Fluid Shear Stress Stimulates Phosphorylation of Akt in Human Endothelial Cells
Involvement in Suppression of Apoptosis

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Abstract—Fluid shear stress alters the morphology and function of the endothelium by activating several kinases. Furthermore, shear stress potently inhibits apoptosis of endothelial cells. Since activation of Akt kinase has been shown to prevent cell death, we investigated the effects of shear stress on Akt phosphorylation. To test the hypothesis that shear stress interacts with the Akt kinase pathway, human umbilical venous endothelial cells were exposed to laminar shear stress (15 dyne/cm²). Western blotting with specific antibodies against the phosphorylated Akt demonstrated a time-dependent stimulation of Akt phosphorylation by shear stress with a maximal increase up to 6-fold after 1 hour of shear stress exposure. The stimulation of Akt phosphorylation by shear stress thereby seemed to be mediated by the phosphoinositide 3-OH kinase (PI3K), as evidenced by the significant inhibition of shear stress–induced Akt phosphorylation by the PI3K inhibitors wortmannin (20 nmol/L) and Ly294002 (10 μmol/L). In addition, pharmacological inhibition of PI3K reduced the antiapoptotic effect of shear stress against growth factor depletion–induced apoptosis. Most important, overexpression of a dominant-negative Akt mutant significantly inhibited the apoptosis-suppressive effect of shear stress against serum depletion–induced apoptosis, thus indicating the direct involvement of shear stress–induced Akt phosphorylation for inhibition of endothelial cell apoptosis. These results define a novel shear stress–stimulated signal transduction pathway, namely, activation of the serine/threonine kinase Akt, which may contribute to the profound changes in endothelial morphology and function by shear stress. (Circ Res. 1998;83:334-341.)

Key Words: protein kinase B ▪ growth factor ▪ integrin ▪ apoptosis ▪ endothelial cell

Mechanical forces play an important role in the cardiovascular system. Especially endothelial cells are continuously exposed to fluid shear stress generated by the flowing blood. Shear stress thereby alters the structure and function of endothelial cells and exerts potent atheroprotective effects. Moreover, shear stress–induced inhibition of endothelial cell apoptosis may contribute to the maintenance of the functional integrity of the endothelial cell.

Alterations in fluid shear stress have been shown to mediate the release of vasoactive mediators and modulate gene expression. Laminar shear stress upregulates the expression of the endothelial NO synthase, the superoxide dismutases, and cyclooxygenase-2, which are potentially atheroprotective. In addition, shear stress modulates the expression of various adhesion molecules, such as vascular cell adhesion molecule-1. The signal transduction regulating gene expression by shear stress is not exactly defined but appears to include the activation of mitogen-activated protein kinases, the c-Jun NH₂-terminal kinase, and modulation of DNA-binding activities of transcription factor activator protein-1 and nuclear factor-κB. Shear stress–induced stimulation of the mitogen-activated protein kinase cascade, which finally leads to extracellular signal–regulated kinase-1 and -2 (ERK1/2) phosphorylation, seems to be mediated by a herbimycin-sensitive tyrosine kinase, likely c-Src. Likewise, c-Jun NH₂-terminal kinase has been described to be activated by shear stress in a similar manner by a Ras-dependent and tyrosine kinase–dependent pathway.11 Shear stress has also been shown to activate focal adhesion kinase, which may account for several cytoskeletal changes observed after shear exposure. Thus, shear stress appears to use signal transduction pathways similar to those involved in integrin-mediated signaling. However, in contrast to the integrin-mediated signal transduction pathways, which are mainly identified, the initial shear stress responsive mechanotransducer and the signal events, which account for the changes of endothelial cellular physiology, still remain ill-defined.

The serine/threonine kinase Akt, also known as protein kinase B or Rac kinase, has been shown to play a key role in matrix adhesion and integrin-mediated signal transduction and in the suppression of apoptotic cell death induced by growth factor deprivation. The activation of Akt seems to be mediated by the phosphoinositide 3-OH kinase (PI3K), which stimulates the phosphorylation of Akt by activating
protein kinase B/Akt kinases (PKD-1 and PKD-2). The downstream targets of Akt include the glycogen synthase kinase-3 and possibly the p70 ribosomal S6 kinase, although neither of these substrates account for the involvement of Akt in cell attachment. Recently, Akt has been shown to phosphorylate the proapoptotic protein Bad, thereby inhibiting its proapoptotic function, which may account for the antiapoptotic effect of Akt.

Thus, the aim of the present study was to investigate the effect of laminar shear stress on Akt phosphorylation and to characterize the upstream signal transduction pathway involved. We demonstrate that shear stress stimulates the phosphorylation of Akt in a time-dependent manner. The shear stress–induced activation of Akt is mediated by PI3K but seems to be independent of tyrosine kinases. Moreover, shear stress–stimulated Akt phosphorylation appears to contribute to the apoptosis-suppressive effect of shear stress against growth factor withdrawal–induced apoptosis. These results define a novel signal transduction pathway, which may importantly contribute to the profound alterations in endothelial morphology and function induced by shear stress.

Materials and Methods

Cell Culture and Shear Stress Exposure

Human umbilical venous endothelial cells (HUVECs) were purchased from Cell Systems/Clonetics and were cultured in endothelial basal medium supplemented with hydrocortisone (1 μg/mL), bovine brain extract (3 μg/mL), gentamicin (50 μg/mL), amphotericin B (50 μg/mL), epidermal growth factor (10 μg/mL), and 10% FCS until the third passage. After detachment with trypsin, 3.5×10^6 cells were grown in 6-cm cell culture dishes for at least 18 hours. Then, HUVECs were exposed to laminar fluid flow in a cone-and-plate brain extract (3 μg/mL) and 5% bovine serum albumin (BSA). After incubation with the second antibody (anti-rabbit, 1:4000) in TBS/0.1% Tween 20/3% NaCl, and 2.5 mmol/L KCl), 0.1% Tween 20, and 3% BSA; antibodies were incubated as follows: phospho-Akt (Biolabs), 1:500, phospho-ERK1/2 (Biolabs), 1:1000 in TBS/0.1% Tween 20/3% NaCl, 10 mmol/L NaVO₄, 10 mmol/L MnCl₂, 10 mmol/L MgCl₂, and 0.5 μmol/L ATP) for 1 hour at 37°C. Samples were loaded onto 9% SDS gels and exposed to autoradiographic films.

Determination of Akt Activation

For determination of the phosphorylated form of Akt, HUVECs were washed 2 times with ice-cold PBS, followed by incubation of the cells with 200 μL cell lysis buffer (20 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na₃VO₄, 1 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride) for 5 minutes on ice. Cells were then scraped off the plates and sonified with a Branson sonifier (3 times for 5 seconds each). The samples were incubated overnight at 65°C. After isolation of DNA by phenol chloroform extraction, the DNA was precipitated with ethanol and resuspended in 100 μL Tris-HCl (pH 7). The resulting pellets were solubilized in TE buffer and the DNA samples were incubated with 5 U Klenow polymerase and 0.5 μCi [32P]dCTP in the presence of 10 mmol/L Tris-HCl (pH 7.5) and 5 mmol/L MgCl₂ for 10 minutes at room temperature according to the method of Rössl et al.

Detection of Apoptosis

For morphological staining of nuclei, cells were centrifuged (10 minutes, 700g), fixed in 4% formaldehyde, and stained with DAPI (0.2 μg/mL in 10 mmol/L Tris-HCl [pH 7], 10 mmol/L EDTA, and 100 mmol/L NaCl) for 20 minutes. Five hundred cells were counted by 2 independent blinded investigators, and the percentage of apoptotic cells per total number of cells was determined.

Transfection

The dominant-negative Akt mutant (Aktmt) (Dr Julian Downward) was cotransfected with pcDNA3.1-iacZ and either pcDNA3.1-Aktmt or pcDNA3.1 control vector lacking an insert. For this purpose, 150 μL medium was mixed with 3 μg plasmids (1 μg pcDNA3.1-iacZ and 2 μg pcDNA3.1-Aktmt or pcDNA3.1) and 30 μl Superfect (Qiagen) and incubated for 10 minutes at room temperature. During the incubation time, the medium was removed from the cell culture plates, and HUVECs were washed once in medium without FCS. Then 1 mL medium was added to the plasmid-Superfect mixture, and HUVECs were incubated for 3 hours at 37°C. After the incubation, culture medium was removed, and HUVECs were incubated for 36 hours to allow protein expression. The transfected cells were identified by β-galactosidase staining. Thereafter, the plates were centrifuged to pellet the detached cells. The cell pellets were then fixed in 2% formaldehyde/0.2% glutaraldehyde, and β-galactosidase activity was determined by incubation of 40 μg/mL X-gal for 6 hours at 37°C. Viable versus dead stained cells were counted by 2 investigators blinded to the experimental conditions, and results were expressed as dead/viable cells×100. In addition, necrotic cell death was excluded by measuring the LDH release, thus indicating that the cell death of the transfected cells is caused by apoptosis.

Statistical Analysis

Data are expressed as mean±SEM from at least 3 independent experiments. Statistical analysis was performed with ANOVA followed by modified least significant difference test (SPSS-Software).
Results

Effect of Shear Stress on Akt Phosphorylation

HUVECs were incubated for 12 hours in the absence of FCS before exposure to laminar flow (15 dyne/cm²) in a cone-and-plate apparatus, and Akt phosphorylation was measured by Western blotting with a phosphospecific Akt antibody. As shown in Figure 1a and 1b, Akt was time-dependently phosphorylated after shear stress exposure. Maximal phosphorylation was obtained after 1 hour and remained enhanced up to 6 hours in the presence of shear stress (Figure 1a and 1b). Prolonged incubation for >12 hours resulted in a decline of Akt phosphorylation to basal levels (Figure 1a and 1b). In addition, enhanced Akt phosphorylation was also detectable by a step-up increase of shear stress. Thus, elevating the shear stress from 5 to 15 dyne/cm² for 1 hour after preexposure of HUVECs to 5 dyne/cm² for 12 hours led to a significant increase of Akt phosphorylation of ~4-fold (n=3) (data not shown).

The specificity of the antibody against the phosphorylated form of Akt was demonstrated by the reaction of the antibody with the phosphorylated form of Akt, whereas no cross-reactivity was observed when nonphosphorylated Akt was loaded (Figure 1a). In addition, reprobe of the Western blot with actin demonstrated equal loading (Figure 1a).

Furthermore, activation of Akt by shear stress was demonstrated by measuring the autophosphorylation of Akt in vitro, which has been shown to correlate with the enzymatic activation of Akt. Therefore, endothelial cells were exposed to shear stress for 1 hour, and autophosphorylation was determined in Akt immunoprecipitates. As shown in Figure 1.
Akt stimulation in other cell systems. Therefore, the effect of PI3K, which has been described to mediate activation of Akt phosphorylation by shear stress, we tested to elucidate the signal transduction pathways underlying the stress–mediated Akt phosphorylation. To further investigate whether shear stress led to a marked increase of Akt autophosphorylation, thus confirming the data obtained by Western blotting using the phospho-specific Akt antibody.

Growth factor addition has been shown to stimulate Akt phosphorylation in neuronal cells. We therefore investigated the effect of serum addition on Akt phosphorylation in HUVECs. As shown in Figure 2a, the incubation of HUVECs with serum also time-dependently stimulated phosphorylation of Akt. To further investigate whether shear stress led to a further stimulation of Akt phosphorylation in the presence of serum, HUVECs were exposed to laminar shear stress in the presence of complete medium containing 10% FCS. Exposure of HUVECs to laminar shear stress also resulted in increased Akt phosphorylation in the presence of serum (Figure 2b). However, whereas Akt phosphorylation increased ∼6-fold in FCS-depleted HUVECs, in the presence of complete medium the increase was only ∼2-fold after 1 hour of shear stress exposure (P<0.05).

Shear stress has been shown to induce the release of growth factors into the medium, which may account for the activation of Akt. However, conditioned medium obtained from HUVECs that were exposed to laminar flow for 1 hour or 12 hours did not stimulate Akt phosphorylation (data not shown), indicating that shear stress–mediated Akt phosphorylation is not due to the release of growth factors into the medium but is mediated by other signaling pathways.

Effect of PI3K and Tyrosine Kinases on Shear Stress–Mediated Akt Phosphorylation

To elucidate the signal transduction pathways underlying the activation of Akt phosphorylation by shear stress, we tested the influence of PI3K, which has been described to mediate Akt stimulation in other cell systems. Therefore, the effect of wortmannin and Ly294002, specific inhibitors of PI3K, on shear stress–induced stimulation of Akt phosphorylation was determined. As shown in Figure 3a, wortmannin at a concentration of 20 nmol/L significantly inhibited Akt phosphorylation stimulated by shear stress exposure (750±50% increase of Akt phosphorylation after 1 hour of exposure to shear stress versus 167±95% in the presence of wortmannin, P<0.05). The inhibition of shear stress–induced Akt activation by wortmannin was confirmed by demonstrating that wortmannin additionally prevented autophosphorylation of Akt (Figure 1c). Similar effects were obtained with Ly294002 (Figure 3a). In addition, wortmannin (Figure 2a) as well as Ly294002 (data not shown) reduced the serum-stimulated Akt phosphorylation, thus demonstrating that PI3K also mediates serum-induced Akt phosphorylation.

Previous studies suggested integrins as initial transducers of shear stress–signaling events. Therefore, integrin binding was blocked by the synthetic peptide GRGDNP, which competitively inhibits fibronectin binding. Preincubation of HUVECs with GRGDNP (0.5 mmol/L) abolished shear stress–induced Akt phosphorylation (Figure 3b), suggesting that the integrin–extracellular matrix interactions are necessary to elicit Akt phosphorylation in response to shear stress.

Having demonstrated that shear stress–induced Akt stimulation depended on integrins, we tried to elucidate the signaling events further downstream. Shear stress has been increased Akt phosphorylation in the presence of serum, HUVECs were exposed to laminar shear stress in the presence of complete medium containing 10% FCS. Exposure to laminar shear stress for 1 hour of shear stress exposure (15 dyne/cm²), and Akt phosphorylation was determined by Western blotting as described above.

Figure 2. Effect of serum on Akt phosphorylation. a, Effect of serum addition on Akt phosphorylation in endothelial cells. HUVECs were starved in medium without supplements for 12 hours. FCS was then added to a final concentration of 10% for the time indicated. Wortmannin (20 nmol/L) was preincubated for 45 minutes. b, Effect of serum on Akt phosphorylation in the presence of FCS. HUVECs were cultured in complete medium and exposed to shear stress for the time indicated, and Akt phosphorylation was determined by Western blotting as described above.

Figure 3. Effect of inhibition of PI3K, integrins, and tyrosine kinases on Akt phosphorylation. a, Effect of wortmannin (20 nmol/L) and Ly294002 (10 μmol/L) on Akt phosphorylation by shear stress. HUVECs were starved for 12 hours. Wortmannin or Ly294002 was then preincubated for 45 minutes before shear exposure for 1 hour (15 dyne/cm²), and Akt phosphorylation was determined by Western blotting with a phospho-specific Akt antibody. The blot was then reprobed with actin to confirm equal loading. A representative blot from at least 3 experiments is shown. Akt-P indicates phosphorylated Akt. b, Effect of PI3K, integrins, and tyrosine kinases on Akt phosphorylation. Shear stress and GRGDNP peptide (0.5 mmol/L) before exposure to laminar shear stress (15 dyne/cm²) for 1 hour. A representative Western blot with a phospho-specific Akt antibody was reprobed with an antibody directed against phosphorylated p42 and p44, which correspond to ERK1/2.
shown to stimulate ERK1/2 phosphorylation via a herbimycin A–sensitive kinase, presumably c-Src. Therefore, the effect of the Src inhibitor herbimycin A on shear stress–induced Akt phosphorylation was determined. As shown in Figure 3b, preincubation of HUVECs with herbimycin (0.5 μmol/L) did not affect the increase of Akt phosphorylation induced by shear stress. However, incubation with herbimycin A completely reversed shear stress–induced phosphorylation of the p42 and p44 proteins corresponding to ERK1/2 (Figure 3b).

Involvement of Akt in the Apoptosis-Suppressive Effect of Shear Stress
Shear stress has been demonstrated to reduce growth factor deprivation–induced apoptosis of human endothelial cells. Since Akt has been shown to prevent neuronal cell death induced by growth factor depletion, we tested whether Akt phosphorylation contributes to the apoptosis-suppressive effect of shear stress. As shown in Figure 4a, depletion of FCS for 18 hours potently induced apoptosis of endothelial cells (2.0 ± 0.5% apoptotic cells) which was significantly suppressed by shear exposure as assessed by morphological analysis of fluorescence-stained nuclei (2.5 ± 0.6% apoptotic nuclei in the presence of shear stress, P < 0.01). Coincubation with wortmannin abolished the protective effect of laminar shear stress, thus indicating that PI3K is involved in the apoptosis-suppressive effect of shear stress. In addition, incubation with Ly294002 slightly increased FCS depletion–induced apoptosis (424 ± 119%) and completely prevented the apoptosis-suppressive effect of shear stress (540 ± 182% versus 123 ± 25% in the presence or absence of Ly294002, respectively; P < 0.05). In addition, apoptotic cell death was detected by demonstration of the typical DNA laddering. As illustrated in Figure 4b, serum depletion–induced DNA laddering was prevented by exposure to shear stress, whereas the further addition of Ly294002 abolished the antiapoptotic effect of shear stress.

To further demonstrate that the PI3K-stimulated Akt phosphorylation accounts for the antiapoptotic effect of shear stress, Akt was specifically inhibited by the expression of a dominant-negative mutant. Thus, HUVECs were cotransfected with β-galactosidase and dominant-negative Akt mutant. Apoptosis was then induced by serum depletion, transfected cells were identified by β-galactosidase staining, and viable versus dead cells were counted (Figure 5a). Dead cells were additionally analyzed under higher magnification to confirm the morphological alterations typical for apoptotic cell death as shown in Figure 5a. Inhibition of Akt by expression of the dominant-negative mutant potently reduced the apoptosis suppression of shear stress (Figure 5a and 5b). Most important, inhibition of Akt by expression of the dominant-negative mutant potently reduced the apoptosis suppression of shear stress (Figure 5a and 5b). In order to further document inhibition of apoptosis suppression, we determined the effect of the dominant-negative Akt mutant on serum depletion–induced DNA laddering. Therefore, HUVECs were transfected with 3 μg plasmid encoding the Akt mutant, and cells were treated as shown before. Analysis of the DNA laddering demonstrated that overexpression of the Akt mutant reduced the apoptosis-suppressive effect of shear stress (Figure 5c), although to a minor extent compared with that shown in Figure 5b, where morphological analysis of only transfected cells was used to quantify the extent of apoptosis. However, this apparent discrepancy might be readily explained by the fact that only ≈24 ± 4% of the cells are transfected; thus, only these cells can be resistant to the antiapoptotic effect of shear stress. Taken together, activation of the serine/threonine kinase Akt by shear stress
Figure 5. Effect of overexpression of a dominant-negative Akt mutant (Aktmt) on the apoptosis-suppressive effect of shear stress. 

a, Effect of the expression of a dominant-negative Aktmt. HUVECs were transiently cotransfected with pcDNA3.1 (vector, 2 μg) or pcDNA3.1-Aktmt (2 μg) and pcDNA3.1-β-galactosidase (1 μg) and incubated for 24 hours to allow protein expression. Apoptosis was then induced by serum depletion in the presence or absence of shear stress (15 dyne/cm²) for 18 hours, and transfected cells were identified by β-galactosidase staining. Panel a (A to E; enlargement, 1:10) shows an overview with arrows indicating the dead cells. Dead cells were additionally analyzed at ×40 magnification as demonstrated in panel a, F. b, Experiments were performed as described above, and cells were exposed to laminar shear stress (SS, 15 dyne/cm²). Dead versus viable stained cells were counted, and results are expressed as percentage of dead cells/total cell number (mean ± SEM, n = 3, *P < 0.05 vs serum depletion + vector + SS). c, Effect of transient transfection of the dominant-negative Aktmt on DNA laddering. Cells were transfected before stimulation of apoptosis by FCS depletion for 18 hours. DNA was then isolated from all cells (transfected and untransfected), and DNA laddering was detected after Klenow labeling. At the top, an ethidium bromide staining to confirm equal loading is shown.
appears to play a major role in the apoptosis-suppressive effect of shear stress in growth factor–depleted HUVECs.

Discussion
The major finding of the present study is that Akt is phosphorylated in response to laminar fluid shear stress in human endothelial cells in a time-dependent manner. Furthermore, the stimulation of Akt phosphorylation seems to be mediated by PI3K, as demonstrated by significant suppression of shear stress–induced Akt phosphorylation by wortmannin or Ly294002, which have been shown to inhibit PI3K but not PI4K, protein kinase A, or protein kinase C.

Moreover, shear stress–induced Akt phosphorylation at least in part accounts for the shear stress–mediated inhibition of serum depletion–induced apoptosis. Taken together, the present study not only demonstrates that shear stress induces Akt phosphorylation but additionally provides evidence for a physiological function of shear stress–triggered Akt activation.

The PI3K-dependent Akt stimulation has been described in neuronal and epithelial cells to be essential for cell attachment and has been shown to be stimulated by integrin receptor and growth factor receptor activation. Indeed, similar results were obtained in the present study using human endothelial cells, where the addition of growth factors stimulated Akt phosphorylation. In addition, we now demonstrate that mechanical stimulation of endothelial cells by laminar shear stress also induces Akt phosphorylation. Shear stress thereby induces Akt phosphorylation in the absence of growth factors to an extent similar to that induced by growth factor stimulation, but shear stress also enhances Akt phosphorylation in the presence of growth factors, indicating an additive effect of shear stress. Thus, these results may support the suggestion that shear stress–mediated signal transduction is linked to integrin-mediated or growth factor receptor–mediated signal events but, in addition, may stimulate further pathways.

The upstream signal transduction pathway leading to Akt stimulation seems to be mediated at least in part by distinct signal transduction pathways compared with the proposed events involved in ERK1/2 activation. Shear stress–induced ERK1/2 phosphorylation has been shown to be dependent on integrin–extracellular matrix binding, followed by the activation of a herbimycin-sensitive tyrosine kinase c-Src. Akt phosphorylation in response to shear stress also seems to require integrin binding, since blocking peptide inhibitors completely prevented shear stress–induced Akt phosphorylation. However, the incubation of HUVECs with the Src inhibitor herbimycin A in concentrations that exert potent inhibitory effects on ERK1/2 activation did not affect Akt phosphorylation stimulated by shear stress. Thus, the initial shear stress transduction seems to be shared by both signaling pathways, but then Akt phosphorylation appears to be independently regulated by a distinct pathway.

The data of the present study further demonstrate a link between shear stress–induced Akt phosphorylation and the antiapoptotic effects of shear stress. PI3K-stimulated Akt phosphorylation has been shown to play a key role in preventing apoptosis induced by serum factor withdrawal.

Indeed, PI3K seems to be involved in the antiapoptotic capacity of shear stress in human endothelial cells, as demonstrated by the reduction of the apoptosis-suppressive effect of shear stress by pharmacological inhibitors wortmannin and LY294002. Most important, however, the overexpression of a dominant-negative Akt mutant significantly reversed the antiapoptotic effect of shear stress, clearly indicating that the downstream target of PI3K, Akt, mediates the apoptosis-suppressive effect of shear stress. The targets of Akt are currently only poorly described. Recently, Akt has been shown to stimulate the phosphorylation of the proapoptotic protein Bad, thereby inhibiting its proapoptotic function.

Thus, the link between Akt and the members of the Bcl-2 family of proteins may contribute to the antiapoptotic function of Akt. A further possible implication might be the posttranscriptional stabilization of proteins by influencing p70S6 kinase, which plays an important role in protein translation. Thus, shear stress might not only interfere with gene expression but might also directly modulate protein translation in endothelial cells via the p70S6 kinase activation. Indeed, preliminary results recently demonstrated that shear stress stimulates the phosphorylation of p70S6 kinase. However, p70S6 kinase has been shown to be activated independent of Akt too,

raising the question whether this pathway contributes to the antiapoptotic effects of Akt.

In summary, the present study defines a novel shear stress–stimulated signal transduction pathway, which may account for several functional and morphological alterations of endothelial cells after exposure to shear stress. In addition, Akt phosphorylation, which preserves cell viability and attachment in other cell types, might contribute to maintain endothelial cell viability in response to shear stress. Indeed, shear stress exerts a potent atheroprotective effect by altering endothelial cell physiology. Further characterization of the physiological consequences of Akt phosphorylation in endothelial cells will provide important information in clarifying the potential antiatherogenic role of shear stress–induced Akt phosphorylation.

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References


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