**REVIEW ARTICLE**

# Cardiovascular Syndrome X and Endothelial Dysfunction

Eugenia Vázquez-Rey and Juan Carlos Kaski

Coronary Artery Disease Research Unit. Department of Cardiological Sciences, St. George's Hospital Medical School. Londres. Reino Unido.

Up to 30% of patients with chest pain who undergo coronary arteriography, have completely normal coronary angiograms. The subgroup with typical angina and a positive response to stress testing is generally included under the diagnosis of cardiovascular syndrome X. Several causes and mechanisms have been investigated in the past twenty years, to explain both chest pain and ischemic angina-like ST segment depression that are commonly observed in these patients. Clinical and pathogenic heterogeneity appears to be the main features of the syndrome. Among the suggested pathophysiological mechanisms, endothelial dysfunction of the coronary microcirculation features prominently.

In this review, we present the available evidence regarding endothelial dysfunction in cardiovascular syndrome X.

**Key words:** *Syndrome X. Endothelial dysfunction. Myocardial ischaemia.*

*Full English text available at: www.revespcardiol.org*

**Síndrome X cardiovascular y disfunción endotelial**

Hasta un 30% de los pacientes que son sometidos a una coronariografía tienen arterias coronarias angiográficamente normales. De estos pacientes, el subgrupo con dolor anginoso típico y ergometría positiva se engloba bajo el diagnóstico de síndrome X cardíaco. Durante años se han investigado múltiples posibles causas y mecanismos para explicar tanto el dolor torácico como las alteraciones electrocardiográficas indicativas de isquemia miocárdica que se observan en estos pacientes. Hoy día parece claro que la heterogeneidad es una de las características principales del síndrome, tanto en lo que se refiere a la fisiopatología como a las manifestaciones clínicas. Entre los posibles mecanismos patogénicos, la disfunción endotelial de la microcirculación coronaria aparece como uno de los más importantes.

En esta revisión se resume la evidencia disponible en la actualidad acerca de la disfunción endotelial como uno de los mecanismos patogénicos implicados en el síndrome X cardíaco.

**Palabras clave:** *Síndrome X. Disfunción endotelial. Isquemia miocárdica.*

## INTRODUCTION

Angina in the setting of normal coronary arteries is a heterogeneous entity, both clinically and pathophysiologically. The most accepted definition of the syndrome includes chest pain with coronary characteristics, a positive stress test (typical ST segment depression), and coronary arteries that appear normal on angiography, with possible extracardiac causes of the chest pain, coronary spasm, arterial hypertension (AHT), and ventricular hypertrophy ruled out.[1]

Over the last 30 years, multiple pathogenic mechanisms have been considered as possible causes; it is likely that these vary from patient to patient (Table 1).[2,3]

Since Kemp introduced the term «syndrome X»[4] in an editorial published in 1973, many groups have studied the possibility of a decrease in the coronary reserve being the cause of this syndrome; it has been noted that at least some of the patients classified as having

TABLE 1. **Physiopathological mechanisms in the cardiac X syndrome**

| |
|---|
| Endothelial dysfunction→microvascular dysfunction→ischemia |
| Change in the perception of chest pain |
| Change in the perception of cardiac pain |
| Metabolic changes in cardiac muscle |
| Hyperfunction of the $Na^+/H^+$ pump |
| Increase in sympathetic tone |

The Dra. Eugenia Vázquez-Rey have got a grant of the Sociedad Española de Cardiología.

Correspondence: Prof. J.C. Kaski.
Director of Coronary Artery Disease Research Unit. Department of Cardiological Sciences. St. George's Hospital Medical School.
London SW17 0RE. United Kingdom.
E-mail: jkaski@sghms.ac.uk

ABBREVIATIONS

AHT: arterial hypertension.
PET: positron emission tomography.
NO: nitric oxide.
ET-1: endothelin 1.
AT-II: angiotensin II.
BK: bradykinin.
Ach: acetylcholine.
eNOS: endothelial nitric oxide synthetase.
sGC: soluble guanylate cyclase.
TNF-α: tumor necrosis factor alpha.
EBP: eNOS binding protein.
vWF: von Willebrand factor.
LBBHB: left branch block of the His bundle.
ACEI: angiotensin-converting enzyme inhibitor.
PARP: poly (ADP-ribose) polymerase.

this syndrome have a limited increase in coronary fluid in response to stimuli such as high-frequency atrial pacemakers or microvascular vasodilators such as acetylcholine, papaverin, adenosine, or dipiridamol. Cannon and Epstein[5] termed this entity, characterized by a decrease in the coronary reserve, «microvascular angina.» Later, several studies identified endothelial dysfunction as one of the pathogenic mechanisms of this type of microvascular dysfunction,[6-8] and even more importantly, some researchers have found, in patients with syndrome X, not only coronary circulation but also peripheral circulation, indicating that the syndrome may involve a generalized vascular disturbance.[9-12]

## PHYSIOPATHOLOGY OF MICROVASACULAR ANGINA

Coronary microcirculation is responsible for regulating coronary flow. It is reasonable to assume, therefore, that a decrease in the coronary reserve may cause myocardial ischemia. Many researchers have questioned, nevertheless, the existence of myocardial ischemia in the cardiac X syndrome due to the absence of hemodynamic or metabolic evidence of ischemia in most patients studied.[13-18]

In patients with heart disease, ischemia is frequently associated with transient abnormalities in myocardial contractility as is shown on echocardiography or isotopic techniques. In patients with the X syndrome, however, only a few studies performed with isotopic techniques have shown a decrease in the ejection fraction during exercise (approximately 30% of patients).[19,20] Some authors have described an association between the perfusion defects evident on gammography and the presence of coronary endothelial dysfunction.[21,22] These changes may be an expression of ischemia or a reflection of the heterogeneity of myocardial perfusion in these patients, which has also been observed in studies performed with positron emission tomography (PET).

Maseri et al[23] proposed the hypothesis that patients with the X syndrome may present with functional and structural abnormalities in microcirculation, prearteriolar in nature, consistent with diffuse or patchy microvascular vasoconstriction (or the absence of appropriate vasodilatation), which would lead to myocardial ischemia that was undetectable by the usual techniques or that was compensated for by hypercontractility in the adjacent territories, which are normally perfused. These changes may help explain the broad spectrum of symptoms that occur in these patients. When greater numbers of vessels are involved, the decrease in the coronary reserve is greater, and the ischemia is greater. When, on the other hand, the involvement is restricted to a few vessels, the freeing of adenosine in the arterioles distal to the diseased prearteriolar vessels could cause chest pain in the absence of ischemia, given the algogenic properties of adenosine, and may even be responsible for changes observed on electrocardiogram (ECG) since adenosine produces a decrease in the action potential duration of myocytes and has a negative chronotropic effect.

Recent studies performed with stress echocardiography, both transthoracic and transesophageal, have established the absence of contractility abnormalities triggered by stress in these types of patients.[14,24-26] In contrast to the echocardiographic data, changes have been identified in $O_2$ saturation, pH, and the production of lactate in coronary chest blood, indicative of ischemia in the subgroup of patients with the X syndrome.[27-29] In a study of 9 patients with the X syndrome and 5 controls who underwent stress testing (induced atrial tachycardia), production of lipid peroxidation products (indicative of ischemia) was documented in the syndrome X patients but not in the controls.[30]

Recently, Buchthal et al[31] studied 35 women with angina whose coronary arteries showed no significant stenosis on angiography (20% or less) and 12 controls, using magnetic resonance (MR) imaging with P-31, which allows direct assessment of high energy phosphates in the myocardium and the metabolic identification of ischemia. The authors found a decrease in the percentage of phosphocreatinine/ATP greater than 2 standard deviations from the mean in 20% of patients during a stress test (handgrip exercise). This result provides new evidence of myocardial ischemia in at least some of the patients with syndrome X (Table 2).

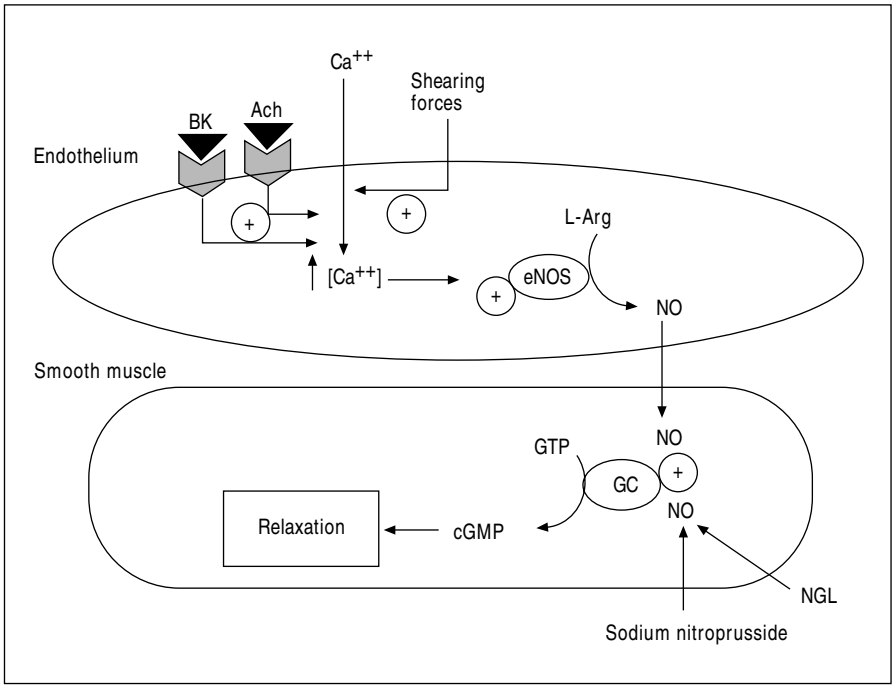


**Fig. 1.** Vasodilatation mediated by NO. The shearing forces, and BK and Ach binding to their respective receptors on the surface of the endothelial cell produce a $CA^{++}$ current that stimulates the eNOS enzyme, resulting in NO synthesis from the L-Arg amino acid. The NO spreads to the adjacent muscle cells, in which the soluble form of GC enzyme is stimulated, causing the synthesis of CGMP which mediates relaxation of smooth muscle cells. Sodium nitroprusside and nitroglycerine liberate NO which relaxes muscle cells via the same mechanism. Ach indicates acetylcholine; BK, bradykinin; $Ca^{++}$, calcium; GC, guanylate cyclase; cGMP, cyclic guanosine monophosphate; NO, nitric oxide; eNOS, endothelial nitric oxide synthetase; NGC, nitroglycerine.

## Coronary endothelium. The physiopathological role of nitric oxide

The vascular endothelium is responsible for important functions, including regulation of capillary permeability, vascular tone, and blood flow. The endothelium has a dual role in the control of vascular tone by segregating, in response to hemodynamic and chemical stimuli, both vasodilatory substances (such as nitric oxide [NO] and prostacycline), and vasoconstrictive substances (endothelin-1 [ET-1] and angiotensin II [AT-II]). An adequate balance between these elements, vasoconstrictors and vasodilators, is essential for maintaining vascular homeostasis. When this balance is upset, the result is an increased predisposition to vasoconstriction of the vessels, leukocyte adherence, plaque activation, mitogenesis, and increases in the oxidative state, thrombosis, activation of coagulation, vascular inflammation, and atherosclerosis.[32-35]

NO plays a key role in the regulation of vascular tone. In addition to being the principal determining factor of the basal tone of smooth vascular muscle, it counteracts the action of AT-II and ET-1. In addition, NO also inhibits the activation of plaque and leukocytes, and has an antiproliferative effect on smooth vascular muscle. NO is synthesized from the amino acid L-Arginine by the NO-synthetase enzyme. The activation of the endothelium by shearing forces or by the binding of bradykinin (BK) or acetylcholine (Ach) to their respective receptors results in a calcium entrance current that stimulates endothelial nitric oxide synthetase (eNOS). The NO that is formed from the L-arginine diffuses the nearby cells of the smooth muscle, stimulating in them the soluble form of the guanylate cyclase (sGC) enzyme, which produces an increase in cyclic guanosine monophosphate (cGMP) which regulates the relaxation of muscle cells (Figure 1).

Initially, eNOS was described an not inducible, but it was later shown that certain stimuli, such as hypo-

TABLA 2. **Studies with findings indicative of ischemia in syndrome X**

| Authors | Number of patients | Type of patients | Technique | Year |
|---|---|---|---|---|
| Crake et al[27] | 10 | Syndrome X | $O_2$ saturation and lactate in coronary chest | 1988 |
| Zeiher et al[21] | 27 | Chest pain and lesions <30% | SPECT $^{201}$Tl and Doppler IC | 1995 |
| Rosano et al[29] | 14 | Syndrome X | pH and lactate in coronary chest | 1996 |
| Hasdai et al[22] | 20 | Chest pain and lesions <50% | SPECT $^{99}$Tc and Doppler IC | 1997 |
| Buchthal et al[31] | 35 | women with chest pain and lesions <20% | NMR with $^{31P}$ phosphocreatinine ratio/ATP | 2000 |
| Buffon et al[30] | 9 | Syndrome X | ROOH and CD in aorta and coronary chest | 2000 |

CD indicates conjugated dienes; IC, intracoronary; ROOH, lipid hydroperoxides; CNMR, cardiac nuclear magnetic resonance.

xia, vigorous exercise, and stages of growth are associated with an increase in the expression of the enzyme in endothelial cells.[36-38] Certain cytokines, such as tumor necrosis factor alpha (TNF-α), reduce the expression of eNOS[39,40] because of the increase of a cystolic protein, called eNOS binding protein (EBP), which binds to the mRNA of the stabilized eNOS.[41,42]

## Microvascular endothelial dysfunction in syndrome X

Patients with cardiovascular syndrome X present with increased resistance of coronary flow,[43-48] which is attributed to endothelial dysfunction in microcirculation. In humans, coronary microcirculation can be studied indirectly by measuring the myocardial flow in response to various vasodilator stimuli. The so-called vasodilatory reserve, or coronary flow reserve, is expressed as the quotient of maximum flow, obtained in response to vasodilators, and basal flow. Among the techniques used to perform these measurements is PET,[49-51] intracoronary Doppler,[7,8,34] blood flow measurement by thermodilution in the coronary chest[8,52] and, more recently, by MR imaging.[31]

Egashira et al[53] reported on endothelial dysfunction in patients with syndrome X and noted that the administration of L-arginine (a precursor of NO) improved endothelium-dependent vasodilation, which indicates a defect in NO synthesis in these patients.[54] Previously, Bellamy et al[11] showed that the oral administration of L-arginine for 4 weeks improved exercise tolerance and reversed the endothelial dysfunction in patients with syndrome X.

Quyyumi et al[8] evaluated the response to Ach and to high-frequency atrial pacemaker in 51 patients with syndrome X, and found a correlation between both responses, indicating that endothelial dysfunction may contribute to a decrease in the coronary reserve during stress and anginous pain in these patients. Nevertheless, the authors also observed that some patients had a normal reaction to Ach.

Endothelial dysfunction can be also be evaluated with serological markers, which are cellular components that are freed into the bloodstream when endothelial cells are damaged or activated. Among these are the von Willebrand factor (vWF), fibrinogen, fibronectin, and alfa-2-macroglobulin. Another type of marker for endothelial activation is the VCAM-1 and ICAM-1 markers, which mediate leukocyte adhesion to the vascular endothelium. Tousoulis et al[45] showed that there is a significant increase in the plasma concentrations of VCAM-1 and ICAM-1 both in patients with syndrome X and in patients with heart disease as compared to healthy controls. Bøtker et al[46] performed a study that compared the vWF values in 3 groups of patients, 1 group with heart disease, 1 with syndrome X, and a control group. The authors found significantly higher vWF values in the patients with heart disease, but not in the other 2 groups. Nevertheless, the considerable overlap of the results from the syndrome X group and the group of patients with heart disease reported in the study by Bøtker et al points out, once again, that the patients with syndrome X are a heterogeneous group; some patients in this group do have elevated concentrations of vWF factor. This heterogeneity may be also be explained by the fact that studies have shown systemic endothelial dysfunction only in some patients with syndrome X, including those patients who had no elevated concentrations of vWF factor. Therefore, some researchers have found a decrease in the endothelium-dependent vasodilatory response on echo-Doppler or plethysmography of the forearm in patients with syndrome X and healthy controls in whom they evaluated the response of myocardial flow to dipiridamol using PET and systemic vasoreactivity using ultrasound of the brachial artery, and in whom they studied the structure and function of subcutaneous resistance vessels in the biopsy specimen obtained from the gluteus. These findings confirmed the presence of both reduced coronary and peripheral vasodilatory capacities; however, they did not find functional or structural abnormalities in the arteries that they isolated.

## The role of endothelin

The endothelins are a family of peptides consisting of 21 amino acids (ET-1, ET-2, ET-3) codified by 3 distinct genes. Endothelins are expressed in various tissues, where they act as moderators of vasomotor tone, cell proliferation, and hormone production. ET-1 is the only one that is produced in endothelial cells, although it is also produced in the smooth muscle cells of the vascular wall.[55,56] It was isolated for the first time by Yanagisawa et al[57] in 1988 from the endothelial cells of pig aorta. ET-1 is a vasoconstrictor and neuromodulator peptide. Recent studies suggest it has an important role in the regulation of basal coronary arterial flow,[58,59] and it has been associated with the pathogenesis of processes such as ischemia-reperfusion damage, cardiac insufficiency, hypertension, and atherosclerosis. Endothelin is the most powerful vasoconstrictor known at the present time (100 times more powerful than noradrenalin).[60] Two receptors (ETA and ETB) have been identified. The ETA receptor is the predominant subtype and is present in vascular wall myocytes. Stimulating the receptor results in vasoconstriction.[61,62] The ETB receptor is present in the endothelial cells, which produce a vasodilation effect, and also in the vascular myocytes, where it exerts a vasoconstrictor effect.[63-65]

ET-1 is not stored in secretory granules in the interior of the cell.[66] Stimuli such as ischemia, hypoxia, and shear stress induce transcription of the gene

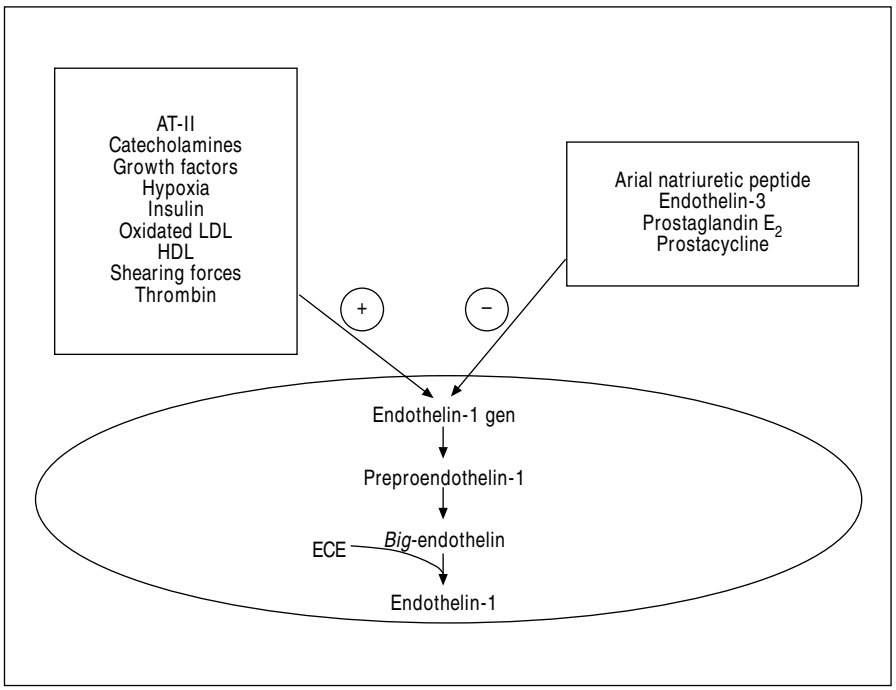


**Fig. 2.** Regulation of the synthesis and secretion of endothelin. AT-II indicates angiotensin II; ECE, endothelin conserving enzyme; HDL, high density lipoproteins; LDL, low density lipoproteins.

(Figure 2) and the synthesis and secretion of ET-1 within minutes.[67-70] Its half-life is 4 to 7 minutes and 80% of it is cleared by the lungs. Nevertheless, after binding to the receptor, the vasoconstrictor effect may last more than 60 minutes. Although its secretion is 75% albumin, acting in a predominantly paracrine manner on the vascular myocytes, its plasma concentrations correlate well with the severity and degree of heart disease,[71,72] unstable angina,[73,74] and cardiac insufficiency, and thus have prognostic value.[75-77] Elevated ET-1 values have also been reported in patients with coronary spasm. Nevertheless, the available evidence shows that vasoconstriction mediated by ET-1 occurs predominantly at the microvascular level,[80] most likely due to the distribution of the various types of receptors in the coronary vasculature.[81] These findings have lead to speculation that increases in ET-1 activity may be implicated in the physiopathology of microvascular angina. In a study by Kaski et al[29] plasma ET-1 values were measured in 40 patients with syndrome X and 21 healthy controls; the authors found that plasma concentrations of ET-1 were significantly higher in patients with syndrome X than in the controls. Even more important, they observed that in patients with syndrome X, the plasma levels of endothelin had a negative correlation with the levels of ischemic pain.[82] In another study, the same group showed an inverse correlation between basal artery ET-1 levels and the coronary reserve in women with syndrome X.[83] In a later study of larger series of patients with syndrome X, the same authors observed that those patients who presented with a left branch block of the His bundle (LBBHB) or who had suffered a previous infarct in particular had the highest ET-1 levels.[84]

Lanza et al[85] measured plasma concentrations of ET-1 in overall circulation and in the coronary chest in 13 patients with syndrome X and 8 controls, at baseline and after stimulation with high-frequency atrial pacemaker. The baseline ET-1 levels, both in the samples obtained from the femoral artery and from the coronary chest, were significantly greater in the patients with syndrome X than in the controls. After stimulation with the atrial pacemaker, the authors found an increase in the ET-1 values in the coronary chest in the patients with syndrome X but not in the control patients.

Other authors, however, have not been able to detect higher plasma concentrations of ET-1 in patients with syndrome X. Newby et al[86] did not observe differences in the ET-1 levels between patients with syndrome X and healthy controls, but found that the peripheral vasoconstrictor response to the infusion of ET-1 had an inverse correlation with its plasma concentrations. This phenomenon may reflect hyperactivity of the ET-1 system and an increase in the number of type A receptors. Desideri et al[87] did not find any differences at baseline in the plasma values of ET-1 or in VCAM-1 or in nitrate values. They did, however, observe significantly higher concentrations of ET-1 in patients with syndrome X than in control patients after glucose overload, which may indicate the endothelium is more susceptible to certain stimuli.

These findings raise important questions regarding the role of ET-1 in the physiopathology of syndrome X. Opherk et al[88] showed that the presence of LBBHB

on ECG is associated with a subgroup of patients with syndrome X who had an increased risk of developing left ventricular dysfunction at a later date. In addition, it has been reported that patients with syndrome X and LBBHB have abnormalities in lactate metabolism during stress.[89] Based on these observations, it has been proposed that patients with LBBHB and syndrome X may have a type of dilated myocardiopathy associated with microvascular ischemia. It has been suggested that the elevated ET-1 values in patients with syndrome X may contribute to the development of microvascular myocardial ischemia, which in serious cases could produce conduction abnormalities and progressive left ventricle dysfunction.[90] A study by our group indicated that among patients with syndrome X who underwent repeated coronary angiography due to the instability of their symptoms, patients with LBBHB had a higher likelihood of a developing coronary angiography disease in than patients who had conduction anamolies.[91]

### Microvascular spasm

The mechanism of microvascular spasm was proposed more than 10 years ago by several researchers.[92,93] Recently, Mohri et al[94] investigated the response to intracoronary Ach in 117 patients with angina and normal or nearly normal coronary arteries (stenosis <50%). The majority of the patients presented with angina at rest, indicative of coronary spasm. During angiography, 29 of the patients presented with typical angina or ischemic changes on ECG, or both, without evidence of spasm in the epicardial coronary arteries. In 11 of the 29 patients in this group the production of lactate, measured in the coronary chest, was also detected. On the other hand, Murakami et al[95] published findings on 3 patients with transient ST segment irregularities and normal coronary angiogram during episodes. All the patients had a positive electric ergometry by ST segment depression; however, coronary stenosis was present on angiogram and testing to provoke coronary spasm was negative. A decrease in the endothelium-dependent coronary reserve was detected in 1 of the 2 patients in whom the test could be performed. The underlying mechanism in this case would be constriction of the arterioles to less than 200 mm in diameter.

## ETIOLOGY OF ENDOTHELIAL DYSFUNCTION IN SYNDROME X

There are a variety of causes that may contribute to endothelial dysfunction in patients with syndrome X, including risk factors such as diabetes, smoking, arterial hypertension (AHT), hypercholesterolemia, and other factors that have recently been implicated in atherogenesis such as homocysteine, $O_2$ free radicals, infections, inflammatory mechanisms, and estrogen deficits.[96]

Several authors have shown that coronary risk factors, such as AHT, hypercholesterolemia, and diabetes are associated with a reduction in NO availability, both at baseline and after stimulation (Ach), in patients with normal coronary arteries on angiogram.[97-102] Even more importantly, other authors have shown that the amelioration of these risk factors,[103-110] as well as angiotensin converting enzyme inhibitors (ACEI),[111] can normalize endothelial function. Similarly, various studies have shown that endothelial dysfunction, produced by classic coronary risk factors, affects not only the epicardial coronary arteries, but also microcirculation and peripheral circulation.

Based on the observation that many patients with syndrome X are menopausal and postmenopausal women, an estrogen deficit has been proposed as a possible factor in the pathogenesis of this syndrome.[112] A relationship has been found between endothelial dysfunction and estrogen deficits,[113] both in asymptomatic and hypertensive women and in women with documented heart disease. Rosano et al[114] performed a study of 107 women with chest pain and normal coronary arteries on angiogram, of whom 95 developed the illness following menopause. Forty-three of the women had undergone a hysterectomy, which is an incidence 4 times higher than that in the general population. In addition, a notable improvement in endothelial function in coronary microcirculation was observed after hormone replacement therapy in women with syndrome X,[115] and improved peripheral endothelial function is observed after estrogen treatment or combined hormone treatment in women with[116] and without[117-119] heart disease. Webb et al[120] showed that the administration of estradiol achieved a notable decrease of ET-1 in postmenopausal women with heart disease; however, they did not observe a change in vasomotor function, in spite of the reduction in ET-1.

There is another syndrome X, a metabolic type that is defined by its epidemiological association with insulin resistance, AHT, dyslipemia, and atherosclerotic heart disease.[121]

Studies have found that patients with angina and normal coronary arteries have insulin resistance, and an increase in the secretion of proinsulin, increased concentrations of triglycerides, and decreased HDL, which also occur in patients with metabolic syndrome X.[122-125] On the other hand, an increase in plasma concentrations of ET-1 has been observed in patients with metabolic syndrome X.[126] Both syndromes are also characterized by endothelial dysfunction and an increase in the $Na^+$-$H^+$ exchange in cell membranes (Table 3).[127,128]

It has been suggested that insulin resistance in patients with angina and normal coronary arteries may result from endothelial dysfunction, due to variations

in microvascular fluid in the skeletal muscle, given that glucose capture is stimulated by insulin by means of an increase in local fluid mediated by NO.[129] Researchers have observed that the response to insulin is associated with capillary density in the skeletal muscle and can be improved by interventions such as physical exercise, which increases the capture of glucose and blood flow. On the other hand, the pancreatic islet beta cell dysfunction, evident in the increase in pancreatic islet beta cells observed in patients with insulin resistance, type 2 diabetes mellitus, and arterial hypertension (AHT),[130,131] as well as in patients with cardiac syndrome X, may be the result of the effect of pancreatic microvascular perfusion. Nevertheless, although endothelial dysfunction has been associated with the presence of insulin resistance in different phases of atherosclerosis,[132] it is not clear whether it is the direct cause.[133]

It has been suggested that a defect in glucose transport in the cell membrane is the most likely mechanism of insulin resistance in patients with cardiac syndrome X.[134] The hyperinsulinemia that is associated with insulin resistance may also be a cause of endothelial dysfunction. Recently, Arcaro et al[135] showed, in a study of healthy volunteers, that moderate hyperinsulinemia, at the same level as that present in patients with insulin resistance, causes serious endothelial dysfunction in macrocirculation. In the same study, the authors reported that a regimen of vitamin C prevented the development of endothelial dysfunction, which indicates a possible mechanism of action that increases oxidative stress. It has been shown that insulin in microcirculation activates both the production of NO and ET1.[136] Endothelin induces the expression of NAD(P)H oxidase in endothelial cells, with the consequent generation of superoxide anion.[137] On the other hand, it has been noted that hyperinsulinemia of exogenous origin activates NAD(P)H oxidase in rat aortic endothelium.[138] Based on these findings, the authors suggested that insulin may cause endothelial dysfunction after an increased availability of ET-1 and a subsequent increase in oxidative stress. In the opinion of some authors, all these changes observed in metabolic syndrome X lead to a vicious circle in which endothelial dysfunction contributes to insulin resistance and vice versa.[139]

A recent study by García-Soriano et al[140] reported that the activation of the (ADP-ribose) polymerase (PARP) enzyme is an important factor in the pathogenesis of endothelial dysfunction in diabetes mellitus. PARP is a nuclear enzyme that has been implicated in the response to AND lesion. These authors found that the destruction of pancreatic islets with streptozotocin in mice induces a state of intense hyperglycemia, the intravascular production of oxidative substances, activation of the PARP, and endothelial dysfunction. In addition, the authors showed that endothelial dysfunction in mice is dependent on PARP. Experiments *in vitro* on pulmonary endothelial cells showed that increased concentrations of glucose are a potent stimulus for the activation of PARP following formation of super oxidized radicals. It has also been shown that the endothelial cells exposed to elevated concentrations of glucose generate oxygen free radicals.[141]

TABLE 3. **Common characteristics of the metabolic and cardiac syndromes X**

| |
|---|
| Insulin resistance |
| Hyperinsulinemia |
| Hypertriglyceridemia |
| Decrease in HDL |
| Increase in ET-1 |
| Endothelial dysfunction |
| Increase in $Na^+$-$H^+$ exchange |

ET-1 indicates endothelin 1; HDL, high density lipoproteins

In accordance with the association observed between insulin resistance and endothelial dysfunction, it has been suggested that agents that increase sensitivity to insulin may also improve endothelial function. Pasceri et al[142] showed that troglitazone (activator of the proliferator-activated receptor gamma) inhibits *in vitro* the expression of VCAM-1 and ICAM-1 in activated endothelial cells. Based on this and other studies,[143-146] clinical trials were performed to study the nonhypoglycemic effects of these new oral antibodies that showed a beneficial effect on endothelial function,[147-149] at least in the short term. Nevertheless, at present, there is no agreement among all researchers authors, as one well-designed study has yielded conflicting results in this regard.[150]

## CONCLUSIONS

Although the long-term prognosis for patients with syndrome X is generally benign, their quality of life frequently deteriorates for years due to the persistence of episodes of precordial pain. In addition, hospital readmissions and repeated diagnostic tests result in a significant consumption of resources.

The physiopathology of syndrome X involves a variety of mechanisms that vary from patient to patient. Among these, microvascular endothelial dysfunction may explain the precordial pain and the electrocardiographic changes in at least 1 subgroup of patients. More importantly, it has recently been shown that endothelial dysfunction in patients with non-obstructive heart disease is a marker for the risk of acute episodes.[151] Nevertheless, the question as to whether this endothelial dysfunction is the cause of myocardial ischemia continues to be controversial, as is whether the ischemia plays a role in the physiopathology of the

syndrome.[152]

A number of authors have investigated the reversibility of endothelial function. It has been shown that short-term treatment with ACEI improves endothelial function in patients with known atherosclerosis. In patients with type 2 diabetes with insulin resistance, studies that evaluated improvement in endothelial function with ACEI have yielded conflicting results. Likewise, in patients with hypercholesterolemia with and without heart disease, an improvement in endothelial function has been reported following hypolipemiant treatment. Replacement hormone therapy and new antidiabetic drugs may also be useful in the treatment of endothelial dysfunction in the cardiovascular syndrome X.

## REFERENCES


1. Kaski JC. Cardiac syndrome X and microvascular angina. In: Kaski JC, editor. Chest pain with normal coronary angiograms. Pathogenesis, diagnosis and management. Massachusetts: Norwell Kluwer Academic Publishers, 1999; p. 1-12.
2. Kaski JC, Russo G. Cardiac syndrome X: an overview. Hosp Pract 2000;35:75-94.
3. Kaski JC, Pérez Fernández R. Angina microvascular y síndrome X. Rev Esp Cardiol 2002;55:10-6.
4. Kemp HG. Left ventricular function in patients with the anginal syndrome and normal coronary arteriograms. Am J Cardiol 1973;32:375-6.
5. Cannon RO, Epstein SE. «Microvascular angina» as a cause of chest pain with angiographically normal coronary arteries. Am J Cardiol 1988;62:1338-43.
6. Motz W, Vogt M, Rabenau O, Scheler S, Luckhoff A, Strauer BE. Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiograms. Am J Cardiol 1991;68:996-1003.
7. Egashira K, Inou T, Hirooka Y, Yamada A, Urabe Y, Takeshita A. Evidence of impaired endothelium-dependent coronary vasodilatation in patients with angina pectoris and normal coronary angiograms. N Engl J Med 1993;328:1659-64.
8. Quyyumi AA, Cannon RO, Panza JA, Diodati JG, Epstein SE. Endothelial dysfunction in patients with chest pain and normal coronary arteries. Circulation 1992;86:1864-71.
9. Bøtker HE, Sonne HS, Sorensen KE. Frequency of systemic microvascular dysfunction in syndrome X and in variant angina. Am J Cardiol 1996;78:182-6.
10. Lekakis JP, Papamichael CM, Vemmos CN, Voutsas AA, Stanatelopoulos SF, Moulopoulos SD. Peripheral vascular endothelial dysfunction in patients with angina pectoris and normal coronary arteriograms, J Am Coll Cardiol 1998;31:541-6.
11. Bellamy MF, Goodfellow J, Tweddel AC, Dunstan FDJ, Lewis MJ, Henderson AH. Syndrome X and endothelial function. Cardiovasc Res 1998;40:410-7.
12. Buus NH, Bøttcher M, Bøtker HE, Sørensen KE, Nielsen TT, Mulvany MJ. Reduced vasodilator capacity in syndrome X related to structure and function of resistance arteries. Am J Cardiol 1999;83:149-54.
13. Camici PG, Marraccini P, Lorenzoni R, Buzzigoli G, Pecori N, Perissinotto A, et al. Coronary hemodinamics and myocardial metabolism in patients with syndrome X: response to pacing stress. J Am Coll Cardiol 1991;17:1461-70.
14. Nihoyannopoulos P, Kaski JC, Crake T, Maseri A. Absence of myocardial dysfunction during stress in patients with syndrome X. J Am Coll Cardiol 1991;18:1463-70.
15. Panza JA, Laurienzo JM, Curiel RV, Unger EF, Quyyumi AA, Dilsizian V, et al. Investigation of mechanism of chest pain in patients with angiographically normal coronary arteries using transesophageal dobutamine stress echocardiography. J Am Coll Cardiol 1997;29:293-301.
16. Bøtker HE, Sonne HS, Bagger JP, Nielsen TT. Impact of impaired coronary flow reserve and insulin resistance on myocardial energy metabolism in patients with syndrome X. Am J Cardiol 1997;79:1615-22.
17. Cannon RO, Curiel RV, Prasad A, Quyyumi AA, Panza JA. Comparison of coronary endothelial dynamics with electrocardiographic and left ventricular contractile responses to stress in the absence of coronary artery disease. Am J Cardiol 1998;82: 710-4.
18. Zouridakis EG, Cox ID, García-Moll X, Brown S, Nihoyannopoulos P, Kaski JC. Negative stress echocardiographic response in normotensive and hypertensive patients with angina pectoris, positive exercise stress testing, an normal coronary arteriograms. Heart 2000;83:141-6.
19. Cannon RO III, Bonow RO, Bacharach SL, Green MV, Rosing DR, Leon MB. Left ventricular dysfunction in patients with angina pectoris, normal epicardial coronary arteries, an abnormal vasodilator reserve. Circulation 1985;71:218-26.
20. Yoshio H, Shimizu M, Kita Y, Ino H, Taki J, Takeda R. Left ventricular functional reserve in patients with syndrome X: evaluation by continuous ventricular function monitoring. J Am Coll Cardiol 1993;22:1465-9.
21. Zeiher AM, Krause T, Schachinger V, Minners J, Moser E. Impaired endothelium dependent vasodilatation of the coronary resistance vessels is associated with exercise-induced myocardial ischemia. Circulation 1995;91:2345-52.
22. Hasdai D, Gibbons RJ, Holmes DR, Higano ST, Lerman A. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. Circulation 1997;96: 3390-5.
23. Maseri A, Crea F, Kaski JC, Crake T. Mechanisms of angina pectoris in syndrome X. J Am Coll Cardiol 1991;17:499-506.
24. Lanzarini L, Previtali M, Fetiveau R, Poli A. Results of dobutamine stress echocardiography in patients with syndrome X. Int J Card Imaging 1994;10:145-8.
25. Picano E, Lattanzi F, Masini M, Distante A, L'Abbate A. Usefulness of a high dose dipyridamole echocardiography test for diagnosis of syndrome X. Am J Cardiol 1987;60:508-12.
26. Panza JA, Laurienzo JM, Curiel RV, Unger EF, Quyyumi AA, Dilsizian V, et al. Investigation of the mechanism of chest pain in patients with angiographically normal coronary arteries using transesophageal dobutamine stress echocardiography. J Am Coll Cardiol 1997;29:293-301.
27. Crake T, Canepa-Anson R, Shapiro L, Poole-Wilson PA. Continuous reading of coronary sinus oxygen saturation during atrial pacing in patients with coronary artery disease or syndrome X. Br Heart J 1988;59:31-8.
28. Hutchinson SJ, Poole-Wilson PA, Henderson AH. Angina with normal coronary arteries: a review. Q J Med 1989;72:677-88.
29. Rosano GM, Kaski JC, Arie S, Pereira WI, Horta P, Collins P, et al. Failure to demostrate myocardial ischaemia in patients with angina and normal coronary arteries. Evaluation by continuous coronary sinus pH monitoring and lactate metabolism. Eur Heart J 1996;17:1175-80.
30. Buffon A, Rigattieri S, Santini SA, Ramazzotti V, Crea F, Giardina B, et al. Myocardial ischemia-reperfusion damage after pacing-induced tachycardia in patients with cardiac syndrome X. Am J Physiol Heart Circ Physiol 2000;279:H2627-H33.
31. Buchthal SD, Den Hollander JA, Merz CNB, Rogers WJ,

Pepine CJ, Reichek N, et al. Abnormal myocardial phosphorus-31 nuclear magnetic resonance spectroscopy in women with chest pain but normal coronary angiograms. N Engl J Med 2000; 342:829-35.
32. Lowenstein CJ, Dinerman JL, Snyder SH. Nitric oxide: a physiologyc messenger. Ann Intern Med 1994;120:227-37.
33. Shah AM, Grocott-Mason RM, Pepper CB, Mebazaa A, Henderson AH, Lewis MJ, et al. The cardiac endothelium: cardioactive mediators. Prog Cardiov Dis 1996;39:263-84.
34. Quyyumi AA, Dakak N, Andrews NP, Gilligan DM, Panza JA, Cannon RO. Contribution of nitric oxide to metabolic coronary vasodilatation in the human heart. Circulation 1995;92: 320-6.
35. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation 2002;105:546-9.
36. Le Cras TD, Xue C, Rengasamy A, Johns RA. Chronic hipoxia upregulates endothelial an inducible NO synthasa gene and protein expression in rat lung. Am J Physiol 1996;270:L164-70.
37. López-Farré A, Sánchez de Miguel L, Caramelo C, Gómez-Macías J, García R, Mosquera JR, et al. Role of nitric oxide in the autocrine control of growth and apoptosis of endothelial cells. Am J Physiol 1997;272:H760-H8.
38. Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise increases coronary vascular nitric oxide production and endothelial nitric oxide synthase gene expression. Circ Res 1994;74: 349-53.
39. Yoshizumi M, Perella MA, Burnett JC, Lee ME. Tumor necrosis factor downregulates and endothelial nitric oxide synthase mRNA by shortening of its half-life. Circ Res 1993;73: 205-9.
40. Mohamed F, Mongue JC, Gordon A, Cernacek P, Blais D, Stewart DJ. Lack of role for nitric oxide (NO) in the selective destabilization of endothelial NO synthase mRNA by tumor necrosis factor-α. Arterioscler Thromb Vasc Biol 1995;15:52-7.
41. Sánchez de Miguel L, Alonso J, González-Fernández F, De la Osada J, Montón M, Rodríguez-Feo JA, et al. Evidence that an endothelial cytosolic protein binds to the 3'-untranslated region of endothelial nitric oxide synthase mRNA. J Vasc Res 1999;36: 201-8.
42. Alonso J, Sánchez de Miguel L, Montón M, Casado S, López-Farré A. Endothelial cytosolic proteins bind to the 3'-untranslated region of endothelial nitric oxide synthase mRNA: regulation by tumor necrosis factor alpha. Moll Cell Biol 1997;17: 5719-26.
43. Epstein SE, Cannon RO III. Site of increased resistance to coronary flow in patients with angina pectoris and normal epicardial coronary arteries. J Am Coll Cardiol 1986;8:459-61.
44. Cannon RO III, Schenke WH, Leon MB, Rosing DR, Urqhart J, Epstein SE. Limited coronary flow reserve after dipyridamole in patients with ergonovine-induced vasoconstriction. Circulation 1987;75:163-74.
45. Tousoulis D, Davies GJ, Asimakopoulos G, Homaei H, Zouridakis E, Ahmed N, et al. Vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 serum level in patients with chest pain and normal coronary arteries (syndrome X). Clin Cardiol 2001;24:301-4.
46. Bøtker HE, Ingerslev J. Plasma concentrations of von Willebrand factor in patients with angina pectoris secondary to coronary atherosclerosis or cardiac syndrome X. Thromb Res 2000; 97:519-23.
47. Geltmen EM, Henes CG, Senneff MJ, Sobel BE, Bergman SR. Increased myocardial perfusion at rest and diminished perfusion reserve in patients with angina and angiographically normal coronary arteries. J Am Coll Cardiol 1990;16:586-95.
48. Galassi AR, Crea F, Araujo LI, Lammertsma AA, Pupita G, Yamamoto Y. Comparison of regional myocardial blood flow in syndrome X and one-vessel coronary artery disease. Am J Cardiol 1993;72:134-9.
49. Rosen SD, Osen NG, Kaski JC, Tousoulis D, Davies GS, Camici PG. Coronary vasodilator reserve, pain perception, and sex in patients with syndrome X. Circulation 1994;90:50-60.
50. Meeder JG, Blanksma PK, Van der Wall EE, Willemsen TM, Pruim J, Anthonio RL, et al. Coronary vasomotion in patients with syndrome X: evaluation with positron emission tomography and parametric myocardial perfusion imagin. Eur J Nucl Med 1997;24:530-7.
51. Bøttcher M, Bøtker HE, Sonne H, Nielsen TT, Czernin J. Endothelium dependent and independent perfusion reserve and the effect of L-arginine on myocardial perfusion in patients with syndrome X. Circulation 1999;99:1795-801.
52. Bøtker HE, Moller N, Ovensen P, Mengel A, Schitz O, Orskov H, et al. Insuline resistance in microvascular angina (syndrome X). Lancet 1993;342:136-40.
53. Egashira K, Katsuda Y, Mohri M, Kuga T, Tagawa T, Kubota T, et al. Role of endothelium-derived nitric oxide in coronary vasodilation induced by pacing tachycardia in humans. Circ Res 1996;79:331-5.
54. Egashira K, Hirooka Y, Kuga T, Mohri M, Takeshita A. Effect of L-arginina supplementation on endothelium-dependent coronary vasodilation in patients with angina pectoris and normal coronary arteriograms. Circulation 1996;94:130-4.
55. Masaki T. The discovery of endothelins. Cardiovasc Res 1998;39:530-3.
56. Levin ER. Mechanisms of disease: endothelins. N Engl J Med 1995;333:353-63.
57. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332: 411-5.
58. Rubanyi GM. Maintenance of «basal» vascular tone may represent a physiological role for endothelin. J Vasc Med Biol 1989; 1:315-6.
59. Luscher TF, Richard V, Tschudi M, Yang ZH, Boulanger C. Endothelial control of vascular tone in large and small coronary arteries. J Am Coll Cardiol 1990;15:519-27.
60. Miyauchi T, Tomobe Y, Shiba R, Ishihawa T, Yanagisawa M, Kimura S, et al. Involvement of endothelin in the regulation of human vascular tonus. Potent vasoconstrictor effect and existence in endothelial cells. Circulation 1990;81;1874-80.
61. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of cDNA encoding en endothelin receptor. Nature 1990;348:730-2.
62. Neubauer S, Ertl G, Haas U, Pulzer F, Kochsiek K. Effects of endothelin-1 in isolated perfused rat heart. J Cardiovasc Pharmacol 1990;16:1-8.
63. Clozel M, Gray GA, Breu V, Loffler BM, Osterwalder R. The endothelin ETB receptor mediates both vasodilation and vasoconstriction *in vivo.* Biochem Biophys Res Commun 1992;186: 867-73.
64. Hirata Y, Emori T, Eguchi S, Kanno K, Imai T, Ohta K, et al. Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J Clin Invest 1993;91: 1367-73.
65. Shetty SS, Okada T, Webb RL, DelGrande D, Lappe RW. Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. Biochem Biophys Res Commun 1993;191: 459-64.
66. Nakamura S, Naruse M, Naruse K, Demura H, Uemura H. Immunocytochemical localization of endothelin in cultured bovine endothelial cells. Histochemistry 1990;94:475-7.
67. Miyauchi T, Yanagisawa M, Tomizawa T, Sugishita Y, Suzuki N, Fujino M, et al. Increased plasma concentrations of endothelin-1 and big endothelin in acute myocardial infarction. Lancet 1989;2:53-4.
68. Rubanyi GM, Vanhoutte PM. Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. J Physiol (Lond) 1985;364:45-56.
69. Yoshizumi M, Kurihara H, Sugiyama T, Takaku F, Yanagisawa

M, Masaki F, et al. Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. Biochem Biophys Res Commun 1989;161:859-64.
70. Malek AM, Izumo S. Control of endothelial cell gene expression by flow. J Biomech 1995;28:1515-28.
71. Omland T, Lie RT, Askvaag A, Aarsland T, Dickstein K. Plasma endotheline determination as a prognosis indicator of 1-year mortality after acute myocardial infarction. Circulation 1994;89: 1573-9.
72. Salomone O, Elliott PM, Calvino R, Holt D, Kaski JC. Plasma inmunoreactive endothelin concentration correlates with severity of coronary artery disease in patients with stable angina pectoris and normal ventricular function. J Am Coll Cardiol 1996; 28:14-9.
73. Wieczorek I, Haynes WG, Webb DJ, Ludlam CA, Fox KAA. Raised plasma endothelin levels in unstable angina and non-Q wave myocardial infarction: relation to cardiovascular outcome. Br Heart J 1994;72:436-41.
74. Bogaty P, Hackett D, Davies G, Maseri A. Vasoreactivity of the culprit lesion in unstable angina. Circulation 1994;90:5-11.
75. Pacher R, Bergler-Klein J, Globits S, Teufelsbauer H, Schuller M, Krauter A, et al. Plasma big endothelin-1 concentrations in congestive heart failure patients with or without systemic hypertension. Am J Cardiol 1993;71:1293-9.
76. Wei CM, Lerman A, Rodeheffer RJ, Mcgregor CG, Brandt RR, Wright S, et al. Endothelin in human congestive heart failure. Circulation 1994;89:1580-6.
77. Stewart DJ, Cernacek P, Costello KB, Rouleau JL. Elevated endothelin-1 in heart failure and loss of normal response to postural change. Circulation 1992;85:510-7.
78. Toyo-oka T, Aizawa T, Suzuki N, Hirata Y, Shin WS, Yanagisawa M, et al. Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris. Circulation 1991;83:476-83.
79. Artigou JY, Salloum J, Carayon A, Lechat P, Maistre G, Isnard R, et al. Variations in plasma endothelin concentrations during coronary spasm. Eur Heart J 1993;14:780-4.
80. Smith TP, Zhang L, Gugino SF, Russell JA, Canty JM. Differential effect of endothelin on coronary conduit and resistance arteries. Circulation 1995;92 (Suppl):I30.
81. Dashwood MR, Timm M, Kaski JC. Regional variations in ETA/ETB binding sites in human coronary vasculature. J Cardiovasc Pharmacol 1995;26:351-4.
82. Cox ID, Salomone O, Brown SJ, Hann C, Kaski JC. Serum endothelin levels and pain perception in patients with cardiac syndrome X and in healthy controls. Am J Cardiol 1997;80: 637-40.
83. Cox ID, Botker HE, Bagger JP, Sonne HS, Kristensen BO, Kaski JC. Elevated endothelin concentrations are associated with reduced coronary vasomotor responses in patients with chest pain and normal coronary arteriograms. J Am Coll Cardiol 1999;34: 455-60.
84. Kaski JC, Cox ID, Crook JR, Salomone OA, Fredericks S, Hann C, et al. Differential plasma endothelin levels in subgroups of patients with angina and angiographically normal coronary arteries. Am Heart J 1998;136:412-7.
85. Lanza GA, Lüscher TF, Pasceri V, Shaw SG, Buffon A, Montenero AS, et al. Effects of atrial pacing on arterial and coronary sinus endothelin-1 levels in syndrome X. Am J Cardiol 1999;84: 1187-91.
86. Newby DE, Flint LL, Fox KA, Boon NA, Webb DJ. Reduced responsiveness to endothelin-1 in peripheral resistance vessels of patients with syndrome X. J Am Coll Cardiol 1998;31:1585-90.
87. Desideri G, Gaspardone A, Gentile M, Santucci A, Gioffrè PA, Ferri C. Endothelial activation in patients with cardiac syndrome X. Circulation 2000;102:2359-64.
88. Opherk D, Schuler G, Wetterauer K, Manthey J, Schwartz F, Kubler W. Four year follow-up study in patients with angina and normal coronary arteriograms («syndrome X»). Circulation 1989;80:1610-9.
89. Greemberg MA, Grose RM, Neuberger N, Silverman R, Strain JE, Cohen MV. Impaired coronary vasodilator responsiveness as a cause of lactate production during pacing-induced ischemia in patients with angina pectoris and normal coronary arteries. J Am Coll Cardiol 1987;9:743-51.
90. Sonnenblick EH, Fein S, Capasso JM, Factor SM. Microvascular spasm as a cause of cardiomyopathies and the calcium channel blocking agent verapamil as a potential primary therapy. Am J Cardiol 1985;55:179-84.
91. Cox ID, Schwartzman RA, Atienza F, Brown SJ, Kaski JC. Angiographic progression in patients with angina pectoris and normal or near normal coronary angiograms who are restudied due to unstable symptoms. Eur Heart J 1998;19:1027-33.
92. Kaski JC, Crea P, Nihoyannopoulos P, Hackett D, Maseri A. Transient myocardial ischemia during daily life in patients with syndrome X. Am J Cardiol 1986;58:1242-7.
93. Clarke JG, Davies GJ, Kerwin R, Hackett D, Larkin S, Dawbarn, et al. Coronary artery infusion of neuropeptide Y in patients with angina pectoris. Lancet 1987;1:1057-9.
94. Mohri M, Koyanagi M, Egashira K, Tagawa H, Ichiki T, Shimokawa H, et al. Angina pectoris caused by coronary microvascular spasm. Lancet 1998;351:1165-9.
95. Murakami H, Urabe K, Nishimura M. Inappropiate microvascular constriction produced transient ST-segment elevation in patients with syndrome X. J Am Coll Cardiol 1998;32:1287-94.
96. Drexler H. Endothelial dysfunction: clinical implications. Prog Cardiovasc Dis 1997;4:287-324.
97. Zeiher AM, Drezler H, Wollschlager H, Just H. Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 1991;83:391-401.
98. Egashira K, Inou T, Hirooka Y, Yamada A, Maruoka Y, Kai H, et al. Impaired coronary blood flow response to acetylcholine in patients with coronary risk factors and proximal atherosclerotic lesions. J Clin Invest 1993;91:29-37.
99. Zeiher AM, Drexler H, Saurbier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. J Clin Invest 1993;92:652-62.
100. Quyyumi AA, Dakak N, Andrews NP, Husain S, Arora S, Gilligan DM, et al. Nitric oxide activity in the human coronary circulation: impact of risk factors for coronary atherosclerosis. J Clin Invest 1995;95:1747-55.
101. Quillen JE, Rossen JD, Oskarsson HJ, Minor RL, López AG, Winnford MD. Acute effect of cigarette smoking on the coronary circulation: constriction of epicardial and resistance vessels. J Am Coll Cardiol 1993;22:642-7.
102. Quyyumi AA, Mulcahy D, Andrews NP, Panza JA, Cannon RO. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia. Comparison of acetylcholine and substance P. Circulation 1997;95:104-10.
103. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481-7.
104. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 1999;33:1506-11.
105. Bijlstra PJ, Smits P, Lutterman JA, Thien T. Effect of long-term angiotensin-converting enzyme inhibition on endothelial function in lpatients with the insulin-resistance syndrome. J Cardiovasc Pharmacol 1995;25:658-64.
106. Egashira K, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Inou T, et al. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 1994;89:2519-24.
107. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower

ME, Koisinski AS, et al. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481-7.
108. Anderson TJ, Meredith IT, Yeug AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy in the endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488-93.
109. Iraculis E, Cequier A, Sabaté M, Pintó X, Gómez-Hospital JA, Mauri J, et al. Mejoría de la función endotelial al reducir las concentraciones lipídicas en pacientes con hipercolesterolemia y arterias coronarias normales. Rev Esp Cardiol 2001;54:685-92.
110. Leung WH, Lau CP, Wong CK. Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolemic patients. Lancet 1993;341:1496-500.
111. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing Endothelial Dysfunction) Study. Circulation 1996;94: 258-65.
112. Kaski JC, Rosano GM, Collins P, Nihoyannopoulos P, Maseri A, Poole-Wilson PA. Cardiac syndrome X: clinical characteristics and left ventricular function. Long term follow-up study. J Am Coll Cardiol 1995;25:807-14.
113. Taddei S, Virdis A, Ghiadoni L, Mattei P, Sudano I, Bernini G, et al. Menopause is associated with endothelial dysfunction in women. Hypertension 1996;28:576-82.
114. Rosano GM, Collins P, Kaski JC, Lindsay DC, Sarrel PM, Poole-Wilson PA. Syndrome X in women is associated with estrogen deficiency. Eur Heart J 1995;16:610-4.
115. Roque M, Heras M, Roig E, Masotti M, Rigol M, Betriu A, et al. Short-term effect of transdermal estrogen replacement therapy on coronary vascular reactivity in postmenopausal women with angina pectoris and normal coronary angiograms. J Am Coll Cardiol 1998;31:139-43.
116. Herrintong DM, Werbel BL, Riley WA, Pusser BE, Morgan TM. Individual and combined effects of estrogen/progestin therapy and lovastatin on lipids and flow mediated vasodilatation in postmenopausal women with coronary artery disease. J Am Coll Cardiol 1999;33:2030-7.
117. Lieberman EH, Gerhard MD, Uehata A, Walsh BW, Selwyn AP, Ganz P, et al. Estrogen improves endothelium-dependent, flow-mediated vasodilation in postmenopausal women. Ann Int Med 1994;121:936-41.
118. Gerhard M, Walsh BW, Tawaakol A, Haley EA, Creager SJ, Seely EW, et al. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation 1998;98:1158-63.
119. De Kleijn MJ, Wilmink HW, Bots ML, Bak AA, Van der Schouw YT, Planellas J, et al. Hormone replacement therapy and endothelial function. Results of a randomized controlled trial in healthy postmenopausal women. Atherosclerosis 2001; 159:357-65.
120. Webb CM, Ghatei MA, McNeill JG, Collins P. 17 beta-estradiol decreases endothelin-1 levels in the coronary circulation of postmenopausal women with coronary artery disease. Circulation 2000;102:1617-22.
121. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
122. Dean JD, Jones CJ, Hutchison SJ, Peters JR, Henderson AH. Hyperinsulinaemia and microvascular angina («syndrome X»). Lancet 1991;337:456-7.
123. Bøtker HE, Moller N, Ovesen P, Mengel A, Schmitz O, Orskov H, et al. Insulin resistance in microvascular angina (syndrome X). Lancet 1993;342:136-40.
124. Goodfellow J, Owens DR, Henderson AH. Cardiovascular syndromes X, endothelial dysfunction and insulin resistance. Diabetes Research Clinical Practice 1996;31(Suppl):163-71.
125. Piatti PM, Fragasso G, Monti LD, Caumo A, Van Phan C, Valsecchi G, et al. Endothelial and metabolic characteristics of patients with angina and angiographically normal coronary arteries. J Am Coll Cardiol 1999;34:1452-60.
126. Piatti PM, Monti LD, Conti M, Baruffaldi L, Galli L, Phan CV, et al. Hypertrigliceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. Diabetes 1996;45: 316-21.
127. Koren W, Koldanov R, Peleg E, Rabinowitz B, Rosenthal T. Enhanced red cell sodium hydrogen exchange in microvascular angina. Eur Heart J 1997;18:1296-9.
128. Gaspardone A, Ferri C, Crea F, Versaci F, Tomai F, Santucci A, et al. Enhanced activity of sodium-lithium countertransport in patients with cardiac syndrome X. A potential link between cardiac and metabolic syndrome X. J Am Coll Cardiol 1998;32: 2031-4.
129. Baron AD. The coupling of glucose metabolism and perfusion in human skeletal muscle. The potencial role of endothelium-derived nitric oxide. Diabetes 1996;45(Suppl 1):105-9.
130. Nagi DK, Hendra TJ, Ryle AJ, Cooper TM, Temple RC, Clark PM, et al. The relationships of concentrations of insulin, intact proinsulin, and 32-33 split proinsulin with cardiovascular risk factors in type II (no-insulin-dependent) diabetic subjects. Diabetologia 1990;33:532-7.
131. Haffner SM, Mykkanen L, Valdez RA, Stern MP, Holloway DL, Monterrosa A, et al. Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome. J Clin Endocrinol Metab1994;79:1806-10.
132. Henderson AH. Endothelial dysfunction: a reversible clinical measure of atherogenic susceptibility and cardiovascular inefficiency. Int J Cardiol 1997;62(Suppl 1):43-8.
133. Utriainen T, Nuutila P, Takala T, Vicini P, Ruotsalainen U, Ronnemaa T, et al. Intact insulin stimulation of skeletal muscle blood flow, its heterogeneity and redistribution, but not of glucose uptake in non-insulin-dependent diabetes mellitus. J Clin Invest 1997;100:777-85.
134. Bøtker HE, Møller N, Schmitz O, Bagger JP, Nielsen TT. Myocardial insulin resistance in patients with syndrome X. J Clin Invest 1997;100:1919-27.
135. Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, et al. Insulin causes endothelial dysfunction in humans. Sites and mechanisms. Circulation 2002;105:576-82.
136. Cardillo C, Nambi SS, Kilkoyne CM, Choucair WK, Katz A, Michael J, et al. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. Circulation 1999;100:820-5.
137. Duerrschmidt N, Wippich N, Goettsch W, Broemme HJ, Morawietz H. Endothelin-1 induces NAD(P)H oxidase in human endothelial cells. Biochem Biophys Res Commun 2000;269:713-7.
138. Kashiwagi A, Shinozaki K, Nishio Y, Maegawa H, Maeno Y, Kanazawa A, et al. Endothelium-specific activation of NAD (P)H oxidase in aortas of exogenusly hyperinsulinemic rats. Am J Physiol 1999;277:E976-83.
139. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev 2001;22:36-52.
140. García-Soriano F, Virág L, Jagtap P, Szabó E, Mabley JG, Liaudet L, et al. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. Nature Med 2001;7: 108-13.
141. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787-90.
142. Pasceri V, Wu HD, Willerson JT, Yeh ET. Modulation of vascular inflamation in vitro and in vivo by peroxisome proliferator-activated-receptor-g activators. Circulation 2000;101:235-8.
143. Cominacini L, Garbin U, Fratta Pasini A, Campagnola M, Davoli A, Foot E, et al. Troglitazone reduces LDL oxidation and lowers plasma E-selectin concentration in NIDDM patients. Diabetes 1998;47:130-3.

144. Cominacini L, Garbin U, Pastorino AM, Campagnola M, Fratta Pasini A, Davoli A, et al. Effects of troglitazone on *in vitro* oxidation of LDL and HDL induced by copper ions and endothelial cells. Diabetologia 1997;40:165-72.
145. Jackson SM, Parhami F, Xi XP, Berliner JA, Hsueh WA, Law RE, et al. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. Arterioscler Thromb Vasc Biol 1999;19:2094-104.
146. Fukunaga Y, Itoh H, Doi K, Tanaka T, Yamashita J, Chun TH, et al. Thiazolidinediones, peroxisome proliferator-activated receptor gamma agonists, regulate endothelial cell growth and secretion of vasoactive peptides. Atherosclerosis 2001;158: 113-9.
147. Murakami T, Mizuno S, Ohsato K, Moriuchi I, Arai Y, Nio Y, et al. Effects of troglitazone on frequency of coronary vasospastic-induced angina pectoris in patients with diabetes mellitus [abstract A8]. Am J Cardiol 1999;84:92.
148. Avena R, Mitchell ME, Nylen ES, Curry KM, Sidawy AN. Insulin action enhancement normalizes brachial artery vasoactivity in patients with peripheral vascular disease and occult diabetes. J Vasc Surg 1998;28:1024-31.
149. Watanabe Y, Sunayama S, Shimada K, Sawano M, Hoshi S, Iwama Y, et al. Troglitazone improves endothelial dysfunction in patients with insulin resistance. J Atheroscler Thromb 2000;7: 159-63.
150. Tack CJ, Ong MK, Lutterman JA, Smits P. Insulin-induced vasodilation and endothelial function in obesity/insulin resistance. Effects of troglitazone. Diabetologia 1998;41:569-76.
151. Suwaidi JA, Hamasaki S, Higani ST, Nishimura RA, Holames DR, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000:101:948-54.
152. Simón A, Castro A, Kaski JC. Avances en el conocimiento de la disfunción endotelial y su aplicación en la práctica clínica. Rev Esp Cardiol 2001;54:211-7.