

Markers of pro-inflammatory and pro-thrombotic state in the diagnosis of metabolic syndrome

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Abstract

The metabolic syndrome refers to the clustering of upper body obesity, atherogenic dyslipidemia, insulin resistance and elevated blood pressure. Both, obesity and metabolic syndrome, have the potential to influence on the incidence and severity of cardiovascular disease with serious implications for worldwide health care systems. Obesity plays a central role in the development of insulin resistance and dyslipidemia through the mediation of a pro-inflammatory and pro-thrombotic state. Adipose tissue has been shown to exert important endocrine and immune functions. Pathogenesis of obesity associated metabolic syndrome is mediated by disturbed production and release of biologically active molecules by fat cells and other cells infiltrating fat tissue. In obese subjects synthesis of several bioactive compounds – adipokines and cytokines/chemokines by adipose tissue cells is dysregulated. Those bioactive molecules participate in regulation of appetite and energy homeostasis, lipid metabolism (tumour necrosis factor α – TNF- α), insulin sensitivity (TNF- α , adiponectin, resistin, visfatin) immunity (monocyte chemoattractant protein-1 – MCP-1, TNF- α , IL-6), angiogenesis, blood pressure and hemostasis (plasminogen activator inhibitor – PAI-1). The effects of major pro-/anti-inflammatory and pro-thrombotic adipokines on several physiological processes will be discussed in this review. Also, an evidence-based approach to the laboratory diagnosis and treatment of metabolic syndrome will be presented.

Key words: obesity, metabolic syndrome, cardiovascular risk, inflammation, thrombosis, laboratory tests.

The metabolic syndrome represents a combined occurrence of atherogenic dyslipidemia, insulin resistance, elevated blood pressure and central adiposity. Pro-inflammatory and pro-thrombotic state contributing to endothelial dysfunction is a common feature of those with metabolic syndrome. Increasing frequency of abdominal obesity, reaching epidemic proportions, enhances the prevalence of metabolic syndrome up to 22% in the adult population of the United States and around 10-15% in Finland [1,2]. Both, obesity and metabolic syndrome, have the potential to influence on the incidence and severity of cardiovascular disease, particularly in the presence of type 2 diabetes mellitus, in men aged over 45 years and women aged 55 years with serious implications for worldwide health care systems.

Metabolic syndrome is defined in various ways. The diagnostic criteria for this disorder have been established by the World Health Organization in 1998, the National Cholesterol Education Program's Adult Treatment Panel III in 2001 and recently by the International Diabetes Federation in 2005. All three definitions are associated with similar risks for cardiovascular diseases and diabetes.

Obesity, particularly in visceral region, is a key component in the development of the metabolic syndrome. It has been found that waist size provides additional information regarding inflammation and insulin resistance. Alterations in visceral fat lipolysis exert direct effects on the liver metabolism. Increased adiposity, leads to greater free fatty acid flux and inhibition of insulin action that may be due to enhanced synthesis and release of TNF- α from fat tissue [3]. In obesity adipose tissue is resistant to insulin which is associated with disturbed glucose metabolism in the muscles and liver. Even mild or moderate degree of obesity with concomitant insulin resistance may be associated with metabolic syndrome. On the other hand, excessive accumulation of abdominal fat may lead to the development of metabolic syndrome independently on degree of insulin resistance.

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It is suggested that chronic mild inflammation constitutes an important underlying factor of metabolic syndrome. Pathogenesis of obesity associated metabolic syndrome is mediated by disturbed production of biologically active molecules in fat tissue. In obese subjects synthesis of several bioactive compounds – adipocytokines, by either adipocytes or adipose tissue infiltrated macrophages, is dysregulated; secretion of pro-inflammatory adipocytokines is elevated while that of anti-inflammatory is reduced. Low-grade inflammation constitutes the bridge linking atherosclerosis with metabolic syndrome and is associated with higher risk for acute cardiovascular syndromes.

Adipose tissue has an important endocrine function involved in inflammatory and thrombotic pathways. Fat cells produce and release more than 50 different compounds into the circulation. All identified adipocytokines form a network linking adipose tissue with skeletal muscle, liver, adrenal cortex, brain and sympathetic nervous system. These compounds participate in regulation of appetite and energy homeostasis (leptin), lipid metabolism (TNF- α cholesterol ester transfer protein – CETP, apoE), insulin sensitivity (adiponectin, resistin, visfatin), immunity (IL-1 β , IL-6, IL-10, IL-8, MCP-1), angiogenesis (vascular endothelial growth factor – VEGF), vascular function (leptin, resistin, apelin, adiponectin), blood pressure (angiotensinogen), hemostasis (PAI-1) and acute phase reaction (CRP, haptoglobin, SAA) [4].

In obese subjects increased expression of several inflammatory cytokine/chemokine genes in adipose tissue was found that affect its metabolism, insulin signaling and endocrine activity. Those pro-inflammatory mediators have been reported to induce insulin resistance in fat tissue and muscles. Dahlman et al. [5] reported that of several cytokines synthesized in adipose tissue of the obese MCP-1 is the only acting as local factor that recruits monocytes contributing to induction of insulin resistance.

Prospective studies have shown that elevated levels of pro-inflammatory indices like CRP or diminished levels of protective anti-inflammatory marker such as adiponectin are important predictors of the development of type 2 diabetes [6].

Inflammatory state is an important component of wide range of the diseases also those associated with aging. Trayhurn and Wood proposed an explanation to the increasing inflammatory response of fat tissue with developing obesity [7]. The authors suggest that in growing poorly vascularized adipose tissue mass, hypoxia is a critical factor. Expression of some cytokines (leptin), chemokines and angiogenic factors (VEGF) to stimulate vascularization may be induced by hypoxia that has been shown recently in different situations and in adipocyte cultures.

Several adipokines are associated to the immune system and inflammation. In obesity expression, synthesis and release of pro-inflammatory adipokines (TNF- α , IL-6, haptoglobin leptin, resistin) is enhanced but that of anti-inflammatory such as adiponectin is decreased [7]. The inflammation state in obese reflects, at least partly, increased release of inflammatory peptides and proteins such as leptin and PAI-1 from adipose tissue as a major source. However, this is not the case for TNF- α , IL-6, resistin and CRP [8].

Leptin

Leptin, is a hormone with divergent activities. This 16-kD cytokine is produced mainly by adipocytes but also in stomach, ovaries, placenta and vascular cells [7]. Leptin remains a key hormone responsible for the regulation of appetite and energy balance by hypothalamus. Acting as a “starvation signal” is a central factor in the elevation of sympathetic activity found in obese hypertensive patients.

Moreover, it has been reported that leptin increases cellular adhesion molecules and affects vessel wall. Leptin stimulates accumulation of cholesterol in macrophages, activates monocytes and rise pro-inflammatory cytokine (TNF- α , IL-6) release. Leptin has been shown to stimulate production of reactive oxygen species by activated monocytes *in vitro*. This adipokine may act also as angiogenic factor. Leptin exerts pro-thrombotic properties by contributing to arterial thrombosis through a platelet leptin receptor [4].

TNF- α

TNF- α is a multipotential cytokine with several immunologic functions. It is produced and released by adipose tissue, primarily from stromal vascular cells, and its enhanced expression associated to induction of insulin resistance was reported in obese subjects [7]. In adipose tissue TNF- α is engaged in the stimulation of lipolysis. TNF- α induces endothelium function changes by raising oxidative stress while adiponectin inhibits this factor. TNF- α may activate transcription factor NF-kappa β that leads to increased production of cytokines and adhesion molecules (ICAM-1, VCAM-1) enhancing monocyte adhesion to the vessel wall. A substantial effect of TNF- α on the expression and release of pro-inflammatory adipokines was confirmed, up to now, only by *in vitro* studies. In human adipocytes differentiated in culture TNF- α increased IL-6, MCP-1, nerve growth factor, VEGF while adiponectin, adipisin, haptoglobin and leptin were decreased [9].

Interleukin 6

Interleukin-6 is a cytokine having multiple effects, secreted by immune cells, fibroblasts, endothelial cells, skeletal muscle and in low amounts by fat tissue. Fat cells produce only about 10% of total IL-6 and regional differences has been observed. Visceral adipocytes produce much more IL-6 than from the subcutaneous depot. This inflammatory cytokine is increased in subjects with obesity and insulin resistance and may be regarded as a predictive factor for type 2 diabetes and myocardial infarction [4]. Induction of insulin resistance by IL-6 could be mediated by suppression of insulin receptor signal transduction in hepatocytes.

Adipsin

Adipsin, a serine protease is known to stimulate glucose transport for triglyceride accumulation in fat cells and to inhibit lipolysis [4]. Obese humans have substantially increased blood adipsin concentration but it is not clear whether high concentration reflects increased activity or resistance to adipsin.

Resistin

Resistin was primarily suggested to be a link between obesity and insulin resistance in humans, however, recent human

Table 1. Interpretation of general and alternative laboratory tests results in the diagnosis of metabolic syndrome (according to <http://www.labtestsonline.org>)

Cholesterol	Total cholesterol should be <200 mg/dl (<5 mmol/L); HDL-cholesterol >40 mg/dl (>1.04 mmol/L) in males, >50 mg/dl (>1.29 mmol/L) in females – good; LDL-cholesterol should be <100 mg/dl (2.6 mmol/L)
Triglycerides	44-100 mg/dl (0.5-1.13 mmol/L) – optimal; 100-150 mg/dl (1.13-1.7 mmol/L) – moderate; >150 mg/dl (>1.7 mmol/L) – high
Fasting glucose	70-99 mg/dl (3.9-5.5 mmol/L) – normal; 100-124 mg/dl (5.6-6.9 mmol/L) – indicates pre-diabetes; ≥125 mg/dl (≥7.0 mmol/L) – indicate type 2 diabetes
Oral glucose tolerance test (with 75 g glucose load)	≤140 mg/dl (≤7.8 mmol/L) at 2 hrs – normal glucose tolerance; 140-200 mg/dl (7.8-11.1 mmol/L) at 2 hrs – impaired glucose tolerance; >200 mg/dl (>11.1 mmol/L) twice – indicates type 2 diabetes
Fasting insulin	≤10 IU/ml – optimal; >10 IU/ml – high
CRP (high sensitive method)	<1.0 mg/L – optimal; 1.0-3.0 mg/L – moderate risk; >3.0 mg/L – high risk
Fibrinogen	Results of this test vary greatly with age, sex and test method. Too high and too low results are problematic
Homocysteine	<6 μmol/L – optimal; >9 μmol/L – high
PAI-1	This test is not yet routinely performed

studies failed to confirm this relation [10]. Adipose-derived resistin is probably expressed in monocytes infiltrating adipose tissue [11]. Since it is produced by blood monocytes its pro-inflammatory activity and contribution to development of endothelial dysfunction has been suggested.

Visfatin

Visfatin, recently discovered in the human visceral fat was suggested to play a role in glucose homeostasis through stimulation of the insulin receptor (insulin-mimetic effects). Another effect of visfatin is to promote adipogenesis by influencing on adipocyte precursors differentiation. At present the relation of visfatin to insulin sensitivity is questioned and the contribution of fat tissue to circulating visfatin concentration remains unknown [12,13].

Adiponectin

Adiponectin has been considered as a key regulator of insulin sensitivity and tissue inflammation. This 30-kD protein synthesized exclusively by white and brown adipocytes is present at very high concentrations in the blood. Its level inversely correlates with the amount of body fat that means adiponectin concentration is higher in non-obese than in obese people [6]. Regional difference exists in adiponectin production in humans, omental adipocytes secrete higher amounts than subcutaneous. Adiponectin level may be a predicting factor of diabetes and cardiovascular disease risk.

In the circulation adiponectin exists as varying molecular weight forms. High molecular weight complexes have the predominant action in the liver but in general trimers exert the highest biological activity.

Adiponectin may act as signaling molecule to regulate insulin action in the liver, improves hepatic insulin sensitivity, and in skeletal muscle, increases fuel oxidation. Two adiponectin receptors have been identified: AdipoR1 is highly expressed in skeletal muscle and promotes lipid oxidation, AdipoR2 is mostly expressed in the liver, enhances insulin sensitivity and reduces

liver steatosis via increased peroxisome proliferator activated receptor- α (PPAR- α) [14]. PPAR- α is a nuclear transcription factor that regulates expression of genes involved in fatty acids beta-oxidation and regulates energy homeostasis [15].

Adiponectin antagonizes many effects of pro-inflammatory TNF- α by inhibition of NF-kappa β pathway; TNF- α in turn suppresses adiponectin production.

In type 2 diabetes adiponectin is significantly reduced [16]. It was shown that administration of adiponectin increased glucose uptake by muscles, improved insulin sensitivity and suppressed gluconeogenesis in the liver cells [6].

Protective role of adiponectin within the vascular system results from suppression of the inflammatory processes such as adhesion, proliferation, phagocytosis and deposition of lipids in monocytes.

In obese people increased gene expression of inflammatory and thrombotic cytokines and decreased expression of protective adiponectin has been reported suggesting a close link between abdominal obesity and other underlying risk factors of metabolic syndrome [17].

Recently it has been shown that in obese postmenopausal women visceral adipose tissue volume inversely correlated with leptin and tended to inversely correlate with adiponectin gene expression [17]. Positive relationship between fasting insulin and visceral adipose tissue TNF- α gene expression was observed in the subgroup of non-diabetic women. Additionally, IL-6 gene expression tended to be positively related to fasting insulin in these women. Expression of adiponectin was much lower in obese women with metabolic syndrome than without. These results suggest that enhanced pro-inflammatory cytokine expression in fat tissue links abdominal obesity with its metabolic disturbances.

Apart from impaired glucose tolerance and insulin resistance, dyslipidemia and hypertension a typical feature in metabolic syndrome is a pro-thrombotic state. The metabolic syndrome is frequently diagnosed in patients with venous

thrombosis. Recent study reported the presence of metabolic syndrome in 50% of patients with deep vein thrombosis [18].

The risk of thromboembolism is significantly increased in abdominal obesity that results from activation and changes of coagulation system [19]. This is reflected by enhanced generation of thrombin which converts fibrinogen to fibrin, diminished fibrinolysis and increased platelet aggregation. Increased levels of fibrinogen, factor VII and VIII that leads to hypercoagulability is characteristic of metabolic syndrome. Simultaneously, enhanced production of PAI-1 decrease fibrinolysis.

Abdominal obesity, mainly accumulation of visceral fat, resulting in low-grade inflammation is related to increased fibrinogen levels. Pro-inflammatory state is also associated with increased levels of coagulation factors: tissue factor and factor VII and thus the risk of activation of coagulation cascade [19].

There are few studies in which interrelations between pro-coagulant factors and anticoagulant proteins were investigated in humans with wide range of body fat. Godsland et al. [20] have found that procoagulant factors VII and X, anticoagulant proteins C and S and PAI-1 correlated directly with total and central body fat but inversely with insulin sensitivity. The authors suggested that procoagulant factors and anticoagulant proteins are the features of the intercorrelated disturbances of the metabolic syndrome.

Also other factors of metabolic syndrome such as: TNF- α and homocysteine has been suggested to contribute to procoagulant state.

Plasminogen activator inhibitor – PAI-1

Fibrin degradation is a process controlled by tissue plasminogen activator – t-PA and PAI-1 balance. Decreased t-PA paralleled by increased plasma level of PAI-1 associated with insulin resistance are common in metabolic syndrome. Chronic inflammation and enhanced lipolysis in adipose tissue, leading to increased free fatty acids – FFA release, stimulate PAI-1 expression and synthesis, decrease conversion of plasminogen to plasmin and in consequence diminish fibrin degradation being the important contributors of hypofibrinolysis.

The relationship between PAI-1 activity, adiponectin and CRP levels, insulin resistance and lipoproteins was studied in overweight and obese women [21]. Interestingly, it was found that, PAI-1 activity inversely correlated with serum adiponectin, independently of the amount of visceral tissue.

The other characteristic feature of metabolic syndrome is endothelial dysfunction often present in insulin resistance and type 2 diabetes mellitus. Excessive lipolysis resulting in chronic elevations of plasma FFA may induce endothelial dysfunction [16]. This is reflected by high levels of markers such as thrombomodulin [19].

Insulin-resistance in obesity and dyslipidemia are associated with excessive platelet activation and aggregation. High levels of VLDL stimulate synthesis of thromboxane A₂ – TxA₂ in platelets from FFA [19]. TxA₂ is known to enhance platelet aggregation.

Laboratory diagnosis of metabolic syndrome

General laboratory tests used in the diagnosis of metabolic syndrome include determination of lipid profile : total cho-

lesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels, glucose concentration, oral glucose tolerance test and glycated hemoglobin level-HbA_{1c} (Tab. 1). Determination of HbA_{1c} was found to be of importance in the diagnosis and prognosis of metabolic syndrome [22,23].

There are other laboratory indicators that may provide additional information and tests that can be used mostly in research setting. Among alternative tests fasting insulin can be measured, however, the values may vary to much to be clinically useful in the diagnosis of metabolic syndrome.

The easiest way to detect a pro-inflammatory state as a part of the cardiac risk assessment is measurement of CRP by a high sensitivity method. If CRP level, measured twice within a few weeks, is above 3 mg/L a pro-inflammatory state is defined. CRP has been confirmed as a clinically important prognostic parameter in the diagnosis of metabolic syndrome [24,25]. The concentration of other inflammatory markers such as IL-6, other adipocytokines or adiponectin are actually assayed only in research setting but in the near future may be very helpful in the diagnosis.

In routine laboratory fibrinogen is used for the assessment of inflammatory processes and pro-thrombotic state while testing for homocysteine and PAI-1 level is not commonly performed yet. Some evidence-based studies indicated an important role of homocysteine in assessing complications in subjects with metabolic syndrome.

Interestingly, the inflammation state in obese people can be partly reversed after weight loss [7]. Whether improving metabolic syndrome by weight loss and physical exercise is a consequence of changes in adipose tissue cytokine gene expression still needs explanation.

Regular physical activity improves insulin sensitivity and correlates inversely with leptin and mild inflammation (IL-6) in adolescents, independently of fat mass and it's localization [26]. However, the beneficial effects of regular physical exercise on metabolic syndrome features cannot be totally explained by changes in adipokine level.

Therapies addressing the treatment of obesity related disorders should focus primarily on modifying the inflammatory profile. It has been found that glitazones significantly increased serum adiponectin while significantly decreased CRP and resistin level [27]. Also slight increase in HDL-cholesterol and favorable effect on LDL particle size was observed. Thiazolidinediones also significantly diminish plasma CRP levels and increase adiponectin in type 2 diabetics [16]. Treatment with thiazolidinediones may decrease the risk of thrombosis in metabolic syndrome; secretion of PAI-1 stimulated by insulin is suppressed by glitazones.

Fibrates have anti-inflammatory and anti-thrombotic effects in the vessel wall in patients with metabolic syndrome. Statins reduce risk for major cardiovascular disease events even in high risk patients and may be used in combination with fibrates.

In primary prevention of arterial thrombosis, antiplatelet agents like low-dose aspirin may be used in the long-term approach. In patients with risk of atherosclerotic cardiovascular disease aspirin seems to be a good therapeutic possibility.

The better understanding of the molecular actions of adipokines is the key issue to better diagnosis and discovery of

effective therapy. Weight loss and pharmacological treatment leading to decrease of pro-inflammatory, pro-thrombotic adipokine level may prevent the metabolic syndrome and type 2 diabetes and in consequence the development of atherosclerosis complications.

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