes in eNOS phosphorylation after acute statin treatment of BAECs. Increased phosphorylation at Ser-1179 was observed as early as 1 min but did not reach maximal levels until 30 min. In addition, statin-stimulated phosphorylation of eNOS at Ser-1179 was blocked by the PI3-kinase/Akt inhibitor wortmannin, which also blocked statin-stimulated NO release. Previous studies have shown that statin treatment of endothelial cells results in an increase in phosphorylation at Ser-1179 in an Akt-dependent manner (3, 15, 21), but none of these studies has reported changes in phosphorylation before 30 min of statin treatment. This may be due to the increased sensitivity of the Western blotting technique used in this study and the overall focus of the other reports on the longer-term effects of statins on eNOS activation and angiogenesis. However, this data is consistent with a more recent report of increased Akt and eNOS phosphorylation (Ser-1179) within 5 min in mouse
hearts perfused with 25 μM ATV (1). Although phosphorylation of Ser-1179 as early as 1 min may play a role in triggering additional mechanisms of eNOS activation, the data suggest that Ser-1179 phosphorylation may play a more important role in long-term statin-stimulated eNOS activation. The effect of wortmannin on Ser-1179 phosphorylation may explain, in part, the effects of wortmannin on statin-stimulated NO release but is more likely due to its effects on another phosphorylation site, Ser-617, as discussed below.

In addition to Ser-1179 phosphorylation, the present study is the first to evaluate the effects of statins on the four other known sites of eNOS phosphorylation: Ser-635, Ser-617, Thr-497, and Ser-116. No change in phosphorylation at Thr-497 and Ser-116 was observed in the present study; however, acute statin treatment resulted in an increase in phosphorylation of both Ser-635 and Ser-617. Previously, we have reported that mimicking phosphorylation of eNOS at either Ser-635 or Ser-617 alters the activity of the enzyme in vitro (18). Mimicking phosphorylation at Ser-635 resulted in a 2.1-fold increase in enzyme activity compared with the wild-type enzyme. Furthermore, we demonstrated that Ser-635 phosphorylation occurred as early as 2.5 min and was sustained as much as 30 min after stimulation of BAECs with BK, which was attenuated by the PKA inhibitor KT-5720. These results correspond well with the current study showing increased phosphorylation of eNOS at Ser-635 within 1 min, sustained as long as 30 min after statin treatment of BAECs. In addition, pretreatment of BAECs with KT-5720 also partially blocked statin-stimulated NO release, suggesting that Ser-635 phosphorylation plays a role in acute statin-stimulated NO release. However, because Ser-635 phosphorylation remained elevated despite the decline in the initial NO release and because PKA inhibition only partially blocked the increased NO release, statin-stimulated phosphorylation at this site may be a secondary event necessary for full activation of the enzyme. In addition, the long-term phosphorylation (up to 30 min) of Ser-635 phosphorylation observed in the present study also suggests that Ser-635 phosphorylation may play a role in the long-term activation of the eNOS.

In contrast to Ser-1179 and Ser-635 phosphorylation, Ser-617 phosphorylation appears to more closely mimic the time course of acute statin-stimulated NO release and may, therefore, play a key role. eNOS phosphorylation at Ser-617 significantly increases Ca2+-CaM sensitivity of the enzyme and is mediated through an Akt-dependent pathway like that of Ser-1179 (18). In addition, Ser-617 phosphorylation is increased transiently between 1 and 2.5 min in BAECs stimulated with either BK or ATP (18). The present study demonstrates a similar effect of statins on eNOS phosphorylation of Ser-617. Statins stimulated an increase in Ser-617 as early as 1 min, peaking around 2.5 min and declining by 5 min. In addition to Ser-1179, pretreatment of BAECs with the Akt inhibitor wortmannin also blocked Ser-617 phosphorylation. The time courses of Ser-617 phosphorylation and NO release measured in this study are very similar, and, coupled with the effects of wortmannin on acute statin-stimulated NO release, suggest that Ser-617 phosphorylation plays an important role in this event.

The relative importance of each of the sites of eNOS phosphorylation in vivo is currently unknown. In addition, the sequence and temporal relationship between the various phosphorylation sites is also unknown; however, it is clear that the pattern of phosphorylation is dependent on the agonist. For instance, although BK and vascular endothelial growth factor (VEGF) both stimulate phosphorylation of eNOS at Ser-1179, Ser-635, and Ser-617, it appears that BK-stimulated NO release is primarily dependent on a transient dephosphorylation at Thr-497, whereas VEGF-stimulated NO release may or may not be (22). Furthermore, it has recently been shown using various phospho- and nonphosphomimetic eNOS mutations that although Ser-1179 phosphorylation results in a more active enzyme, simultaneous dephosphorylation of Thr-497 results in even greater enzyme activity but also increased superoxide generation compared with wild-type or Ser-1179 phosphomimetic eNOS mutant (16). In the present study, statins were shown to increase Ser-1179 phosphorylation without a concomitant increase in Thr-497 phosphorylation, which may lead to a slight uncoupling of the enzyme. This may provide a potential explanation for the acute statin-stimulated increase in superoxide production previously reported in concert with increased NO production (6, 14). It has also recently been shown that shear stress increases eNOS phosphorylation at both Ser-1179 and Ser-635 but causes no change in Thr-497 phosphorylation. Furthermore, phosphorylation of Ser-635 results in a shift to an active enzyme that is not dependent on changes in intracellular Ca2+ (2). These data support the hypothesis that statin-stimulated phosphorylation at Ser-635 may play a more important role in the long-term activation of the enzyme. As various single and multiple phospho-mimetic mutations of eNOS are developed, it may be possible to further discriminate the relative importance of the various eNOS phosphorylation sites in statin-stimulated NO release. The present study, however, provides additional evidence that multiple eNOS phosphorylation sites play a role in both acute and long-term statin-stimulated NO release.

Inhibition of acute statin-stimulated NO release and phosphorylation by the pharmacological inhibitors wortmannin and KT-5720 suggest that acute statin treatment of endothelial cells results in activation of PI3-kinase and PKA, respectively. It is unknown how statins activate PI3-kinase and PKA in the endothelium. PI3-kinase can be activated by interaction with receptors and their adaptor proteins, which contain phosphorylated tyrosine residues, such as growth factor receptors (24). PKA is activated by cAMP, produced by adenylyl cyclase associated with the inner face of the plasma membrane. Adenylyl cyclase activation is coupled to cell surface receptors by G proteins. It is possible, therefore, to speculate that statins may stimulate a cell surface receptor to initiate the rapid phosphorylation signaling cascade observed herein. This cell surface receptor may involve tyrosine autophosphorylation, leading to PI3-kinase activation and/or small G proteins that can activate adenylyl cyclase, increasing cAMP and activating PKA. It is unlikely, however, that statin-induced prenylation of small G proteins such as Ras and Rho (19) plays a role in the acute statin-stimulated NO release observed in this study because the addition of mevalonic acid had no effect.

Another potential explanation for the increase in NO release observed in this study and previous reports (6, 13, 14) is the potential superoxide scavenging effects of statins (20). However, this explanation is unlikely to account for the increase in NO release observed in our study and others (6, 13, 14). First, previous reports of the antioxidant effects of statins have focused on long-term (hours to days) statin treatment (20) and
have shown effects to be dependent on HMG-CoA reductase inhibition as evidenced by reversal of the effects when the downstream product of HMG-CoA reductase, mevalonic acid, is restored (23). NO release in our study was unaffected by treatment with mevalonic acid. Furthermore, the two previous studies reporting acute statin-stimulated NO release from endothelial cells also report a concomitant increase in superoxide production, but no decrease (6, 14). Another study has confirmed these findings, showing that initial treatment of endothelial cells with ATP results in increased superoxide production within the first few minutes and is followed by a drop in superoxide production after treatment of 6 h and beyond (23). Therefore, it appears that although the antioxidant effects of statins may play a role in the observed increase in NO release after statin treatments of hours to days, they do not play a role in the acute NO release reported herein.

It is interesting to note that there also appear to be some differences in the degree of NO release and the response to the various inhibitors depending on the statin used. In the present study, PRA resulted in slightly less, but not statistically significant, NO release compared with LOV (Fig. 5). In addition, inhibition of PKA with KT-5720 appeared to reduce NO release but, unlike LOV, was not statistically significant (Fig. 5). Previously, it has been demonstrated that the various statins have different pharmacokinetics and efficacies in terms of lipid lowering (4). Furthermore, PRA has been shown to be more effective than SIM in stimulating acute NO release and vasorelaxation (13). In addition, using microsensors, Dobrucki et al. (6) demonstrated that although PRA resulted in a slight ~14% increase in NO release compared with LOV, PRA also resulted in an ~87% increase in superoxide compared with LOV. Therefore, the ratio of NO to superoxide was significantly greater after treatment with LOV (6.56) compared with PRA (4.17) and may account for the differences in NO release observed using our reporter cell technique. As novel uses of statins continue to develop, it is important to evaluate more than one statin to determine whether the effects are limited to a particular statin or whether there may be any differential effects depending on the statin utilized that prove useful in refining the development of new statins for alternative uses.

Finally, this was the first study reporting statin-stimulated NO release from endothelial cells within the first few minutes (6, 13, 14) to examine and report that this effect is independent of HMG-CoA reductase inhibition. It is unlikely that statins would have an appreciable effect on eNOS regulation and reactive oxygen species via events downstream of HMG-CoA reductase inhibition, such as inhibition of isoprenoid synthesis (19), due to the short time course. However, to verify that the acute statin-stimulated NO release was, in fact, not due to HMG-CoA reductase inhibition, we examined the effects of statins in the presence of the downstream product of HMG-CoA reductase, mevalonate. As stated above, no change in acute statin-stimulated NO release was observed in the presence of mevalonic acid.

In summary, the present study suggests that increased eNOS phosphorylation at Ser-1179, Ser-635, and Ser-617 is a potential mechanism through which statin treatment of endothelial cells increases NO release within the first few minutes independent of HMG-CoA reductase inhibition. Furthermore, because of the transient increase in phosphorylation observed at Ser-617, this study suggests that Ser-617 may play a more important role in acute statin-stimulated NO release. In addition, this study is the first to suggest that because of the sustained increase in Ser-635 phosphorylation, this site, like Ser-1179, may also be an important factor in long-term regulation of statin-stimulated NO release. Future studies should focus on the relative importance of the various sites of phosphorylation on eNOS, their influence on each other, and their influence on the complex array of eNOS regulatory factors. Understanding the mechanisms involved in the acute or short-term statin-stimulated NO release may help explain the recent reports of improved outcomes after immediate administration of statins to patients with acute coronary syndromes (20) and may lead to the development of a new generation of statins or a new criteria for therapeutic use.

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