Physiological fluid shear stress causes downregulation of endothelin-1 mRNA in bovine aortic endothelium

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Malek, Adel, and Seigo Izumo. Physiological fluid shear stress causes downregulation of endothelin-1 mRNA in bovine aortic endothelium. *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C389–C396, 1992.—We report here that the level of endothelin-1 (ET-1) mRNA from bovine aortic endothelial cells grown in vitro is rapidly (within 1 h of exposure) and significantly (fivefold) decreased in response to fluid shear stress of physiological magnitude. The downregulation of ET-1 mRNA occurs in a dose-dependent manner that exhibits saturation above 15 dyn/cm^2. The decrease is complete prior to detectable changes in endothelial cell shape and is maintained throughout and following alignment in the direction of blood flow. Peptide levels of ET-1 secreted into the media are also reduced in response to fluid shear stress. Cyclical stretch experiments demonstrated no changes in ET-1 mRNA, while increasing media viscosity with dextran showed that the downregulation is a specific response to shear stress and not to fluid velocity. Although both pulsatile and turbulent shear stress of equal time-average magnitude elicited the same decrease in ET-1 mRNA as steady laminar shear (15 dyn/cm^2), low-frequency reversing shear stress did not result in any change. These results show that the magnitude as well as the dynamic character of fluid shear stress can modulate expression of ET-1 in vascular endothelium.

gene regulation; mechanical stress; dynamic forces; vasoactive substances; growth substances; vessel structure; atherosclerosis

MECHANICAL FORCES determine and regulate the structure of animal cells and tissues during development and as a compensatory mechanism in physiological and pathological situations. One example is the vascular endothelial cell. Furthermore, the endothelium has been shown in recent years to elaborate a number of growth factors, such as transforming growth factor-β (TGF-β), basic fibroblast growth factor (22), platelet-derived growth factor A- and B-chain (20), vasodilators such as endothelium-derived relaxing factor (EDRF) and prostacyclin (prostaglandin I_2; PGI_2) (6), and mitogenic vasoconstrictors such as angiotensin II (ANG II) and endothelin-1 (ET-1) (23), and play a central role in regulating the growth and functional phenotype of vascular smooth muscle and fibroblast cells.

In most large-size vessels, one can estimate the magnitude of the fluid shear stress (τ), $\tau = 4\mu Q/\pi r^3$ (Hagen-Poiseuille law), as being proportional to viscosity (μ) and flow rate (Q) and inversely proportional to the third power of the internal vessel radius (r) (10). This relationship indicates that, by controlling the tone of the underlying smooth muscle cells and thereby changing internal vessel radius, the endothelial cell can control the magnitude of fluid shear stress to which it is exposed at any given flow rate.

A number of reports have clearly demonstrated that long-term fluid shear stress in arterial vessels is maintained constant at ~15 dyn/cm^2, regardless of species and vessel caliber and despite externally induced blood flow changes at a constant pressure (12, 13). Murray (17) hypothesized that maintenance of a constant fluid shear stress at the vessel wall throughout the arterial vasculature was a consequence of the "optimization of an energy cost function." Functional and structural changes in the endothelial cells have been reported to be dependent on fluid shear stress. The endothelium responds to fluid shear stress in vitro by changing its shape from polygonal to spindlelike and aligning itself in the direction of the shear (21), rearranging its actin microfilament network and elaborating actin stress fibers (7). Increased PGI_2 release upon exposure to step changes in shear stress (6) and increased tissue-type plasminogen activator secretion in response to shear stress exceeding a threshold magnitude have also been reported (5). The endothelial cell has also been shown to be sensitive to the dynamic nature as well as to the magnitude of shear stress since turbulent, but not laminar, shear stress was found to induce entry into the cell cycle (4) and fluid-phase pinocytosis was found to be increased as a result of step changes, but not periodic variations, in shear (3).

Taken together, these findings point to the existence of a relationship between structure and function that is locally regulated and provide strong evidence to suggest the possibility that the endothelium senses local shear stress and controls vessel structure. Among the potential effectors that may be important in modifying structure in response to external mechanical forces, ET-1 (28) has been shown to be the most potent vasoconstrictor described to date. This 21-amino-acid peptide has also been shown to induce c-*fos*, c-*myc*, mitogenesis in smooth muscle cells and fibroblasts (16), and hypertrophy in the cardiac myocyte (25). ET-1 expression and release have been shown to be induced by thrombin, TGF-β, ANG II, calcium ionophore, and interleukin-1 (see Refs. 11, 26 for review).

Although the initial report describing the isolation of ET-1 mentioned (but data not shown) that ET-1 expression was decreased by shear (28), a later finding by the same group (29) using aortic endothelial cells reported a transient increase by shear that peaked at 2 h and returned to baseline by 16 h. Because fluid shear stress is likely to be the major physiological regulator of expression of the ET-1 gene, we have systematically evaluated the effects of well-defined levels of steady and time-varying fluid shear stress on endothelial levels of ET-1 mRNA. We have found that ET-1 mRNA levels and ET-1 peptide release are downregulated by physiological levels of laminar shear stress in a time- and dose-dependent manner that is sensitive to the dynamic nature of the stimulus.

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MATERIALS AND METHODS

Cell culture. Bovine aortic endothelial (BAE) cells were isolated from freshly obtained aortas (Arena, Hopkinton, MA) as previously described (9) by using partial collagenase treatment. Endothelial purity of the cell population was assessed by using the acetylated/low-density lipoprotein (Biomedical Technologies, Stoughton, MA) uptake method (18) and was found to be greater than 98% (data not shown). Cells were grown in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 10% calf serum (GIBCO), 4 mM L-glutamine, 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.4, 10,000 U/ml of penicillin, and 10 mg/ml of streptomycin at 37°C, 5% CO_2 in a humidified incubator. Cells were passaged every 6–7 days by using 0.5% trypsin-0.53 mM EDTA-4 mM Na for 2–3 min followed by dilution in the growth media and seeding in tissue culture plates (Falcon Labware, Oxnard, CA). Confluent cells were used from passages 6–15 and subjected to shear stress 24–48 h following the last change of culture medium. Where indicated, cells were cultured with media containing low serum (0.5% calf serum) or serum-free media [supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), and ascorbic acid (100 μM)].

Shear-stress apparatus. A cone-plate viscometer similar to the one described by Sdougos et al. (24) but modified to accept polystyrene tissue culture plates was used to subject BAE cells to fluid shear stress. Briefly, this consists of a cone of shallow angle (α) rotating at angular velocity (ω) on top of a tissue culture plate on which the endothelium is grown and subjected to fluid shear stress of controlled magnitude. Figure 1*A* shows a schematic diagram along with the relationship ($\tau = \mu\omega/\alpha$) between shear-stress magnitude and the various parameters in the case of steady laminar flow.

Flatness of the tissue culture plates once in the device as measured with a depth indicator (±0.0005-in. accuracy) was found to be within 5% of the gap between the cone and the tissue culture plate. Ink studies and measurement of the angle formed by the streamline between the radial and tangential components of shear stress at the surface of the tissue culture plate under conditions of secondary flow were used to demonstrate that the fluid-mechanical properties of the device were within 10% of the theoretical predictions (24).

Two cone angles were used, 1° to attain laminar flow and 5° for turbulent flow (chaotic variations of shear stress in magnitude and direction) (4). The magnitude of the fluid shear stress at the tissue culture plate in the various regimens was computed along the radius of the tissue culture dish and the average was obtained by using the equations derived previously (4).

Sinusoidally varying shear stress was obtained in two ways. *1*) Pulsatile fluid shear stress of frequency 2.5 Hz and magnitude 12–18 dyn/cm^2 was obtained by introducing a wobble effect at the level of the cone as previously described (3). *2*) Reversing sinusoidal fluid shear stress of magnitude ±21 dyn/cm^2 and frequency 0.25 Hz was obtained by varying the angular velocity of the cone, while maintaining the rates of cone rotational acceleration low enough to ensure transmission of the shear forces to the tissue culture plate as described by Sutera and Nowak (27). Notice that the parameters for the two above conditions were chosen so as to maintain the same mean value of the absolute shear-stress magnitude as for the laminar and turbulent conditions (15 dyn/cm^2). The various time profiles under the four different flow conditions are shown in Fig. 1*B* (the time profile for turbulent shear is simply shown for illustration and is not exact).

The tissue culture media was supplemented in some experiments with 5% uncharged dextran (mol wt 70,000, Sigma Chemical, St. Louis, MO) to increase medium viscosity by 2.5-fold, thereby allowing the use of a lower cone rotational and fluid velocity without affecting the magnitude of fluid shear stress.

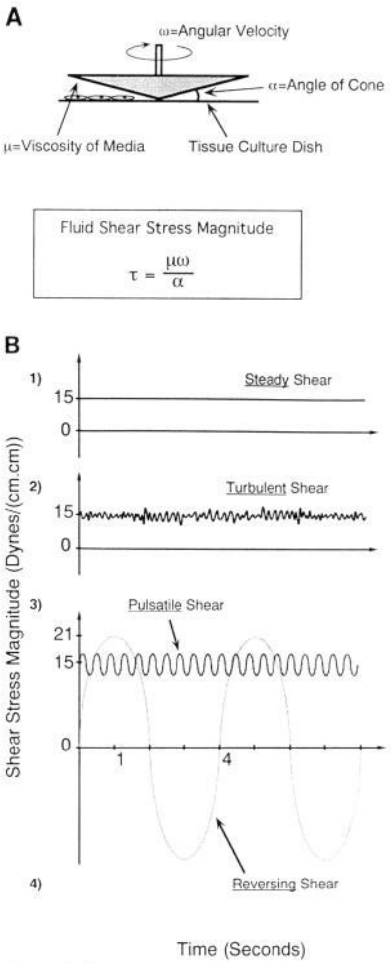


Fig. 1. Description of shear-stress apparatus. *A*: schematic figure of cone-plate viscometer used to apply fluid shear stress to endothelial cells plated in tissue culture dishes. *B*: representation of 4 regimens of fluid shear stress to which endothelial cells were exposed: *1*) steady laminar shear, *2*) turbulent shear, *3*) pulsatile laminar (2.5 Hz frequency) shear (12–18 dyn/cm^2), and *4*) reversing laminar (0.25 Hz frequency) shear (±21 dyn/cm^2). Note that all 4 regimens have same absolute mean value as a function of time of 15 dyn/cm^2.

Lactate dehydrogenase measurements of the supernatant of cells exposed to shear stress for a period of 24 h revealed no significant increase compared with cells maintained under static conditions (data not shown), indicating a lack of cell injury.

Dynamic stretch apparatus. Bovine aortic endothelial cells were grown to confluence on silicone sheet precoated with rat-tail type I collagen (Biomedical Technologies, Stoughton, MA). After the last feeding with media containing 10% calf serum (36–48 h), confluent cells were mounted on a dynamic stretch apparatus (courtesy of Dr. Thomas Kulik) and their substratum was linearly stretched by 20% cyclically at a rate of 20/min. Cells were then lysed for RNA isolation.

RNA isolation and Northern analysis. Total cellular RNA was obtained by using the acid guanidium thiocyanate phenol chloroform method (2). RNA concentration and relative purity were quantified by measuring absorbance at 260 nm and the ratio of the absorbance at 260 nm relative to that at 280 nm. Twenty-five micrograms of RNA were loaded per well and separated on 1.5% agarose gels containing 6% formaldehyde, 0.02 M 3-(*N*-morpholino)propanesulfonic acid, 0.005 M sodium acetate, and 0.001 M Na^{2+}·EDTA. RNA was transferred onto GeneScreen

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membranes (New England Nuclear, Boston, MA) by capillary blotting using 10× standard saline citrate (SSC; 1.5 M sodium chloride, 0.15 M sodium citrate, pH 7) and immobilized by ultraviolet irradiation. The membranes were prehybridized at 42°C in 50% formamide, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficoll, 0.05 M tris(hydroxymethyl)-aminomethane·HCl (pH 7.5), 1.0 M NaCl, 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, 100 μg/ml of denatured salmon sperm DNA and 1 μg/ml each of poly(A) and poly(C). Hybridization was carried out in the same solution containing a ^{32}P-labeled 1.9 kb *Eco*R I cDNA specific for bovine preproendothelin-1 (kind gift of Dr. Thomas Quertermous) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). After overnight incubation, the membranes were washed in 2× SSC-0.3% SDS at room temperature for 15 min, in 0.2× SSC-0.3% SDS for 30 min at room temperature, and in 0.2× SSC-0.3% SDS for 1 h at 55°C and then exposed to X-ray film (Kodak X-Omat-AR) at −80°C.

Densitometry. Autoradiograms exposed in the linear range of the X-ray films were scanned in two dimensions. Densitometry of individual bands of interest was carried out on the stored digital image by subtraction of the local background and two-dimensional integration. To control for RNA loading and transfer to the filter, the hybridization signals of ET-1 mRNA were then normalized for each sample with respect to the density of the corresponding GAPDH mRNA signal. GAPDH was chosen as internal control because the concentration of GAPDH mRNA per microgram of total RNA did not change significantly with or without shear stress (Fig. 3 and data not shown)

Radioimmunoassay for ET-1 peptide. A radioimmunoassay kit for bovine ET-1 peptide (RIK 6901, Peninsula Laboratories, Belmont, CA) was used. Five microliters of supernatant were mixed with 45 μl of assay buffer, and the samples were processed according to the manufacturer's instructions. Cross-reactivity was 7% to ET-2 and ET-3 and 35% to Big ET-1. Samples were measured in triplicate, and the concentration was obtained by reading off a cubic spline fit through the standard curve obtained from triplicate standard samples.

RESULTS

Endothelial cells align in direction of fluid shear stress. Figure 2 shows BAE cells that have been subjected to steady laminar shear stress of magnitude 15 dyn/cm^2 in our cone-plate viscometer for 0, 3, 6, 12, 18, and 24 h. Cells at 6 h show little shape change but progressively begin to appear more aligned by 12 h and eventually complete their shape change by 18–24 h. This is in agreement with the results obtained by Remuzzi et al. (21) and suggests that our device using plastic tissue culture dishes

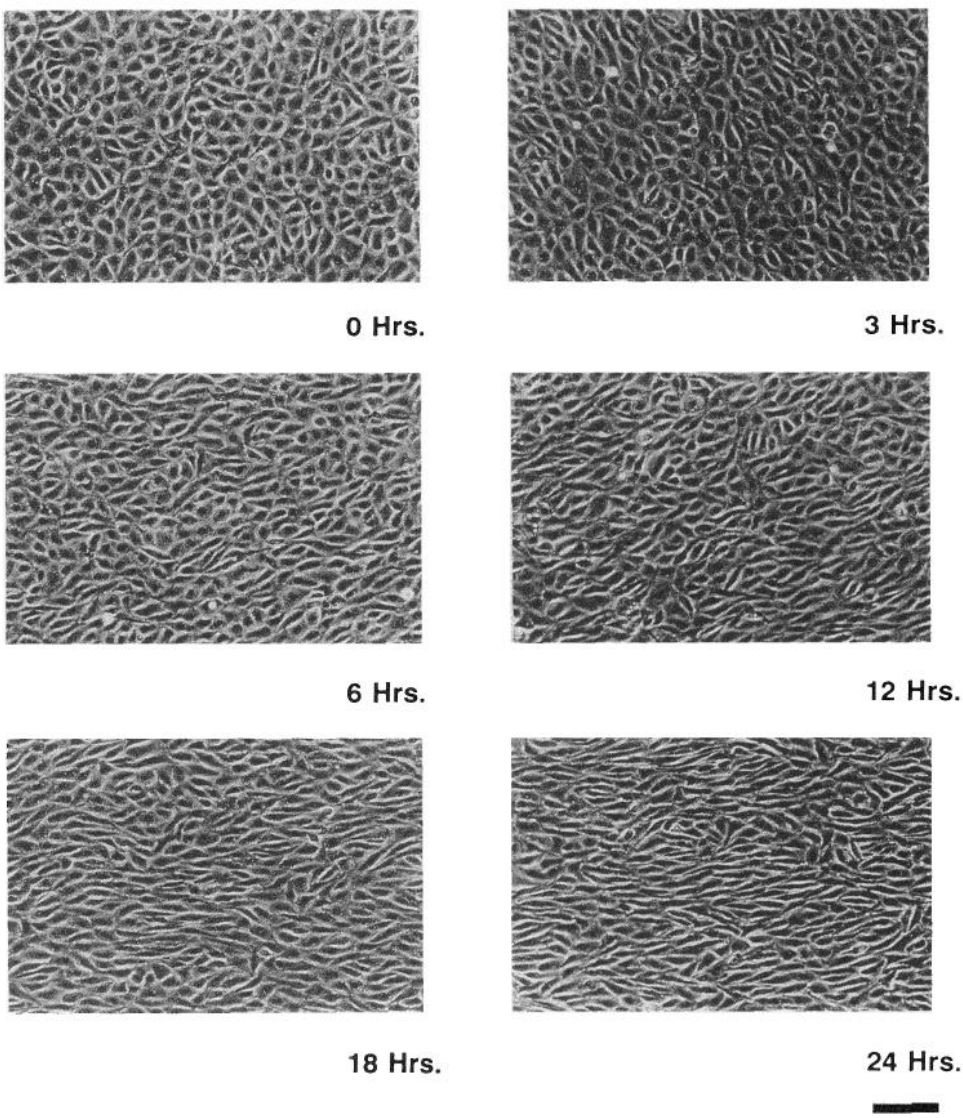


Fig. 2. Bovine aortic endothelium shape and alignment change in response to steady laminar shear stress. Representative phase-contrast micrographs of bovine aortic endothelial (BAE) cells exposed to steady laminar shear stress of magnitude 15 dyn/cm^2 for 0, 3, 6, 12, 18, and 24 h. Direction of flow and fluid shear stress is from left to right. Bar, 100 μm.

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produces similar effects to those previously described with cells grown on microscope slides in parallel-plate and cone-plate viscometers.

Fluid shear stress of physiological magnitude downregulates ET-1 mRNA and ET-1 peptide secretion in BAE cells. We have analyzed RNA from BAE cells subjected to steady laminar fluid shear stress (15 dyn/cm^2) and have observed a significant downregulation of ET-1 mRNA that is shown in Fig. 3*A*. Densitometry of multiple Northern blots normalized to GAPDH mRNA signals revealed a sustained four- to fivefold decrease (Fig. 3*B*). This shear-induced downregulation was evident as soon as 1 h following the onset of shear-stress application and was complete by 2–4 h. The change in ET-1 mRNA occurred prior to any observable changes in the shape of the endothelium as seen by phase-contrast microscopy (compare Fig. 2 and Fig. 3*B*) and persisted following completion of cell shape change.

We supplemented the culture media with 5% uncharged dextran to induce a 2.5-fold increase in kinematic viscosity with a corresponding decrease in the fluid velocity at the same level of shear stress and observed the same phenomenon, occurring with a similar time course and magnitude (data not shown). The latter finding confirms that it is fluid shear stress rather than fluid flow velocity which is the mechanical factor that is inducing the downregulation of ET-1. Our finding of endothelial functional and biochemical changes prior to cellular shape changes is similar to that of Franke et al. (7), who have documented actin stress fiber changes as soon as 3 h following onset of low-magnitude shear stress before the observation of any cell shape changes.

We have verified that the changes in mRNA level of ET-1 were reflected by changes in ET-1 peptide by collecting aliquots of supernatant from cells that were fed with fresh media at the onset of shear (15 dyn/cm^2) and measuring ET-1 immunoreactive peptide. Figure 3*C* shows that cells exposed to shear stress secreted significantly lower levels of ET-1 peptide at 24 and 48 h compared with cells maintained under static conditions. This decrease seems smaller in magnitude than that observed with ET-1 mRNA and may reflect the potential presence of a difference in the rate of translation, processing, or secretion of the peptide or in its rate of degradation or conversion in the media between shear stress and static conditions.

To assess the importance of serum concentration in the culture media on the downregulation of ET-1 by fluid shear stress, cells were cultured with low serum (0.5%) and serum-free media before exposure to shear. Shear stress induced strong ET-1 mRNA downregulation in these cells similar to the one seen in cells grown in 10%

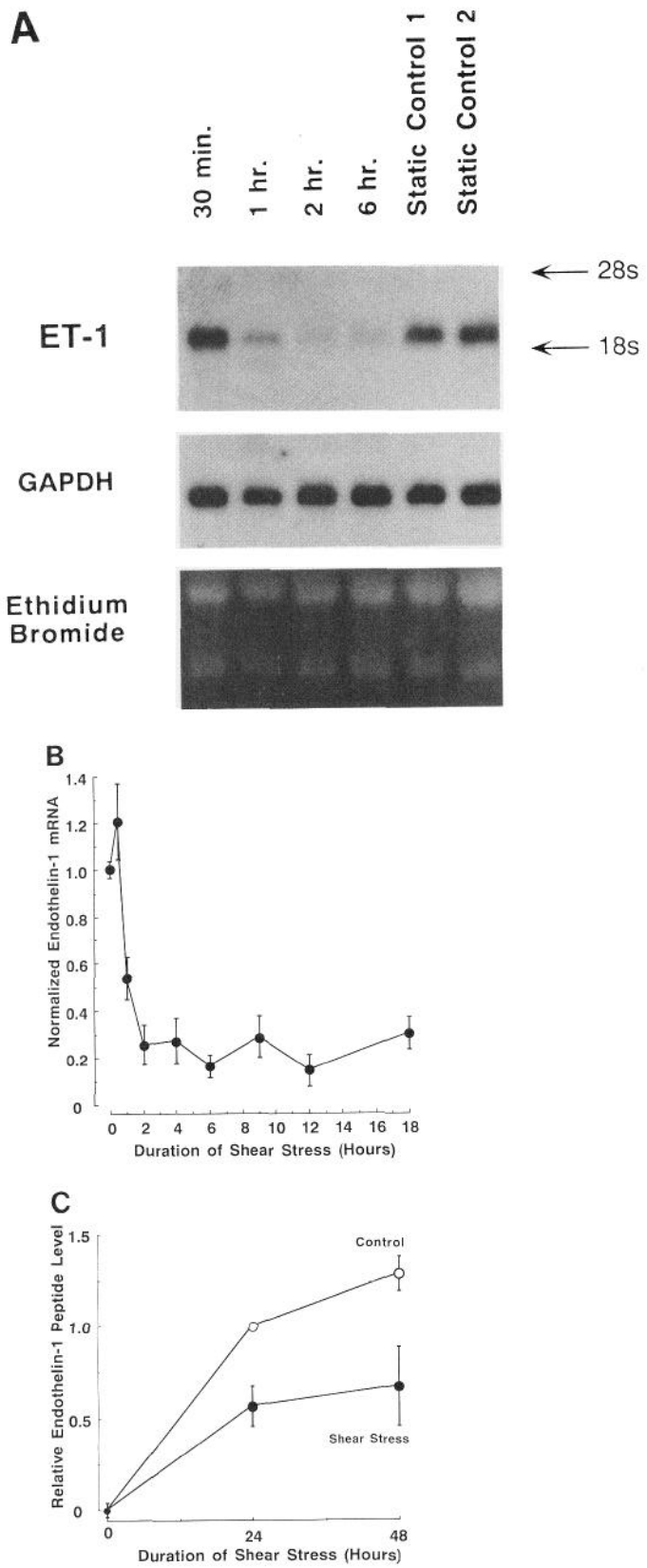


Fig. 3. Steady laminar fluid shear stress (15 dyn/cm^2) induces downregulation of endothelin-1 (ET-1) mRNA levels and peptide release in a time-dependent fashion. *A*: Northern analysis of mRNA obtained from BAE cells exposed to steady laminar fluid shear stress of magnitude 15 dyn/cm^2 for increasing times (30 min, 1 h, 2 h, and 6 h) hybridized with bovine cDNA for preproendothelin-1 (*top*), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; *middle*), and ethidium bromide staining of gel (*bottom*). Static control *lanes 1* and *2* indicate levels in control cells not exposed to fluid shear stress. *B*: time course of fluid shear stress-induced downregulation of ET-1 mRNA using steady laminar fluid shear stress of magnitude 15 dyn/cm^2. Abcissa represents density of ET-1 band normalized with respect to density of GAPDH band in linear range of X-ray film. Bars indicate SE (n = 6, except for 18 h where n = 4). *C*: normalized results of ET-1 peptide levels by radioimmunoassay. Data were normalized with respect to value of control cells at 24 h. Bars indicate SE (n = 4). No immunoreactivity was detected in fresh culture medium containing 10% calf serum at *time 0*.

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calf serum (data not shown).

ET-1 downregulation is dependent on fluid shear-stress magnitude. To determine the presence of any threshold effect of the shear-stress magnitude, we measured the level of ET-1 mRNA after 4 h of applying laminar shear stress of increasing magnitude (Fig. 4). The dose-response curve showed a slight and nonsignificant decrease at shear-stress magnitude of 3 dyn/cm^2 and a steep sigmoidal shape with an asymptote between 15 and 20 dyn/cm^2. This indicates that ET-1 is downregulated at physiological shear-stress levels and that cells exposed to shear-stress levels <15 dyn/cm^2 have a higher level of ET-1 mRNA. Experiments conducted at the higher shear stress of 45 dyn/cm^2 after 6 h of shear (using 5% dextran) showed similar four- to fivefold downregulation (data not shown).

Turbulent shear is indistinguishable from laminar shear in downregulating ET-1. We studied the effect of disturbed fluid shear stress on shear-induced ET-1 downregulation, particularly in light of the findings reported by Davies et al. (4) showing that turbulent but not laminar shear stress of same magnitude caused confluent endothelial cell monolayers in vitro to enter the cell cycle without any loss of contact in the monolayer. To determine whether turbulent shear had a differential effect on ET-1 mRNA downregulation compared with laminar shear, we exposed endothelium to turbulent shear stress by using a 5° cone. Figure 5*A* shows BAE cells exposed to fluid shear stress of magnitude 15 dyn/cm^2 for 18 h in the laminar and in the turbulent regimen. The shape of the cells exposed to turbulent flow is more similar to that of control cells than to that of cells exposed to laminar shear stress, but it does show a mild alignment in the general direction of the flow. We found, however, that shear stress in the turbulent regime caused a downregulation of ET-1 mRNA that was statistically indistinguishable from that resulting from exposure to laminar shear stress of the same magnitude (Fig. 5*B*).

Pulsatile shear stress is indistinguishable from laminar shear stress in downregulating ET-1. We have simulated pulsatile fluid shear stress, such as is found in peripheral arteries, with a resulting sinusoidally varying shear stress between 12 and 18 dyn/cm^2 at a frequency of 2.5 Hz (Fig. 1*B*). Our results show that pulsatile shear stress of physiological level induces a similar downregulation to steady shear stress (Fig. 6). This indicates that the mechanism of shear-induced ET-1 is not sensitive to small variations of physiological frequency in the presence of a mean shear amplitude that is sufficient to induce downregulation.

Low-frequency reversing shear stress and cyclical stretch fail to affect ET-1 mRNA. To verify that the observed downregulation is not due to axial strain of the endothelium, we grew cells on elastic silicone membranes and subjected them to cyclical strain of 20% at a rate of 20/min. Cyclical stretching of the endothelium did not induce a change in ET-1 mRNA (Fig. 6). We subjected endothelium to sinusoidally direction- and time-varying shear stress of mean magnitude of 15 dyn/cm^2 at a frequency of 0.25 Hz (Fig. 1*B*) and found no significant change. This indicates that the mean magnitude of shear is not the only important determinant of shear stress-induced downregulation. This result also further argues against the possibility that shear-induced ET-1 downregulation is simply the result of culture media disturbance.

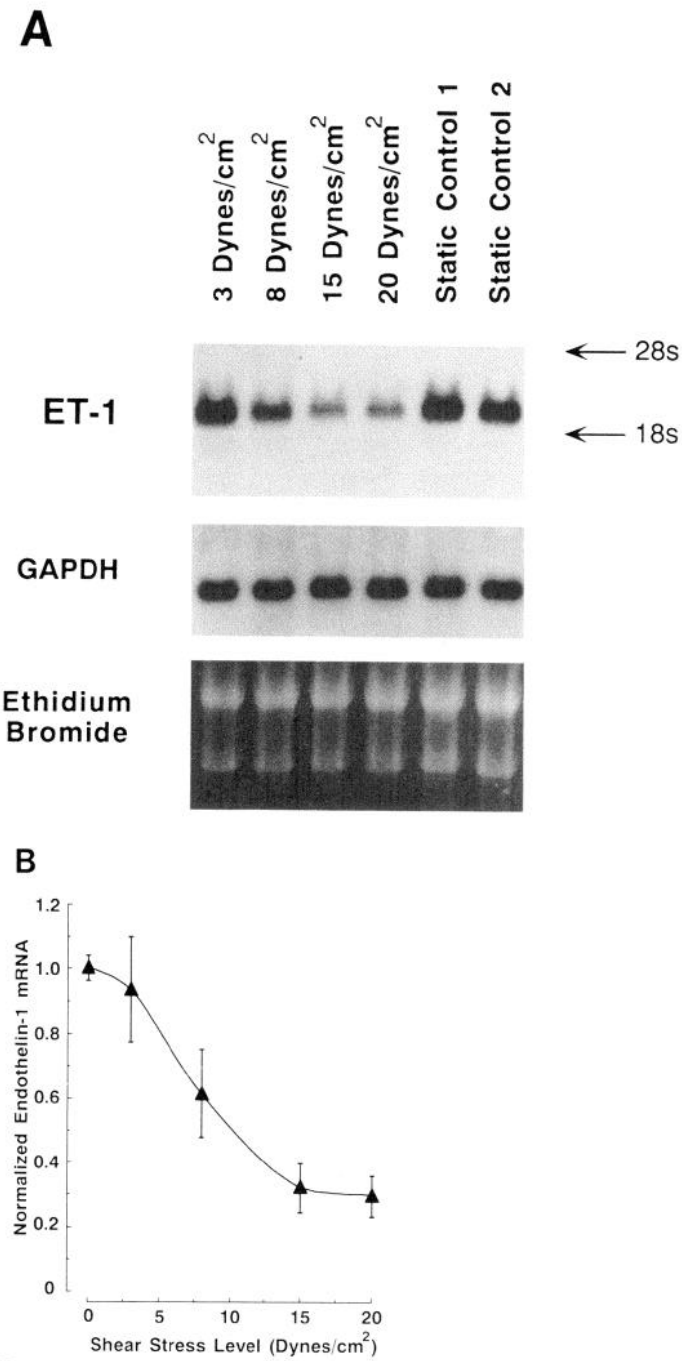


Fig. 4. ET-1 mRNA downregulation in response to fluid shear stress is sensitive to magnitude of applied fluid shear stress in a dose-dependent fashion. *A*: Northern analysis of mRNA obtained from BAE cells exposed to increasing magnitude of steady laminar fluid shear stress for 4 h and increasing magnitude (3, 8, 15, 20 dyn/cm^2), hybridized with bovine cDNA for preproendothelin-1 (*top*), GAPDH (*middle*), and ethidium bromide staining of gel (*bottom*). mRNA from control cells not exposed to fluid shear stress appears as static control *1* and *2*. *B*: dose response of fluid shear stress induced downregulation of ET-1 mRNA after 4 h of applying steady laminar fluid shear stress of magnitudes 3, 8, 15, and 20 dyn/cm^2. Abcissa represents density of ET-1 band normalized with respect to density of GAPDH band in linear range of X-ray film. Bars indicate SE (n = 4). Note saturation above 15 dyn/cm^2.

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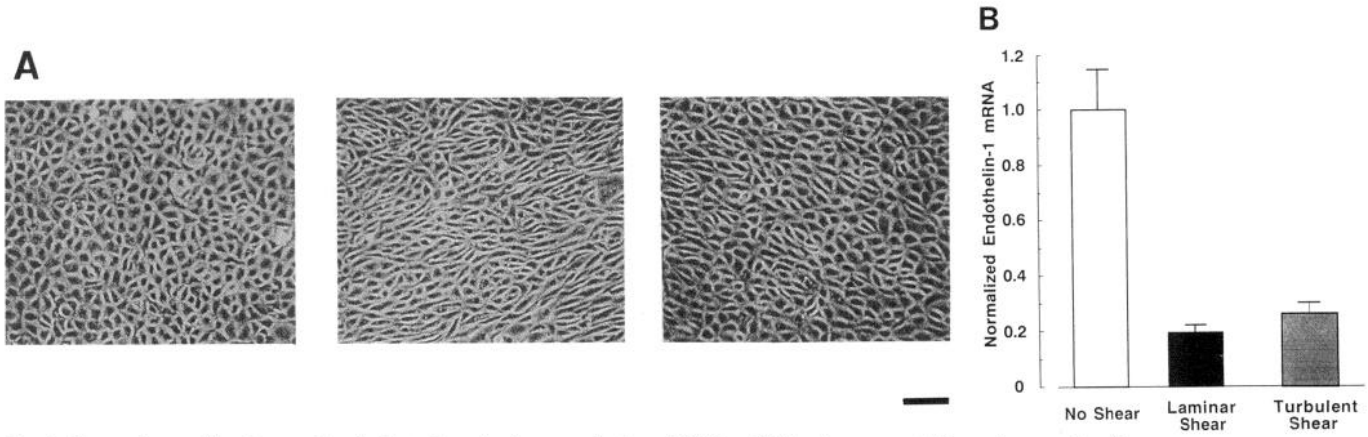


Fig. 5. Comparison of laminar and turbulent shear in downregulation of ET-1 mRNA. *A*: representative micrographs of bovine aortic endothelial cells under static conditions (*left*), 18 h of laminar shear stress at 15 dyn/cm^2 (*middle*), and 18 h of turbulent shear stress at 15 dyn/cm^2 (*right*). Direction of flow and fluid shear stress is from left to right. Bar, 100 μm. *B*: comparison of ET-1 mRNA levels after application of fluid shear stress of magnitude 15 dyn/cm^2 in laminar and turbulent regimens for 6 h. Bars indicate SE ($n = 6$).

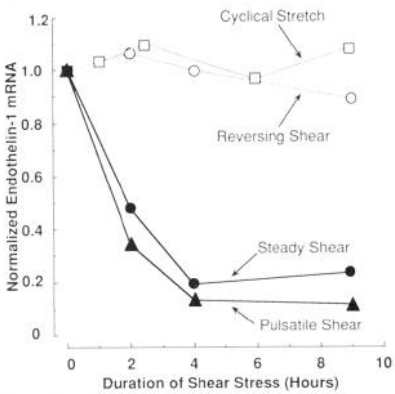


Fig. 6. Effect of cyclic stretch and importance of dynamic character of fluid shear stress in ET-1 downregulation. Time course of ET-1 mRNA downregulation in cells subjected to steady laminar shear stress of magnitude 15 dyn/cm^2 (●), cells subjected to cyclic stretch of 20% at a rate of 20/min (□), and cells subjected to pulsatile (▲) and reversing shear (○). All shear stress stimuli have same absolute mean magnitude of 15 dyn/cm^2. Note that pulsatile shear induces ET-1 downregulation similar to that of steady shear whereas reversing shear does not.

DISCUSSION

We have shown that fluid shear stress of physiological magnitude induces the downregulation at the mRNA and peptide level of ET-1 in a dose-dependent fashion that is also sensitive to the dynamic nature of the shear stimulus. The responsiveness of ET-1 mRNA levels to fluid shear stress did not discriminate between steady shear stress, the high-frequency time variations of turbulent shear stress, and the intermediate frequency (2.5 Hz) of pulsatile shear stress. ET-1 mRNA did, however, fail to decrease when low-frequency reversing shear stress or cyclic linear stretch was applied. These findings are important in characterizing the transduction mechanism of shear stress into ET-1 mRNA levels and in highlighting the potential differences in ET-1 expression in regions of the vasculature where endothelium is exposed to flow reversal and pulsatility (1, 10).

Interestingly, the dose-response relationship (Fig. 4*B*) showed saturation at shear-stress levels above 15 dyn/cm^2, a behavior similar to the saturation observed by Olesen et al. (19) in the shear-induced outward potassium current. By increasing the viscosity of the culture media through supplementation with dextran, we were able to subject the BAE cells to lower fluid velocity at the same level of fluid shear stress and demonstrate that downregulation of ET-1 mRNA is sensitive to shear stress and not to flow velocity. This is in contrast to the changes in free cytoplasmic intracellular [Ca^{2+}] reported by Mo et al. (15) in response to applied ATP, a phenomenon that was found to be dependent on flow magnitude alone as a result of a convective-diffusive effect.

Numerous review articles have cited that ET-1 mRNA is transiently upregulated by mechanical forces and shear stress based on a single report (29) despite the statement to the contrary made by the same group of authors in the original report (28). Recently, a preliminary report by McIntire et al. (14a) has confirmed our findings. Using a parallel-plate flow viscometer, they showed that application of steady laminar shear stress of 25 dyn/cm^2 for 24 h on human umbilical vein endothelial cells caused a decrease of ET-1 mRNA levels. The report of the similar finding presented here using a different type of shear stress apparatus and endothelial cells from a different species strengthens the hypothesis that ET-1 mRNA downregulation in response to fluid shear stress is an intrinsic feature of endothelial cells regardless of species or vascular origin. The reasons for the difference between our results and those of Yoshizumi et al. (29) are not clear at present; however, our data are consistent with that group's initial statement (28).

The central importance of the endothelium as a regulator of vascular structure and function has been demonstrated by two types of studies. The first type of experiments consists of artificially altering levels of blood flow without any significant changes in blood pressure in defined arteries either by inducing an arteriovenous shunt (12) or by clamping upstream of the site of study (13). This has shown that blood vessels remodel in response to an increase or decrease in shear stress by increasing or decreasing their internal diameter so as to maintain the shear-stress level around 15 dyn/cm^2. This flow-induced remodeling has been shown to be entirely dependent on the presence of intact endothelium (13). Although plasma

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levels or local concentrations of ET-1 have not been measured in these experiments, our finding may have implications on the possible interpretation of these findings because we have shown the existence of a relationship (see Fig. 4*B*) between the magnitude of shear stress to which the endothelial cell is exposed and the level of ET-1 mRNA. This relationship offers a potential link in the form of a stable negative feedback loop between shear stress and the tone and growth of the underlying vascular smooth muscle cells that may help interpret the in vivo findings described above. An example of how such a loop could be operating in vivo is in the response of arterial endothelium to a decrease in blood flow such as the one induced by Langille and O'Donnell (13). The reduction in blood flow would induce an immediate decrease in fluid shear stress on the endothelium with a resulting increase in ET-1 expression from its previously low level. This increase in ET-1 expression, together with a decrease in EDRF release resulting from the decrease in flow (8), would potentially induce a contraction of underlying smooth muscle cells and a long-term remodeling that would result in a decrease in the internal radius of the vessel, thereby raising the value of shear stress back to its previous level (see the Hagen-Poiseuille equation) with an ensuing decrease of ET-1 expression back to the level prior to the flow decrease. Although this hypothetical scheme would be in accordance with the in vivo observations of endothelium-dependent vessel remodeling in response to decreases or increases in blood flow in the absence of significant changes in blood pressure, one must be cautious in extrapolating from our data obtained in vitro to the in vivo situation where interactions between endothelium, smooth muscle cells, and other blood-borne elements may be playing an important role. The data presented here used endothelium of aortic origin, a conduit vessel, and may not be applicable to endothelium of resistance vessel origin. Additional experiments measuring local production of ET-1 or in situ hybridization studies in shunted arteries, in regions of low shear stress, or in isolated vascular preparations are required to elucidate the actual mechanism occurring in vivo.

In the second type of experiments, numerous reports have shown a strong and unique correlation between the location of initial and later atherosclerotic foci and shear stress of low magnitude and of oscillatory nature with flow reversal (1, 30), both in the carotid bifurcation and in the coronary arteries. Our findings that ET-1 expression is elevated under static conditions, low shear-stress magnitude, and in some cases of time-varying shear stress may have implications on this correlation with atherosclerotic plaques. Such atherosclerotic foci, because of the nature of the local shear forces, would, if our findings extend to endothelium in vivo, be expressing higher amounts of ET-1, a growth factor to smooth muscle cells, than would areas exposed to laminar shear stress of higher magnitude (>8 dyn/cm^2). Although Lerman et al. (14) have recently reported higher levels of circulating ET-1 in patients with atherosclerosis as well as ET-1-like immunoreactivity in atherosclerotic sites, these measurements were obtained in patients with already-established disease, making it difficult to directly implicate ET-1 as a participant in the disease process rather than simply a marker. Furthermore, one cannot neglect the effect of elevated blood pressure on ET-1 expression, an effect that we have not directly evaluated.

The present study offers a dynamic model system that allows the molecular and cellular study of the endothelium in vitro under conditions that closely resemble in vivo fluid shear stress. The sensitivity of the ET-1 gene to fluid shear stress presents a model of how mechanical forces are transduced into gene regulation and, ultimately, into cellular phenotype. The system presented here offers the possibility of dissecting the mechanisms of sensing and signaling at multiple levels from second messenger to cytoskeletal and gene expression by the endothelial cell in response to the ever-present fluid shear forces with which it has evolved over time.

We thank Thomas Kulik for access to the cyclical stretch apparatus, Thomas Quertermous and Kenneth Bloch for providing ET-1 cDNA plasmids, Richard Ahlquist for skillful technical assistance, and Seth Alper and Adam Greene for critical reading of the manuscript. The prototype of the shear stress apparatus was initially designed and constructed while A. Malek was in Dr. Victor J. Dzau's laboratory at the Brigham and Women's Hospital, Boston, MA.

This work was supported by Johnson & Johnson (through the Harvard-Massachusetts Institute of Technology HST Division) and the Whitaker Foundation.

A. Malek is supported by the National Institutes of Health Medical Scientist Training Program. S. Izumo is an Established Investigator of the American Heart Association.

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Received 23 December 1991; accepted in final form 12 March 1992.

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