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Hemostasis alterations in metabolic syndrome (Review)

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Abstract. Metabolic syndrome (MS) is characterized by the presence of at least three of the following alterations: enlargement of the waist diameter, higher levels of arterial pressure, low density lipoprotein cholesterol and glycemia, and reduction of high density lipoprotein cholesterol. The prevalence of MS reaches 23% in young adults, a percentage that increases with age. People with MS have a greater risk of suffering from cardiovascular disease (CVD). The physiopathologic alterations now found to exist in MS are diverse; among them is endothelial dysfunction, which triggers atherogenic lesions and hypercoagulability characterized by alterations of the coagulation factors and the regulatory proteins of fibrinolysis such as the plasminogen activator inhibitor (PAI-1). The increase in oxidative stress and/or the reactive oxygen species in patients with MS is partially related to the oxidation state of the lipoproteins, especially of the low density lipoproteins. This fact favors atherogenesis. Moreover, the oxidative stress produces alterations in the production of adipokines, cytokines secreted by the adipose tissues. The abnormality in the transport of lipoprotein diminishes the catabolism of the very low density lipoprotein (VLDL) and increases the catabolism of the high density lipoprotein (HDL), which creates insulin resistance. This process is associated with a lower concentration of adiponectin that in turn regulates the catabolism of VLDL and HDL; consequently increasing the flow of fatty acids from the adipose tissue to the liver and muscles. The proinflammatory cytokines, among them tumor necrosis factor α (TNF-α), are of great importance in MS regulating different processes and molecules such as PAI-1. PAI-1 is controlled by the group of transcription factors peroxisome proliferator-activated receptor (PPAR), especially by PPAR γ and α ligands. In summary, MS includes multiple alterations related to insulin resistance at several levels: hepatic, muscular, adipose and vascular tissue (endothelium). The exact mechanism that underlies the relationship between MS and CVD are not sufficiently known yet; pathogenic explanations are lacking for the mechanisms relating metabolic factors to insulin resistance and the association with the development of atherosclerosis and thrombosis. MS alterations and the main aspects related to homeostasis alterations are examined in this report.

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1. General background

Cardiovascular disease (CVD) is the main cause of death in the world representing around 30% of all deaths (1). It also constitutes an important factor in morbidity and incapacity (2). There are several CVD risk factors, both classic and emergent (3). Metabolic syndrome (MS) is characterized by a series of alterations that increase endothelial damage and atherothrombosis (4-6). MS includes multiple alterations related to insulin resistance involving hepatic, muscular, vascular (endothelial) and fatty tissue (7). The exact mechanisms of MS and CVD are not yet sufficiently well-known; they lack pathogenic explanations that relate the metabolic factors to insulin resistance, atherosclerosis development and thrombosis (risk of CVD).

The National Cholesterol Education Program (NCEP), in the year 2001, redefined the diagnostic approaches to MS in the Adult Treatment Panel III (ATP III) demanding the presence of at least 3 of the 5 following conditions (8): abdominal obesity (waist circumference: men, > 40 inches; women, >35 inches), hypertriglyceridemia (>150 mg/dl), low levels of HDL (high density lipoprotein) cholesterol (c-HDL) (<40 mg/dl), arterial hypertension (>130/85 mm Hg), and hyperglycemia (>100 mg/dl). Besides these conditions, other alterations have been described for MS: hyperuricemia, coagulation alterations and high levels of plasminogen activator inhibitor type 1 (PAI-1).

The prevalence of MS varies according to the definition used to determine it, as well as age, sex, ethnic origin and lifestyle. In Chile, the National Survey of Health (2003) showed a prevalence of 22.6%, without differences between men and women, but differing according to age: 17-24 years (4.6%),

25-44 years (17.9%), 45-64 years (36.5%) and over 65 years (48%).

Individuals with glucose intolerance, abdominal fat, hypertriglyceridemia, and low levels of c-HDL have a greater risk of suffering CVD (9-11). This group of factors is part of MS, which is also denominated syndrome X, insulin resistance syndrome, cardiovascular dysmetabolic syndrome and plurimetabolic syndrome (12-14). A high risk of CVD is associated with the rapid development of atherosclerosis which favors thrombosis (4-6).

Obesity is associated with an increase in free fatty acids and subsequently with increased glucogenolysis and hepatic gluconeogenesis, which leads to hyperglycemia. This in turn provokes hyperinsulinemia and, subsequently, insulin resistance.

Fatty tissue is at the moment considered an endocrine tissue that secretes several adipokines. Among these cytokines secreted by the adipose are adiponectin, leptin, resistin, tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), IL-6 and PAI-1. These molecules regulate metabolic aspects of carbohydrates and lipids, the endothelium, inflammatory processes, hemostasis, and others (15,16). Thus, for example, some increase the secretion of proatherogenic cytokines, such as TNF-α, while others such as adiponectin lower the synthesis of other cytokine protectors (7).

From a physiopathologic point of view, MS is characterized by dyslipidemia, endothelial dysfunction and alteration of coagulation or fibrinolysis.

2. Endothelial dysfunction

The vascular endothelium synthesizes vasoactive peptides, growth factors and cytokines. It regulates the balance between vasoconstriction and vasodilation, coagulation and fibrinolysis, platelet adhesion and aggregation, proliferation and apoptosis, and leukocyte adhesion and diapedesis (17). MS is associated with changes in the proliferation of smooth musculature cells and endothelial dysfunction (18). The endothelium is thus an important point of union between CVD and the metabolic pathways because hyperinsulinemia favors endothelial dysfunction (19) with which atherosclerosis is associated (20,21).

Some cytokines, as previously indicated, favor endothelial function (22). The TNF-α that is increased in MS (23) inhibits lipoproteinlipase action, and activates oxidative stress and the high synthesis of proteins at the acute phase (24). IL-6 inhibits insulin signaling and activates endothelial cells (25), thus moderating inflammation; it also participates in the immune response and the increased expression of tissue factor (TF) in monocytes (26).

Cellular adhesion molecules are secreted on the surface of endothelial cells (EC) and, thus, can bind leukocytes which cross the intima (27,28). VCAM-1 (vascular cell adhesion molecule-1) is not expressed constituently on the endothelium, but it can be regulated *in vitro* in response to TNF-α, IL-4 and interferon-γ (IFN-γ), cytokines that are synthesized by the adipose as well as other tissues. Soluble VCAM-1 (sVCAM-1) is in the serum of healthy people and an elevation of this is observed in such pathologies as autoimmune diseases, infections and inflammatory processes (29), and in patients with non-compensated hypertension (30). ICAM-1 (intercellular adhesion molecule-1) is expressed in EC when they are stimulated with IL-1, TNF-α and IFN-γ (31). E-Selectin is expressed in EC only after activation by inflammatory cytokines such as IL-1ß, TNF-α or endotoxines.

Nitric oxide is a vasodilation molecule synthesized by the EC. The insulin increases the synthesis of endothelial NO synthase (eNOS), and the bioavailability of NO also increases, which is translated into an antiatherogenic action (32). Endothelial damage diminishes the synthesis of NO.

Endothelin-1 (ET-1) is synthesized by the endothelium, monocytes and macrophages; it produces vasoconstriction (33). It is increased in obesity, insulin resistance (34,35), diabetes mellitus (36), HTA (37) and coronary disease associated with MS (38,39).

Intracellular signals play an important role in endothelial activation. Nuclear factor κ B (NFκB) is one of the most important mechanisms of endothelial activation (40). This and other transcription factors can be activated by tumor necrosis factor α (TNF-α), IL-1, hyperglycemia, shear force, LDLox and oxidative stress, among other factors. NFκB can regulate the expression of genes that code for cytokines, eNOS, cyclooxygenase-2, CAMs, immune response, IL-8, acute phase proteins and metalloproteinase (41-43).

3. Alterations in hemostasis and fibrinolysis

The alterations of the hemostasis of MS that favor a prothrombotic state include modifications in the coagulation system, fibrinolytic system and platelets. Individuals with MS present an increase in the levels of factor VII, fibrinogen and von Willebrand Factor (FVW), increasing the risk of CVD (44).

In arterial thrombosis the platelets, tissue factor (expressed in monocytes and activated platelets) and microparticles that express anionic phospholipid (45) take on important actions.

The monocytes stimulated by oxidative stress can express TF (46) and they contribute in this way to the thrombogenic process increasing the risk of CVD. In the atheromatosus lesions the TF is located around the necrotic center rich in lipids, for it is assumed that low density lipoprotein cholesterol (c-LDL) and the oxidized lipids induce the expression of TF (47).

Microparticles (MP) are small membrane pieces, liberated from sanguine cells or EC during cellular activation or apoptosis (48-50), which anionic phospholipids and TF can expose with the activation of the coagulation system (51,52). MP is bound to the cells via specific receptors inducing cellular activation with the liberation of TF and cytokines (53-55). In patients with diabetes type 2 with microvascular complications, an increase in derived platelet MP has been observed (56).

The alteration of fibrinolysis in MS is due fundamentally to an increment in the serum level of PAI-1. The proinflammatory cytokines participate in their overexpression, particularly in the adipose tissue (16). The increase of the concentration of PAI-1 can lead to arterial thrombosis increasing CVD in individuals with MS (57-59). Abdominal obesity is related to an increase in PAI-1 concentration, as much antigenic as functional (60). On the other hand, a healthy diet and loss of weight diminish these levels (60,61).

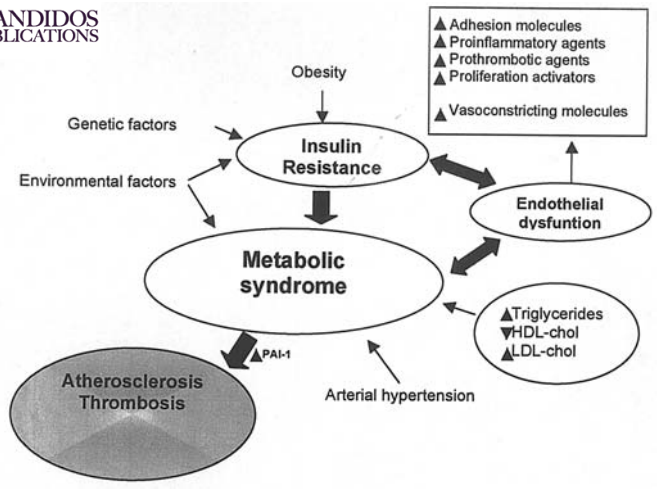


Figure 1. Factors that favor the prothrombotic state in Metabolic Syndrome. Ethiologic factors involved in metabolic syndrome and some of its consequences, where atherogenesis and thrombosis have a central role.

Lately, there have been attempts to integrate atherogenic and thrombogenic processes. Research has been centered on LDL, the molecule that initiated the atherosclerotic process. The platelets possess many receptors, among them, CD36 and oxidized LDL receptor-1 (LOX-1) (62), through which the oxidized LDL (LDLox) would produce platelet activation for phosphorylation of protein kinase p38 (p38MAPK) (63). LOX-1 are also expressed in endothelial cells, allowing the entrance of LDL to the intimal layer and the activation of these by means of the activation of NFκB (64). This receptor is also present in the monocytes where it would allow entrance by the LDL and consequently the formation of foamy cells (65).

LDLox is recognized by the scavenger receptors, CD36 and lectin-like LOX-1 (64) in the macrophages, giving origin to the foamy cells.

LDLox can activate the lymphocytes T and B generating immune response (66). LDLox can unite with the ß$_2$ glycoprotein I (ß$_2$GPI), generating new immunogenic epitopes and, subsequently, an autoimmune response that can increase the atherosclerosis and, finally, favor thrombosis (67,68). ß$_2$GPI is a fundamental part of the antiphospholipid syndrome in which such antibodies as anticardiolipin are detected, which present a crossed reaction with LDLox (69,70) being able to increase even more the atherogenic potential of the mentioned lipoprotein. These antibodies have been associated with acute myocardial infarction (AMI) (71), ischemic disease (72), arterial and venous thrombosis (73) and found in patients with systemic lupus erythematosus (74).

The increase of coagulation factors and tissue expression factor.

Factor VII and VIII. Individuals with MS have increased levels of factor VII, fibrinogen and von Willebrand factor (FVW), increasing the risk of CVD (44). The hyperfibrinogenemia is related to hyperinsulinism, suggesting a relationship independent from the inflammatory process. The loss of weight is associated with a decrease in the levels of factor VII, which is probably associated with a reduction of the TG.

Factor XIII. Positive correlation has been observed between the increase in the concentration of factor XIII, specifically subunit B, and MS, which could represent a CVD risk factor (75). Some association between the increased levels of FXIII and the polymorphism Val34Leu (75) have been demonstrated. However, it has been seen that the distribution of this polymorphism is similar in both patients with AMI and controls (76).

Expression of TF in monocytes and microparticles. The activity of the monocytes increases in endothelial inflammation in patients with CVD. In MS the superoxide radicals are associated with endothelial inflammation and the consequent activation of the monocytes (77). Microparticles (MP) are small membrane pieces, liberated from blood cells or EC during cellular activation or apoptosis (48-50). MP can expose anionic phospholipids, CD62P, CD42a and also TF, with the activation of the coagulation system (51,52). MP bind to the cells via specific receptors that induce cellular activation with the liberation of TF and cytokines (53-55).

In the atheromatous lesions TF is located around the necrotic center rich in lipids, for it is assumed that c-LDL and the oxidized lipids induce the expression of TF (47). In contrast, PCR associated with inflammatory processes also induces the expression of TF on the cellular surfaces (78).

Type 2 diabetes is associated with atherosclerosis (79,80). In patients with type 2 diabetes with poor metabolic control and microvascular complications an increment of derived platelet MP has been described (56). The TF expressed in microparticles, possibly of white cell origin, could be involved in different coagulation processes, such as signal transduction or angiogenesis (81).

TF can activate coagulation and it plays an important role in atherothrombosis; increased levels of TF have been associated with an increment in CVD risk (82). The expression of TF in the adipose tissue induced in obese mice deficient in leptin (ob/ob) has been demonstrated in addition to an association with an increase in the mRNA of the transforming growth factor ß (TGFß) (83,84). In obese patients the thrombogenic state results from an increase in the levels of PAI-1 (85), factor VII and fibrinogen (86,87). However, the role of circulating TF in relation to the death of obese patients has not been well studied. The increase in TF levels has been reported in patients with microvascular complications in diabetes and atherosclerosis favoring an increased risk of CVD (88-90).

Alterations of natural anticoagulants. The TF inhibitor (tissue factor pathway inhibitor, TFPI) is produced by EC (91) and it inhibits the complex FT/VIIa (91). The activity of TFPI has been associated with MS (92) and body mass index (BMI) (93). The activity of TFPI in patients with glucose intolerance and diabetes type 2 is found at high levels (94).

Decrease of fibrinolysis. In the course of diabetes intracellular hyperglycemia causes changes in the blood flow and an increase in vascular permeability. This is reflected by a decrease in NO activity and an increase in the activity of angiotensin II and ET-1 95. The angiotensin II produces an acute vasoconstriction that increases the blood pressure. ET-1 is also an important regulator of the vascular tone and it has been implicated in the pathogenesis of atherosclerosis. This

in addition to the abnormalities in the extracellular matrix contribute to an irreversible increase in vascular permeability. Hyperglycemia does not only diminish the endothelial production of NO, which is an anti-atherogenic molecule, but it also increases the production of a potent fibrinolysis inhibitor, PAI-1 (95).

PAI-1. The atherothrombotic processes in MS are attributable to metabolic abnormalities, inflammation and alteration in the fibrinolysis; in the latter case by an increase in the levels of PAI-1. The synthesis of PAI-1 in adipocytes is regulated by insulin, glucocorticoides, angiotensin II, some fatty acids and mainly by adipokines such as TNFα and TGFß; catecholamines may inhibit their production. The proinflammatory cytokines have an important role in the oversecretion of PAI-1, particularly in the adipose tissue (16). Patients with MS present increased atherosclerosis and hypofibrinolysis (4,11,96). The serum levels of PAI-1 are increased in patients with diabetes type 2 (97), AMI and stroke (98-100). High concentrations of PAI-1 and tPA reflect dysfunction of the fibrinolytic system that can lead to arterial thrombosis, increasing the risk of CVD in individuals with MS (57-59). Due to the complexity of the methods for measuring the function of the fibrinolytic system, the study of it has not been carried out on a large scale (101). There is evidence indicating that obesity, specifically abdominal obesity, is related to the increase in levels of PAI-1, as much antigenic as functional (60). Several pharmacological agents such as the tiazolidinediones, metformin and AT (1)-receptor antagonists reduce the production of PAI-1 in the adipocytes. A healthy diet, loss of weight, and lifestyle modification decrease the inflammation and the levels of PAI-1 (60,61).

There are 5 identified PAI-1 polymorphisms: two common polymorphisms, -765 4G/5G and -844 A>G, not located in the promoter; and three related, Ala15Thr (located in the peptide signal), Val17Ile and Asn195Ile (located in the ß structure). In obese non-diabetic individuals, the first two polymorphisms are associated with high concentrations of glucose (p=0.006 and p=0.0004, for -765 4G/5G and -844 A>G, respectively) and insulin (p=0.05 and p=0.008, for 765 4G/5G and -844 A>G, respectively). However, the -844 A>G was associated with hypotriglyceridemia (p=0.002) and high levels of c-HDL (p=0.02). The promoter's two polymorphisms and the polymorphism Ala15Thr also showed association with CVD in diabetic persons (-765 4G/5G: 0.56/0.51, p=0.05; -844 A>G: 0.63/0.57, p=0.02; Ala15Thr: 0.91/0.88, p=0.04). In summary, these polymorphisms increase the risk of CVD in diabetic individuals (102).

TAFI. Positive correlation has been observed between the levels of insulin and TAFI (thrombin-activatable fibrinolysis inhibitor) (103) and positive correlation has been observed between the levels of TAFI in diabetic patients v/s normal individuals (104); however, studies demonstrating these associations are scarce.

Although, previous studies have documented that the fibrinolysis markers are abnormal in MS, it is not clear whether these abnormalities reflect a fibrinolytic dysfunction or they are simply a response to the vascular damage (14). The presence and degree of the fibrinolytic dysfunction in people with MS, and whether this dysfunction can explain the increase in CVD in this group, must be determined.

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