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Heart Failure, Redox Alterations, and Endothelial Dysfunction

Antonio López Farré, Santos Casado

Abstract—Heart failure is characterized by neurohumoral alterations, such as activation of the sympathetic nervous system, stimulation of the renin-angiotensin system, increased activity of the endothelin system, increased production of norepinephrine, and increased circulating levels of cytokines. Oxidative stress is associated with the formation of reactive oxygen species (ROS). The myocardium has enzymes that stimulate ROS generation and enzymes with antioxidant effects. Several studies have suggested that ROS are increased in the failing heart. ROS may contribute to the pathophysiology of heart failure by initiating myocyte apoptosis and exerting direct negatively inotropic effects through the reduction of cytosolic intracellular free calcium. However, mechanisms such as endothelial dysfunction and inflammation have also been involved in the progression of heart failure. Antioxidants (eg, vitamin C) seem to improve endothelial functionality and reduce the inflammatory response in patients with heart failure. Therefore, in this review, we analyzed the involvement of ROS in the cellular and molecular mechanisms associated with endothelial dysfunction in heart failure. (*Hypertension*. 2001;38:1400-1405.)

Key Words: endothelium ■ endothelium-derived factor ■ free radicals ■ heart failure ■ inflammation

Heart failure may be viewed as a progressive disorder that is initiated after an index event either damages the heart muscle, with a resultant loss of cardiac myocytes, or disrupts the ability of the myocardium to generate force, thereby preventing the heart from contracting normally. The impaired emptying of the left ventricle, which characterizes heart failure, may result from a variety of cardiac diseases, including myocardial ischemia or infarction (which alters regional function), cardiomyopathies (which alter global function), and pressure or overload states (which lead to hypertrophy and dilatation of the chamber). Efforts to enhance the contractile force of the left ventricle have involved strategies for the management of heart failure for the next generation.

Heart failure is also characterized by a number of neurohormonal abnormalities. These include the sympathetic nervous system, as indicated by an elevated plasma norepinephrine level, stimulation of the renin-angiotensin system (which increased plasma levels of aldosterone), increased activity of the endothelin system, increased production of the arginine vasopressin levels, and increased circulating levels of cytokines. The clinical observation that heart failure can progress independently of the hemodynamic status of the patient has focused interest on the potential spectrum of mechanisms responsible for disease progression in the failing heart.

Oxidants and Antioxidants in the Myocardium

Oxidative stress is usually associated with increased formation of reactive oxygen species (ROS). Three major ROS, superoxide

anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot), are responsible for oxidative stress in heart failure.

The enzymatic sources of superoxide production within the vascular wall include NADPH oxidase, xanthine oxidase, and the endothelial NO synthase (eNOS) enzyme. Cells also contain different mechanisms against oxidative stress.^{1,2} The main intracellular enzymatic mechanisms are superoxide dismutase (SOD), catalase, and glutathione peroxidase.^{1,2} SOD catalyzes the dismutation of superoxide radical into H_2O_2 and oxygen. In the heart, this enzyme is present in 2 isoforms: Mn-SOD, which is expressed in the mitochondrial matrix, and Cu/Zn-SOD, the cytosolic form. H_2O_2 generated from this reaction is hydrolyzed by catalase (present in tissue peroxisomes) and by glutathione peroxidase (mainly present in the cytoplasm). A decrease in the antioxidant defense mechanisms in myocytes can be seen to promote oxidative stress. In this sense, Dhalla and Singal³ have shown that production of superoxide in cardiac tissue is increased as a consequence of the reduced antioxidant reserve in heart failure.

Iron-binding proteins, iron-transport protein, and albumin present in plasma are examples of extracellular antioxidants, because they lower the free iron concentrations that, if present in free form, can promote lipid peroxidation.⁴ Lipid-soluble α -tocopherol (vitamin E) and water-soluble ascorbic acid (vitamin C) protect against lipid peroxidation and act as cytosolic or extracellular antioxidants, respectively.

Several data have suggested increased ROS in patients with heart failure. The plasma and pericardial fluid of patients

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with heart failure contain elevated levels of thiobarbituric acid-reactive substances, which are markers of ROS activity.^{5,6} Ellis et al⁷ have also recently shown, by electron paramagnetic resonance spectroscopy, that lipid-derived ROS were significantly higher in patients with chronic heart failure than in control subjects. ROS may contribute in an important manner to the pathophysiology of heart failure by initiating myocyte apoptosis through nuclear factor (NF)- κ B and exerting direct negatively inotropic effects through depressed calcium uptake and reduced calcium-stimulated magnesium-dependent adenosine triphosphate activity of the cardiac sarcoplasmic reticulum.^{8,9} Oxidative stress may also contribute to endothelial dysfunction in heart failure, a pathology with growing interest in the setting of heart failure. The purpose of this review is to analyze the involvement of ROS in cellular and molecular mechanisms associated with endothelial dysfunction in heart failure.

Endothelial Dysfunction in Heart Failure

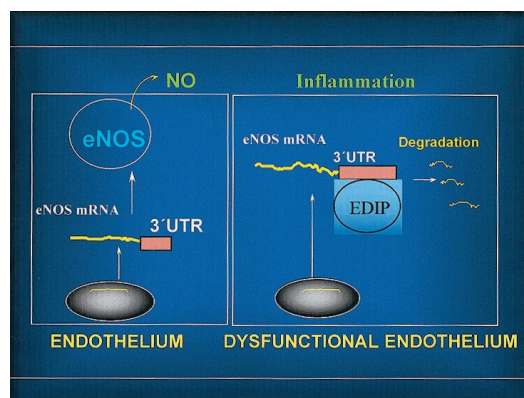
Investigators have increasingly recognized the importance of the endothelium as a central regulator of vascular homeostasis. Heart failure has been associated with a reduced cardiac output and excessive peripheral vasoconstriction. It has been shown that endothelium-dependent vasodilation is abnormal in both experimental animals and humans with compromised cardiac function.^{10,11}

It is also now appreciated that NO is the main molecule derived from endothelium that (in addition to the regulation of vascular tone) inhibits platelet activity, vascular smooth muscle cell growth, and adhesion of inflammatory cells to the endothelial surface.¹²⁻¹⁴ The combination of loss of endothelium and NO-dependent vasorelaxation, which is termed endothelial dysfunction, plays a critical role in the pathogenesis of a number of heart disease risk factors, including atherosclerosis and hypertension, and in the genesis of the acute coronary syndromes.¹⁵ Flow-mediated dilation has also been demonstrated to be impaired in patients with chronic heart failure and does not differ between patients with ischemic etiologies and those with nonischemic etiologies of chronic heart failure.^{7,16} Endothelial dysfunction of the peripheral vasculature contributes to the elevated peripheral vascular resistance in patients with heart failure. Endothelial dysfunction in these patients may also limit cardiac and skeletal muscle blood flow (particularly on exercise), impairing exercise capacity and pulmonary blood flow regulation (resulting in regulation-perfusion mismatching), and lead to impaired diastolic cardiac performance.

The most proposed mechanism for endothelial dysfunction related to ROS activity in heart failure is the enhanced biodegradation of NO by ROS. Elevated levels of ROS deplete bioavailable NO and exacerbate local oxidant stress by reacting directly with NO to form peroxynitrite, which, in turn, imparts further oxidative injury to the endothelium.¹²

Molecular Mechanisms Implicated in Endothelial Dysfunction: Regulation of eNOS mRNA Stability

In addition to reducing the bioavailability of NO, ROS may also be involved in the regulation of the expression of eNOS,



Under inflammatory conditions in the cytosol of endothelium, there appears a 60-kDa protein that binds to 3'-UTR of eNOS mRNA. The binding of the 60-kDa cytosolic protein, termed EDIP, was associated with eNOS mRNA destabilization and, therefore, with downregulation of eNOS protein.

the enzyme that generates NO in the endothelium. The so-called endothelium-dependent vasorelaxing response is attributed to the NO released by eNOS activation expressed in the endothelium. Therefore, to maintain an adequate eNOS activity and, therefore, the ability of endothelium to produce NO, it is essential to preserve the expression of the eNOS enzyme. Endothelial hyporesponsiveness in the coronary circulation and attenuated expression of the eNOS protein have been described in the heart-failure model of ventricular pacing in the dog.

Although, initially, eNOS protein was defined as constitutive, we have begun to discover that there are complex mechanisms that regulate the level of eNOS expression. In this regard, we^{17,18} and other authors¹⁹ have recently demonstrated that cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β reduce eNOS expression. The mechanism by which cytokines reduce eNOS expression appears to be linked to a reduction in the half-life of the eNOS RNA messenger. In this regard, we recently performed several experiments to analyze the mechanism by which cytokines reduce eNOS mRNA stability. The regulation of mRNA stability has emerged as an important mechanism in the control of cellular mRNA levels. In some mRNAs, there are sequences involved in the mRNA stability mainly located in the 3'-untranslated region of the messenger (3'-UTR). In this region, specific sequences can be recognized by specific proteins that ultimately regulate the stability of the messenger and, therefore, its half-life. The eNOS mRNA possesses a 3'-UTR extreme, and we have recently described the existence of endothelial cytosolic proteins that interact with the 3'-UTR of eNOS mRNA.^{17,18}

Incubation of endothelial cells with cytokines increased the binding activity of endothelial cytosolic proteins, particularly a 60-kDa cytosolic protein, to 38 nt within the 3'-UTR of eNOS mRNA, which was associated with eNOS mRNA destabilization^{18,20} (Figure). Therefore, the prevention of the binding activity of the 60-kDa cytosolic protein to the 3'-UTR of eNOS mRNA could protect the endothelial functionality in inflammatory-related cardiovascular diseases, including heart failure. This 60-kDa endothelial cytosolic

protein has been referred to as an endothelial dysfunctional inducer protein (EDIP).

ROS and eNOS Expression

Despite ROS-reduced NO bioactivity, it has recently been described that exposure of cultured bovine aortic endothelial cells to H₂O₂ paradoxically causes an increase in eNOS mRNA stability.²¹ Accordingly, we have observed that H₂O₂ reduces the binding activity of EDIP to the 3'-UTR of eNOS mRNA, which was associated with the downregulation of eNOS expression (authors' unpublished data, 2001). Taken together, we may speculate that ROS reduce the bioavailability of NO, although increased eNOS protein expression could be a compensatory response.

It is not surprising that ROS could regulate the expression of eNOS protein, inasmuch as the involvement of ROS in the regulation of the expression of several other proteins has been reported. For example, different adhesion proteins, such as monocyte chemotactic protein and intercellular adhesion molecule-1 (ICAM-1), expressed in cultured human umbilical vein endothelial cells by shear stress and cyclic strain, have been reported to be inhibited by the antioxidant *N*-acetylcysteine and catalase.^{22,23} The phenolic antioxidant probucol and vitamin E have been also shown to inhibit the oxidized LDL-mediated induction of ICAM-1 and vascular cell adhesion molecule-1 expression. α -Tocopherol decreases the influx of leukocytes in the vasculature by downregulating the expression of E-selectin, decreasing interleukin-1 secretion from monocytes, and attenuating the activation of NF- κ B.²⁴

ROS could also increase eNOS protein expression by acting on the eNOS gene. In this regard, eNOS promoter regions contain putative binding sites for redox-sensitive factors, including activator protein-1, specific promoter-1 transcription factor, and antioxidant-responsive elements. Therefore, in addition to protecting eNOS mRNA stability, ROS could stimulate the eNOS promoter, thus enhancing eNOS expression, as has been demonstrated in cultured endothelial cells incubated with H₂O₂.

Despite the *in vitro* evidence suggesting that ROS increases eNOS protein expression, several studies have suggested that heart failure leads to a decline in the expression of eNOS protein.^{25,26} Therefore, in the setting of heart failure, other factors, such as cytokines, could counterbalance the effect of ROS on eNOS expression, inducing endothelial dysfunction.

Heart Failure and Inflammation

Several inflammatory markers have been found to be elevated in patients with heart failure. The present interest in understanding the role of proinflammatory cytokines, such as TNF- α , in patients with heart failure arises from the simple observation that many aspects of the syndrome of heart failure can be explained by the known biological effects of these molecules.

The cytokine hypothesis does not imply that cytokines cause heart failure, *per se*, but that to potentially be involved in the genesis of this pathology, the overexpression of cytokines contributes to the progression of heart failure once

left ventricle dysfunction ensues. Recent studies in transgenic mice that overexpress TNF- α in the cardiac compartment have shown that these animals develop progressive left ventricular dilation and dysfunction.²⁷ Elevated levels of TNF- α have consistently been detected in patients with heart failure. Moreover, a trend toward increasing mortality seems to exist with increasing levels of TNF- α in the Studies of Left Ventricular Dysfunction (SOLVD).²⁸

The heart generates TNF- α in response to a variety of agents and forms of injury. Several works have shown that TNF- α mRNA and protein are present in failing human hearts, whereas neither TNF- α mRNA nor protein is detectable in nonfailing human hearts.^{28,29} These findings suggest that the myocardium represents an important source for TNF- α production in heart failure. In addition to stimulating NADPH oxidase in the myocardium, TNF- α exerts direct effects on the regulation of eNOS expression. As described above, TNF- α reduces the half-life of eNOS mRNA in the endothelium through activation of the binding of EDIP to the 3'-UTR of eNOS mRNA.^{18,20} An eNOS-like protein has been identified within the heart in the endothelium of both the endocardium and the coronary vasculature (including capillary and venular endothelium), in cardiac myocytes, and in specialized cardiac conduction tissue (including sinoatrial and atrioventricular nodal tissue), as well as in some formed elements of the blood, including neutrophils, monocytes, and platelets.^{30,31} Therefore, as in the vascular endothelium, TNF- α could also act by downregulating eNOS protein expression in the myocardium through the interaction of EDIP to the 3'-UTR of eNOS mRNA, favoring myocardial dysfunction.

Based on these observations, different strategies have been attempted to suppress cytokines in patients with heart failure. A potentially important pharmacological method for inhibiting TNF- α production is the use of agents that elevate cAMP levels, such as dobutamine, amrinone, and adenosine, which block mRNA accumulation by blocking the transcriptional activation of TNF- α . However, this point of view was not supported by a recent full-length publication, in which treatment with either intravenous dobutamine or milrinone had no effect in terms of decreasing circulating TNF- α levels.³² In a preliminary study of a small number of patients with advanced heart failure treated with a soluble TNF- α receptor antagonist that neutralizes the biological effects of circulating TNF- α , an improvement in the functional status and quality of life has been found.³³ Accordingly, the levels of TNF- α and other cytokines (interleukin-6, vascular cell adhesion molecule-1, and ICAM-1) have been detected as being elevated in the plasma and in the myocardium of patients with heart failure; these elevated levels have been suggested to be correlated with the severity of heart failure.^{34,35}

Neutrophils and Heart Failure

A close relationship between the activation of neutrophils and the inflammatory response has been postulated. Neutrophils are source of both ROS and cytokines. The superoxide-generating capacity has been shown to be increased in neutrophils from patients with heart failure but not in patients

being treated with ACE inhibitors.⁷ The activation of neutrophils and migration of these cells from the circulation to areas of inflammation may play a significant role in the immunologic response of chronic heart failure.

Vitamin C reduced the ability of neutrophils from patients with chronic heart failure to produce ROS.⁷ We have observed that carvedilol, which also improves superoxide-induced endothelial dysfunction,³⁶ reduces the ability of neutrophils to release superoxide (authors' unpublished data, 2001), suggesting a close relationship between endothelial functionality and neutrophil activation. In this regard, we have recently demonstrated that neutrophils express an eNOS-like protein, as has been previously demonstrated in the endothelium, and that the downregulation of eNOS protein expression by TNF- α is associated with the presence of EDIP in the cytosol of human neutrophils.³¹ This fact has also been observed in patients during acute myocardial infarction, a cardiovascular pathology associated with marked endothelial dysfunction.³¹ These findings support a relationship between endothelial dysfunction and neutrophil activation.

Pharmacological Perspectives

Several cardiovascular drugs have been considered as antioxidant supplements when used in patients suffering from different cardiovascular diseases. Propranolol, a nonselective β -adrenergic receptor blocker, is highly lipophilic and is known for its membrane-stabilizing activity, antiperoxidative activity, and antiradical activity.³⁷ Carvedilol, a nonspecific β -antagonist with α -blocking activity, has antioxidant properties.³⁸ Calcium channel blockers have also been reported to have antioxidant properties, inasmuch as they inhibit lipid peroxidation by scavenging peroxy radicals.³⁹ Captopril, an ACE inhibitor, has also been reported to be an OH⁻ scavenger because of its sulfhydryl content in addition to reduced angiotensin II, which is a potent activator of NADPH oxidase.⁴⁰ Losartan, an angiotensin II type 1 receptor antagonist, has also demonstrated antioxidant properties.⁴¹ Aspirin has also been shown to be an inhibitor of neutrophil NADPH oxidase.⁴²

Antioxidants may offer the therapeutic benefit of protecting endothelial function. α -Tocopherol improved endothelial function in patients with type I diabetes mellitus, those with spastic angina, hypercholesterolemic patients who smoke cigarettes, and hyperhomocysteinemic subjects.^{43,44} Vitamin C administration also improves endothelium-dependent vasodilation in patients with hypertension, hypercholesterolemia, diabetes mellitus, coronary artery disease, and hyperhomocysteinemia (see reviews^{43,44}). In the same line of evidence, it has been postulated that smokers have lower levels of plasma vitamin C than do nonsmokers; this difference has been associated with endothelial dysfunction. Even children exposed to environmental tobacco smoke have exhibited reduced levels of ascorbic acid.⁴⁵ Horning et al¹⁶ have recently shown that both short-term and long-term treatment with vitamin C improves endothelial dysfunction in patients with congestive heart failure, which has been further confirmed by others.⁷

It is noteworthy that improvement of flow-mediated dilation in patients with chronic heart failure after long-term oral treatment with vitamin C (1 month) was not correlated with reductions in oxidative stress,⁷ which could be related to the above-mentioned additional effects of vitamin C. A recent study in vitamin C-treated patients with coronary artery disease demonstrated a marked improvement in endothelial function with no detectable change in oxidative stress. This may support the involvement of other factors in addition to ROS in the genesis of endothelial dysfunction related to heart failure and also support other effects of vitamin C in addition to ROS depletion. In this regard, vitamin C increased the ability of eNOS to generate NO by increasing tetrahydrobiopterin availability.⁴⁶ Although the main product of eNOS activity is NO, paradoxically, this enzyme may also generate superoxide, a NO scavenger. eNOS will switch to generate superoxide rather than NO in conditions in which intracellular tetrahydrobiopterin levels are reduced.⁴⁶

It has also been shown that vitamin C increases eNOS gene expression.^{46,47} There are several data that support the inhibitory effect of antioxidants on inflammation and indirectly suggest the role of ROS as an activator of the inflammatory response. α -Tocopherol helps decrease the influx of leukocytes in the vasculature by downregulating the expression of E-selectin, decreasing interleukin-1 secretion from monocytes, and attenuating the activation of NF- κ B.⁴⁸ Captopril and aspirin, drugs that have been used in patients with heart failure to reduce ROS production, have also demonstrated anti-inflammatory properties in leukocytes and smooth muscle cells.^{49,50}

Physical training program is used as therapeutic treatment in patients with heart failure, with demonstrated beneficial effects. Daily exercise has been shown to enhance the expression of eNOS in normal animals and to improve endothelium-dependent vasodilation in patients with chronic heart failure.^{51,52} The increase in cardiac output that occurs during exercise increases endothelial shear stress, which stimulates eNOS expression. It is noteworthy that the eNOS promoter contains a shear stress-responsive element that stimulates eNOS gene transcription. Moreover, shear stress has been shown to enhance Cu/Zn-SOD expression in human aortic endothelial cells.⁵³ Interestingly, training that has been demonstrated to enhance eNOS expression also improves exercise tolerance, which is correlated with the attenuation of the inflammatory process in patients with chronic heart failure, indicating that both endothelial functionality and inflammation may contribute to the impaired exercise capacity seen in chronic heart failure.³⁵

Conclusions

The present review summarizes recent clinical and experimental data suggesting the involvement of ROS and endothelial dysfunction in heart failure. ROS are increased in the plasma of patients with heart failure. An increased production of ROS has been also demonstrated in neutrophils from patients with heart failure. Several cardiovascular drugs, in addition to vitamin C and α -tocopherol, have antioxidant properties, including the α - and β -blocker carvedilol, angiotensin I-converting enzyme inhibitors, angiotensin II type 1

receptor antagonists, and aspirin. Antioxidants improve endothelial functionality in patients with heart failure by mechanisms in addition to the reduction of NO biodegradation.

Inflammation is also closely related to oxidative stress and has a main role in the genesis of endothelial dysfunction by downregulating eNOS expression in the endothelium and by destabilizing eNOS mRNA in neutrophils. A 60-kDa cytosolic protein termed EDIP has been associated with downregulation of eNOS expression in the endothelium and neutrophils.

Physical training programs and drugs that modulate eNOS expression, inflammation, and ROS may represent a new therapeutic strategy for the treatment of patients with heart failure.

References

1. Yu BP. Cellular defenses against damage from reactive oxygen species. *Physiol Rev.* 1994;74:139–162.
2. Fridovich I. Superoxide anion radical (O_2^-), superoxide dismutases, and related matters. *J Biol Chem.* 1997;272:18515–18517.
3. Dhalla AK, Singal PK. Antioxidant changes in hypertrophied and failing guinea pig hearts. *Am J Physiol.* 1994;266:H1280–H1285.
4. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause or consequence? *Lancet.* 1994;344:721–724.
5. McMurray J, McLay J, Chopra M, Bridges A, Belch JFF. Evidence for enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease. *Am J Cardiol.* 1990;65:1261–1262.
6. Singh N, Dhalla AK, Seneviratne C, Singal PK. Oxidative stress and heart failure. *Mol Cell Biochem.* 1995;147:77–81.
7. Ellis GR, Anderson RA, Lang D, Blackman DJ, Mornis RHK, Morris-Thurgood J, McDowell FW, Jackson SK, Lewis MJ, Frenneaux MP. Neutrophil superoxide anion-generating capacity, endothelial function and oxidative stress in chronic heart failure: effects of short- and long-term vitamin C therapy. *J Am Coll Cardiol.* 2000;36:1474–1482.
8. Liao F, Andalibi A, Qiao JH, Allayee H, Fogelman AM, Lussis AJ. Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J Clin Invest.* 1994;94:877–884.
9. Rowe GT, Manson NH, Caplan M, Hess ML. Hydrogen peroxidase and hydroxyl radical mediation of activated leukocyte depression of cardiac sarcoplasmic reticulum: participation of the cyclooxygenase pathway. *Circ Res.* 1983;53:584–591.
10. Drexler H, Hayoz D, Munzel T, Horning B, Just H, Brunner HR, Zelis R. Endothelial function in chronic congestive heart failure. *Am J Cardiol.* 1992;69:1596–1601.
11. Kubo SH, Rector TS, Bank AL, Williams RE, Heifetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation.* 1991;84:1589–1596.
12. Moncada S, Higgs A. L-Arginine–nitric oxide pathway. *N Engl J Med.* 1993;329:2002–2012.
13. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endothelial modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A.* 1991;88:587–590.
14. López-Farré Caramelo C, Esteban A, Alberola ML, Millás I, Montón M, Casado S. Effects of aspirin on platelet-neutrophil interactions: role of nitric oxide and endothelin-1. *Circulation.* 1995;91:2080–2088.
15. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol.* 1999;34:631–638.
16. Horning B, Arakawa N, Kohler C, Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation.* 1998;97:363–368.
17. Arriero MM, Rodríguez-Feo JA, Celdrán A, Sanchez de Miguel L, Gonzalez-Fernandez F, Fortes J, Reyero A, Frieyro O, de la Pinta JC, Franco A, Pastor C, Casado S, López-Farré A. Expression of endothelial nitric oxide synthase in human peritoneal tissue: regulation by *Escherichia coli* lipopolysaccharide. *J Am Soc Nephrol.* 2000;11:1848–1856.
18. Alonso J, Sanchez de Miguel L, Montón M, Casado S, López-Farré A. Endothelial cytosolic proteins bind to the 3'-untranslated region by tumor necrosis factor- α . *Mol Cell Biol.* 1997;17:5719–5726.
19. Yoshizumi M, Perrella MA, Burnett JC, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. *Circ Res.* 1993;73:205–209.
20. Sanchez de Miguel L, Alonso J, Gonzalez-Fernandez F, de la Osada J, Montón M, Rodríguez Feo JA, Guerra JJ, Arriero MM, Rico L, Casado S, López-Farré A. Evidence that endothelial cytosolic protein binds to the 3'-untranslated region of endothelial nitric oxide synthase mRNA. *J Vasc Res.* 1999;36:201–208.
21. Drummond GR, Cai H, Davis ME, Ramasamy S, Harrison DG. Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. *Circ Res.* 2000;86:347–354.
22. Chin JJ, Wang BS, Shyy JY, Hsieh HJ, Wang DL. Reactive oxygen species are involved in shear stress induced intercellular adhesion molecule-1 expression in endothelial cells. *Arterioscler Thromb Vasc Biol.* 1997;17:3570–3577.
23. Wung BS, Cheng JJ, Hsieh HJ, Shyz YJ, Wang DL. Cyclic strain-induced monocyte chemotactic protein-1 gene expression in endothelial cells involves reactive oxygen species activation of activator protein-1. *Circ Res.* 1997;81:1–7.
24. Erl W, Weber C, Wardemann C, Weber PC. Alpha-tocopherol succinate inhibits monocytic cell adhesion to endothelial cells by suppressing NF-kappa B mobilization. *Am J Physiol.* 1997;273:H634–H640.
25. Wang J, Seyedi N, Xu XB, Wolins MS, Hintze TH. Defective endothelium-mediated control of coronary circulation in conscious dogs after heart failure. *Am J Physiol.* 1994;266:H670–H680.
26. Smith CJ, Sun D, Hoegler C, Roth DS, Chang X, Chao G, Xu XB, Kobari Y, Pritchard K, Sessa WC, et al. Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. *Circ Res.* 1996;78:58–64.
27. Bryant D, Becker L, Richardson J, Shelton J, Franco F, Pechock R, Thompson M, Giroir B. Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor- α (TNF). *Circulation.* 1998;97:1375–1381.
28. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). *J Am Coll Cardiol.* 1996;27:1201–1206.
29. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor severe chronic heart failure. *N Engl J Med.* 1990;223:236–241.
30. Kelly RA, Balligand JL, Smith TW. Nitric oxide and cardiac function. *Circ Res.* 1996;79:363–380.
31. de Frutos T, Sanchez de Miguel L, Farré J, Gomez J, López-Farré A. Expression of an endothelial-type nitric oxide synthase isoform in human neutrophils: modification by tumor necrosis factor- α and during acute myocardial infarction. *J Am Coll Cardiol.* 2001;37:800–807.
32. Milani RV, Mehra MR, Endres S, Eiger A, Cooper S, Lavie CJ, Ventura HO. The clinical relevance of circulating tumor necrosis factor- α in acute decompensated chronic heart failure without cachexia. *Chest.* 1996;110:992–997.
33. Deswal A, Seta Y, Blosch CM, Mann DL. A phase I trial of tumor necrosis factor receptor (p57) fusion protein (TNFR: F₂) in patients with advanced heart failure. *Circulation.* 1997;96(suppl I):I-1802. Abstract.
34. Deswal A, Peterson NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone Trial (VEST). *Circulation.* 2001;103:2055–2059.
35. Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, Koniavitou K, Coats AJ, Kremastinos DT. Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. *Eur Heart J.* 2001;22:791–797.
36. López BL, Christopher TA, Yue TL, Ruffolo R, Fenerstein GZ, Ma XL. Carvedilol, a new beta-adrenoreceptor blocker antihypertensive drug, protects against free-radical induced endothelial dysfunction. *Pharmacology.* 1995;51:165–173.
37. Khaper N, Rigatto C, Seneviratne C, Li T, Singal PK. Chronic treatment with propranolol induces antioxidant changes and protects against ischemia-reperfusion injury. *J Mol Cell Cardiol.* 1997;29:3335–3344.
38. Cagnoni A, Ceconi C, Bemocchi P, Boraso A, Parrinello G, Curello S, Ferrari R. Reduction of oxidative stress by carvedilol: role in maintenance of ischaemic myocardium viability. *Cardiovasc Res.* 2000;47:556–566.
39. Sevanian A, Shen L, Ursini F. Inhibition of LDL oxidation and oxidized LDL-induced cytotoxicity by dihydropyridine calcium antagonists. *Pharm Res.* 2000;17:999–1006.
40. Hayek T, Attias J, Smith J, Breslow JL, Keider S. Antiatherosclerotic and antioxidative effects of captopril in apolipoprotein E-deficient mice. *J Cardiovasc Pharmacol.* 1998;31:540–544.

41. Khaper N, Signal PK. Modulation of oxidative stress by a selective inhibition of angiotensin II type I receptors in myocardial infarction rats. *J Am Coll Cardiol*. 2001;37:1461–1466.
42. López-Farré A, Riesco A, Digiuni E, Mosquera JR, Caramelo C, de Miguel LS, Millas I, de Frutos T, Cerradas MR, Monton M, et al. Aspirin-stimulated nitric oxide production by neutrophils after acute myocardial ischemia in rabbits. *Circulation*. 1996;94:83–87.
43. Berry C, Brosnan MJ, Frennell J, Hamilton CA, Dominiak AF. Oxidative stress and vascular damage in hypertension. *Curr Opin Nephrol Hypertens*. 2001;10:247–255.
44. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens*. 2000;18:655–673.
45. Strauss RS. Environmental tobacco smoke and serum vitamin C levels in children. *Pediatrics*. 2001;107:540–542.
46. Huang A, Vita JA, Venema RC, Keaney JF. Ascorbic acid enhances endothelial nitric oxide synthase activity by increasing tetrahydrobiopterin. *J Biol Chem*. 2000;275:17399–17406.
47. Mizutani A, Maki H, Torii Y, Hitomi K, Tsukagoshi N. Ascorbate-dependent enhancement of nitric oxide formation in activated macrophages. *Nitric Oxide*. 1998;2:235–241.
48. Devaraj S, Li D, Jialal I. The effects of alpha-tocopherol supplementation on monocyte function: decreased lipid oxidation, interleukin 1 β secretion, and monocyte adhesion to endothelium. *J Clin Invest*. 1996;98:756–763.
49. Zhao SP, Xie XM. Captopril inhibits the production of tumor necrosis factor- α by human mononuclear cells in patients with congestive heart failure. *Clin Chim Acta*. 2001;304:85–90.
50. Sanchez de Miguel L, de Frutos T, Gonzalez-Fernandez F, del Pozo V, Lahoz C, Jimenez A, Rico L, García R, Aceituno E, Millas I, Gomez J, Farre J, Casado S, López-Farré A. Aspirin inhibits inducible nitric oxide synthase expression and tumor necrosis factor- α release by cultured smooth muscle cells. *Eur J Clin Invest*. 1999;29:93–99.
51. Katz SD, Yuen J, Bijou R, LeJemtel TH. Training improves endothelial-dependent vasodilation in resistance vessels of patients with heart failure. *J Appl Physiol*. 1997;82:1488–1492.
52. Hambrecht R, Fiehn E, Weigl C, Gielen S, Hamann C, Kaiser R, Yu J, Adams V, Niebauer J, Schuler G. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation*. 1998;98:2709–2715.
53. Inoue N, Ramasamy S, Fukai T, Nerem RM, Harrison DG. Shear stress modulates expression of Cu/Zn superoxide dismutase in human aortic endothelial cells. *Circ Res*. 1996;79:32–37.