Assessment and Treatment of Endothelial Dysfunction in Humans

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The endothelium plays a key role in vascular homeostasis through the release of a variety of autocrine and paracrine substances (1). In addition to vasodilation, a healthy endothelium is antiatherogenic because of effects that include inhibition of platelet aggregation and adhesion, smooth muscle cell proliferation and leukocyte adhesion. Dysfunction of endothelial cells is a systemic process and the initiating event in atherosclerosis, and is important in the ischemic manifestations of the disease process as well.

Endothelial cell dysfunction occurs in the presence of atherosclerosis or its risk factors, particularly hypercholesterolemia (2). Over the past five years, new methodology has allowed more widespread assessment of endothelium-dependent vasodilation in patients in a variety of research settings. This review will focus on the assessment of endothelial function in humans and the therapeutic options that are now available for treating abnormalities in vascular function.

ROLE OF THE ENDOTHELIUM IN HEALTH

Local vascular control depends on a balance between dilators and constrictors, with endothelium-dependent nitric oxide (NO) being the best characterized and probably the most important (1,3). Nitric oxide is stimulated by a variety of stimuli that serve as the basis for the assessment of endothelium-dependent vasodilation (Table 1). The major opposition to the vasodilator substances is endothelin, a 21-amino acid peptide and a potent vasoconstrictor. A local renin-angiotensin system exists in several tissues, including the vascular endothelium, heart and monocytes (4), and angiotensin II, another vasoconstrictor, is very important in local vascular control.

Vasomotion. In healthy people, the predominant effect of stimulation of the endothelium is vasodilation. It is well established in animals that basal release of NO exists in both conduit and resistance vessels (5). This is true in the human coronary circulation as well (6). Basal tone is probably also mediated by endothelin (7), angiotensin II (8) and prostacyclin (9). In the coronary circulation, endothelium-dependent stimulation causes vasodilation of epicardial vessels (10). Acetylcholine-induced vasodilation can be blocked by NO synthase (NOS) inhibition with N-monomethyl-L-arginine (L-NMMA) (6). Resistance vessel function is determined in vivo by measuring blood flow. Administration of endothelium-dependent agonists result in an increase in blood flow in the human peripheral and coronary circulations (11). Whereas this effect has also been shown to be NO dependent (6), other mediators probably are involved in resistance vessel dilation and the control of coronary blood flow, particularly to metabolic stimuli (12,13).

Leukocyte adhesion. Antiinflammatory properties of the healthy endothelium are important in the prevention of atherosclerosis and ischemic coronary syndromes (14). Multiple families of "adhesion molecules" provide trafficking signals for the interaction between leukocytes and the endothelium. A detailed description of the events of monocyte adhesion is reviewed elsewhere (15). Leukocyte adhesion is not only important during the early stages of atherogenesis, but may contribute significantly to plaque instability and rupture (16).

Atherosclerosis and its risk factors result in upregulation of cell adhesion molecules by the nuclear factor-$\kappa\beta$-regulating gene expression through redox-sensitive mechanisms (17). This pathway can be mediated by a number of cytokines including interleukins, tumor necrosis factor-alpha, monocyte chemotactic protein and interferon. Nitric oxide has been shown to inhibit leukocyte adhesion (18). Soluble forms of adhesion molecules can be measured in blood and serve as markers of endothelial activation (19). It was recently shown in the Physicians Health Study that sICAM-1 (soluble intercellular adhesion molecule-1) was predictive of future myocardial infarction (20).

Platelet function. Nitric oxide is antithrombotic via potent antiaggregating and antiadhesive properties (21). Nitric oxide inhibits expression of P-selectin on platelets and suppresses the calcium-dependent conformational change in the glycoprotein (GP) IIb-IIIa receptor required for fibrinogen binding (22). Platelet activation occurs in conditions associated with impairment of endothelium-dependent va-

> **Abbreviations and Acronyms**
> | | | |
> |---|---|---|
> | ACE | = | angiotensin-converting enzyme |
> | EDHF | = | endothelium-derived hyperpolarizing factor |
> | FMD | = | flow-mediated vasodilation |
> | GP | = | glycoprotein |
> | LDL | = | low-density lipoprotein |
> | L-NMMA | = | N^G-monomethyl L-arginine |
> | NO | = | nitric oxide |
> | NOS | = | nitric oxide synthase |

sodilation (23). Platelet function can be assessed by standard aggregation techniques. More recently, flow cytometry has been used to quantify the expression of P-selectin and GP IIb/IIIa receptors, markers of platelet activation (24).

Platelet activation is particularly important in unstable coronary syndromes and coronary intervention where blockade of the GP IIb/IIIa receptor is becoming a mainstay of therapy (25). Because P-selectin is present on both platelets and the endothelium, it plays an important role in the interaction with platelets, leukocytes and the endothelium (26). It was recently demonstrated that platelets from patients with unstable coronary syndromes release less NO (27). The release products of activated platelets mediate vasoconstriction at sites of endothelial dysfunction (28). Other functions of the healthy endothelium are shown in Table 2.

ASSESSMENT OF ENDOTHELIAL FUNCTION IN HUMANS

Intracoronary studies. Ludmer et al. (2) were among the first to demonstrate that acetylcholine (up to 10^{-6} M) could be safely infused selectively into the coronary circulation to assess conduit vessel vasomotion. This has served as the gold standard for endothelial function testing for the last decade. Acetylcholine-induced vasoconstriction is one of the earliest manifestations of endothelial dysfunction (29), occurring before abnormalities with other endothelium-dependent stimuli (cold presor testing, flow-mediated vasodilation [FMD]). L-NMMA can be selectively infused to assess basal NO activity in the coronary circulation. Resistance vessel function in the coronary circulation can now be readily assessed by measuring coronary blood flow with

Table 1. Vasodilator Stimuli to Assess Endothelial Function

Acetylcholine	Blood flow (shear stress)
Bradykinin	Catecholamines
Serotonin	A 23187
ADP, ATP	Calcium gene-related peptide
Histamine	Platelet-activating factor
Thrombin	Substance P

ADP = adenosine diphosphate; ATP = adenosine triphosphate; A 23187 = calcium ionophore.

Table 2. Function of the Healthy Endothelium

Role	Substances
Vasoregulation	NO, EDHF, PGI_2, ET-1, Ang II, TXA_2
Coagulation	PGI_2, TXA_2, vWF, fibrinogen, thrombomodulin, TF
Fibrinolysis	t-PA, PAI-1
Inflammation	P and E selectin, VCAMs and ICAMs, NF-$\kappa\beta$
Erythrocyte adherence	Integrins
Permeability	RAGE
Vasculogenesis/angiogenesis	VEGF, PDGF, TGF-β

Ang II = angiotensin II; EDHF = endothelium-derived hyperpolarizing factor; ET-1 = endothelin-1; ICAM = intercellular adhesion molecule; NF-$\kappa\beta$ = nuclear factor kappa beta; NO = nitric oxide; PAI-1 = plasminogen activator inhibitor; PDGF = platelet-derived growth factor; PGI_2 = prostacyclin; RAGE = receptor for advanced glycosylated end-products; TF = tissue factor; TGF-β = transforming growth factor-beta; t-PA = tissue plasminogen activator; TXA_2 = thromboxane; VCAM = vascular cellular adhesion molecule; VEGF = vascular endothelial growth factor; vWF = von Willebrand's factor.

intracoronary Doppler wires (30). Coronary blood flow increases in response to infused agonists, such as acetylcholine, and the magnitude of this increase can be used as a quantitative measure of endothelial function. The change in coronary blood flow is due to both the direct effect of the infused agonist and the resulting FMD. When compared with conduit vessel responses, the measurement of coronary blood flow is somewhat more difficult with greater variability and expense. Coronary blood flow has also been assessed by coronary sinus thermodilution techniques in laboratories equipped to do this.

Positron emission tomography. Quantitative assessment of myocardial blood flow and metabolic activity can be made by positron emission tomography scanning (31,32). Both basal flow and hyperemic flow (usually to intravenous dipyridamole) can be obtained to calculate coronary flow reserve (33). Because the increase in myocardial flow is related to adenosine-induced increases and flow-mediated vasodilation, it is in part a measure of endothelial function. This technique is noninvasive and has the advantage of the potential for multiple tests per patient; however, it is very expensive and limited to a small number of laboratories (34,35).

Impedance plethysmography. Hokanson et al. (36) described electrically calibrated plethysmography for direct measurement of limb blood flow. The apparatus is relatively inexpensive and versatile because direct intraarterial infusions of methacholine or acetylcholine assess endothelial function (37,38). Because forearm blood flow (ml/min/100 ml) is measured, venous occlusion plethysmography reflects resistance vessel function in the forearm. Although ideally suited for one-time measurements, there is some concern about day-to-day variability, and as such, it has not been extensively used for long-term intervention studies.
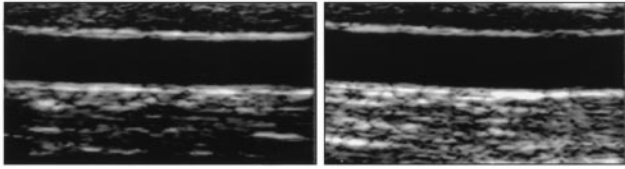
Figure 1. High-resolution ultrasound image of brachial artery at baseline and 1 min after upper-arm occlusion cuff release and subsequent flow-mediated vasodilation.

Brachial ultrasound. Celermajer et al. (39) were the first to describe a noninvasive assessment of flow-mediated vasodilation in the brachial or femoral artery. Upper-arm occlusion for 5 min results in reactive hyperemia after the cuff is released (blood flow increases five- to sevenfold), and this increase in shear stress results in FMD (Fig. 1). The variability is acceptable (about 2%), and the measurements are reproducible in a good laboratory (40). Brachial artery FMD has been shown to correlate with measures of coronary endothelial function (41). The main advantages of this approach are the noninvasive nature and the ability to repeat multiple tests in the same patient or the study of large numbers of patients. Some laboratories are now using direct tracking ultrasound technology that may improve the precision of the technique (Drexler H, personal communication, 1998).

Venous studies. Dorsal hand vein compliance can be assessed with the linear variable differential transducer technique (42). This technique uses local infusions of agonists into the hand veins with an assessment of conduit vessel compliance. In terms of invasiveness and utility, it would be similar to impedance plethysmography, except that venous conduit vessels are studied instead of forearm resistance arterial vessels.

ENDOTHELIAL DYSFUNCTION

The term endothelial dysfunction is most often used to denote impairment of endothelium-dependent vasodilation, but probably encompasses those conditions leading to endothelial activation with abnormalities in endothelial interactions with leukocytes, platelets and regulatory substances (43–46) (Table 3).

Atherosclerosis. It is the general consensus of vascular biologists that endothelial injury with resulting dysfunction is the initiating event in atherosclerosis (47) and plays an important role in the ischemic manifestations of coronary disease. Ludmer et al. (2) were among the first to recognize this in human coronary arteries. In health, acetylcholine causes vasodilation as a result of endothelium-dependent release of NO; in disease, the effect of NO is decreased, and unopposed muscarinic smooth muscle cell activation leads to vasoconstriction. Atherosclerosis also impairs acetylcholine-induced increases in coronary blood flow, despite the fact that resistance vessels are rarely affected by the physical

Table 3. Conditions Associated With Impaired Endothelium-dependent Vasodilation

Atherosclerosis	Type I and type II diabetes mellitus
Hypercholesterolemia	Hyperglycemia
Low HDL cholesterol	Acute postprandial hypertriglyceridemia
High Lp(a)	Active and passive cigarette smoking
Small LDL particles	Dilated cardiomyopathy
Susceptibility of LDL to oxidation	Chagas disease
Hypertension	Heart failure—any cause
Hyperhomocysteinemia	Family history of coronary disease
Aging	Postmenopausal status
Vasculitic conditions	Post-Kawasaki's disease
Transplantation atherosclerosis	Pregnancy-induced hypertension/ preeclampsia
Syndrome X and variant angina	Pulmonary hypertension
Insulin resistance	Methionine loading

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

presence of atherosclerosis (11). Part of the dysfunction in atherosclerosis is related to a decrease in NOS activity (48).

In balloon-injury models of atherosclerosis, there is emerging evidence that angiotensin II blockade or ACE inhibition decreases intimal proliferation; however, this has not translated into a decrease in restenosis rates in studies of angioplasty in humans. Depending on the animal model, angiotensin II appears to be an important mediator of intimal proliferation and vasomotion (49). Increased endothelin activity also appears to play a role in endothelial dysfunction in atherosclerosis (50).

Cardiovascular risk factors. Endothelial dysfunction, a systemic disturbance of function, precedes the physical presence of atherosclerosis (51,52). Oxidative stress appears to play a pivotal role in the alteration of endothelial function that characterizes all risk factors (Fig. 1). Steinberg and others have advanced the concept that oxidative modification of low-density lipoprotein (LDL) is central to the development of atherosclerosis, and recent work has suggested that oxidized LDL plays an important role in abnormal endothelial vasorelaxation (53). The detrimental effects of oxidized LDL on endothelial function are likely modulated through lysophosphatidylcholine (54), protein kinase C (55) and G proteins (56). In addition, oxygen-free radicals impair endothelial function through direct inactivation of NO (57). Oxidized LDL also increases the production of endothelin in cultured cells and intact blood vessels (58).

Tetrahydrobiopterin is a cofactor for NOS, the rate-limiting enzyme responsible for the conversion of L-arginine to NO. activation of NOS at suboptimal concentrations of tetrahydrobiopterin leads to the production of hydrogen peroxide instead of NO (59). Relative deficiency of tetra-

Table 4. Treatment Associated With Improvement of
Endothelial Dysfunction in Humans

Acute	Chronic
LDL lowering with pheresis	LDL lowering with statins, resins
ACE inhibition	ACE inhibition
Antioxidants (Vitamin C and E + C)	Antioxidants (probucol with lovastatin)
Estrogen	Estrogen
L-arginine, D-arginine	Estrogen + progesterone
Tetrahydrobiopterin, methyltetrahydrofolate	L-arginine
Deferoxamine	Exercise
Glutathione	
Calcium channel blockers	

ACE = angiotensin-converting enzyme; LDL = low-density lipoprotein.

hydrobiopterin has been implied as contributing to endothelial dysfunction in some conditions (60).

Hypercholesterolemia induces a number of changes on vascular homeostasis, including a decrease in NO bioactivity, an increase in superoxide production, an increase in endothelin immunoreactivity (61), an increase in adhesion molecules (19) and attenuation of endothelium-dependent vasodilation (37). The combination of diabetes and hypertension appears to have an additive effect on monocyte adhesion (62). It seems that cholesterol-induced endothelial dysfunction is related to the degree of LDL oxidation and not LDL concentration itself (63,64). Acute elevations of free fatty acids and triglycerides can attenuate vasodilator responses over the course of several hours (65,66). Acute hyperglycemia (6 h) can also impair vasomotor responses (67), adding evidence to the belief that it is the milieu of the artery that determines vascular function, and this can be modulated over minutes to hours.

Attenuation of endothelium-dependent responses is an important manifestation in many other cardiac and noncardiac conditions. Detailed discussion is beyond the scope of the current review (38,68–72) (see Table 3).

TREATMENT OF ENDOTHELIAL DYSFUNCTION

In the 1990s, studies have demonstrated that endothelial dysfunction in both animal models and humans can be attenuated by a variety of interventions (Table 4).

Cholesterol lowering. Ohara et al. (73) demonstrated that cholesterol feeding increased the production of superoxide from the endothelium of rabbit aorta, and that dietary lowering of cholesterol not only improved endothelium-dependent vasodilation, but also normalized endothelial superoxide production (74). The finding that cholesterol lowering was associated with an improvement in endothelium-dependent vascular relaxation is in keeping with prior animal studies (75).

We studied the effect of LDL lowering and the combi-

nation of LDL lowering and antioxidant therapy on coronary epicardial endothelial function (76). Whereas LDL lowering resulted in an improvement in endothelial function, the combination of lovastatin and probucol resulted in near normalization in endothelial function. The acetylcholine response at follow-up, or the improvement in endothelial response over the study period, was closely related to the resistance of LDL oxidation (63). Whereas we treated patients for one year, Tamai et al. (77) recently demonstrated that forearm blood flow can be modulated within hours after LDL pheresis, demonstrating the dynamic nature of this process. Studies with other modalities to lower cholesterol have shown improved function as well, suggesting that it is the cholesterol-lowering effect and not the pleiotrophic effect of the statins that are important (31,78).

Antioxidants. Animal studies by Keaney et al. (79,80) have demonstrated beneficial effects of probucol and antioxidant vitamin therapy beyond protection of LDL. Recently, studies have demonstrated that vitamin C acutely improves endothelium-dependent responses in the forearm circulation in patients with a variety of risk factors (81–84). Long-term studies on vascular function in humans with vitamin C have not been reported.

Although alpha-tocopherol has been shown to protect LDL against oxidation in humans (85), longer-term studies using antioxidant vitamins have not shown any effect on endothelium-dependent vasodilation (86,87). Recently, two weeks of vitamin E treatment was shown to decrease P-selectin in patients with hypercholesterolemia, suggesting attenuation of endothelial activation (19).

Angiotensin-converting enzyme (ACE) inhibition. Angiotensin II has been shown to increase superoxide production via membrane-bound NADH/NADPH (88). Although it is unclear whether angiotensin II blockers result in improved endothelial function, these data are available for ACE inhibitors (8,89). The ACE inhibitors could potentially improve endothelium-dependent vasodilator responses through decreased levels of angiotensin II, increased levels of bradykinin and NO.

In humans, acute administration of ACE inhibitors augmented endothelium-dependent vasodilation in both the coronary and peripheral circulation (90,91). Mancini et al. (92) recently reported that the tissue-specific ACE inhibitor quinapril attenuated coronary endothelial dysfunction in patients with coronary artery disease.

To assess the potential differences in tissue specificity among ACE inhibitors (93) and the importance of the bradykinin effect, we recently compared quinapril, enalapril, losartan and amlodipine in a cross-over design in 80 patients with coronary artery disease. Over an 8-week treatment period, improvement in brachial artery FMD was only seen in the quinapril group, suggesting potential differences among vasoactive medications (94). Two recent studies using enalapril or lisinopril reported negative results as well
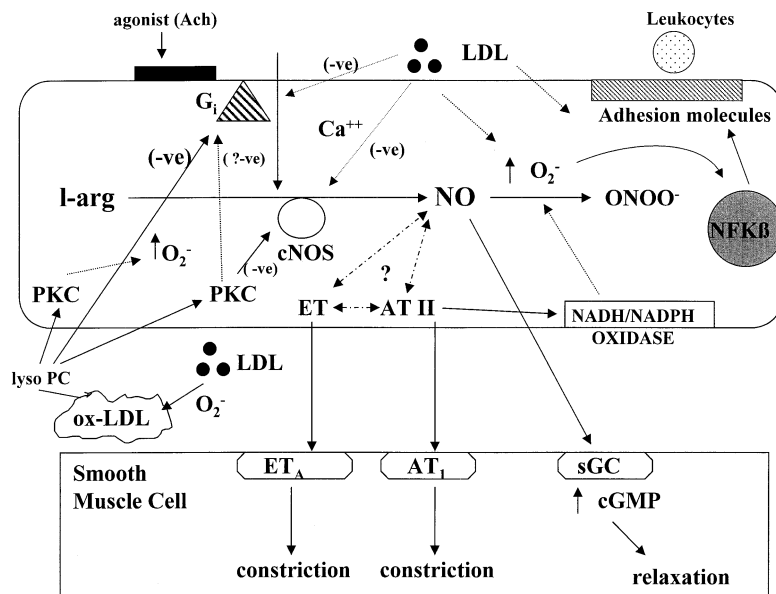
Figure 2. Oxidative stress leads to attenuation of endothelium-dependent vasodilation by: 1) decreased production of NO through oxidized LDL-mediated mechanisms, and by 2) increased destruction of NO by superoxide. See text for details. G_i = pertussis sensitive G protein; O_2^- = superoxide anion; $ONOO^-$ = peroxynitrite anion; PKC = protein kinase C; cNOS = constitutive form of nitric oxide synthase; ET = endothelin; AT II = angiotensin II; ox-LDL = oxidized LDL; lyso PC = lysophosphatidylcholine; sGC = soluble guanylate cyclase; cGMP = cyclic guanosine monophosphate; NFKB = nuclear factor kappa beta.

(95,96). The effect of lowering blood pressure by other means on endothelial function is less clear, but it appears that ACE inhibitors have unique vascular protective properties.

Hormone replacement therapy. Hormone replacement with estrogen has been shown to improve endothelium-dependent vasorelaxation acutely in a number of animal models, including primate coronary arteries (97). It is likely that benefit is both NO dependent and independent (98).

Sack et al. (99) demonstrated that estrogen attenuates the susceptibility of LDL to oxidation in women. Studies in postmenopausal women demonstrated that acetylcholine-induced coronary vasoconstriction (100,101), but not metabolic vasodilation (102), can be attenuated in as little as 10 min by either intracoronary or intravenous administration of estrogen. Guetta et al. have demonstrated that the acute beneficial effects of estrogen on blood flow are NO related because this can be attenuated with L-NMMA (103). Improved endothelial vascular responses have been demonstrated in the peripheral circulation after nine weeks of estradiol therapy (104), and these effects were not attenuated by the coadministration of progesterone (105). Medroxyprogesterone, which is commonly used in combination with estrogen, probably has a detrimental effect on endothelial responses (106). Long-term studies evaluating the effect of hormone replacement on coronary endothelium-dependent vasodilation are underway.

Other interventions. Augmentation of NO production by L-arginine supplementation has been shown to improve vascular relaxation acutely in certain conditions (107). A recent study demonstrated improved brachial artery FMD in hypercholesterolemic subjects after four weeks of oral L-arginine supplementation (108). It is interesting to note that Quyyumi (109) more recently demonstrated augmentation of Ach-induced changes in forearm blood flow acutely with both L- and D-arginine, suggesting other mechanisms of action of arginine.

A variety of other interventions that have been shown to modulate vasomotor responses in humans are shown in Table 4. Most of these were administered acutely, and further studies are required to assess their long-term use.

PERSPECTIVE

The clinical manifestations of coronary disease depend on a multitude of interrelated pathophysiologic processes, of which endothelial dysfunction is only one. A wealth of indirect evidence argues that the endothelium plays a vital role in atherosclerosis and its manifestations. Endothelial dysfunction is generally believed to be the inciting event in atherosclerosis (47), and is probably important in ischemic manifestations as well. We demonstrated that endothelial dysfunction was associated with the development of atherosclerosis as assessed by intravascular ultrasound in patients' postcardiac transplantation (110). Those participants with normal vasodilator responses to acetylcholine developed atherosclerosis at a rate one-third that of those with endothelial dysfunction. Studies of cholesterol lowering, antioxidant therapy and ACE inhibition also suggest that atten-

uation of endothelial dysfunction provides the link between basic scientific observations and the decrease in cardiac events observed in the megatrials. However, it is not clear at this stage whether this is causal and if improvement in endothelium-dependent vasodilation is clinically relevant.

However, we are currently at a stage where the importance of measuring endothelial function in individual patients is unknown. The noninvasive technique is well suited for group studies in laboratories that are expert in its application and measurement (Fig. 2). Even in these laboratories, the day-to-day variability is at least 20% to 25% (just as it is with other measures of endothelial function). In laboratories that have not done a lot of testing and do not use careful off-line measurements, the variability will be at least twice that. Thus, before we openly embrace endothelial function testing, significantly more research has to be done. Standard methodology needs to be established. Large population studies over the next decade are required to determine if a single measure of vasoreactivity in an individual patient predicts the development of atherosclerosis or its complications, and such studies are now in the planning stages. In addition, the relationship between vasomotor responses and other measures of endothelial activation (platelet and leukocyte markers) needs to be established. Finally, intervention studies would need to be done to determine if intervening is prudent and effective in those patients at increased risk because of vascular dysfunction. Whether endothelial dysfunction in a primary prevention population can be used as a marker for participants at higher risk of developing atherosclerosis is yet to be proved, and this remains a very important goal of the clinical vascular biologist over the next two decades.

CONCLUSION

Endothelial function can now be readily measured in humans and is a very useful research tool to assess the effect of risk factors and their treatment on vascular function. A growing list of therapeutic modalities have been shown to modulate endothelial dysfunction, which has important implications for the treatment of participants at risk of developing atherosclerotic complications. For measurement of endothelial function to become a clinically useful tool, much work needs to be done. However, it is probable that endothelial function testing will assume a prominent role in the evaluation and treatment of patients at risk of developing coronary atherosclerosis and its sequelae.

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