

6. PRO-INFLAMMATORY AND PROTHROMBOTIC FACTORS AND METABOLIC SYNDROME

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The metabolic syndrome represents combined occurrence of atherogenic dyslipidemia, insulin resistance, elevated blood pressure and central adiposity. Pro-inflammatory and prothrombotic 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. 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.

Visceral obesity is a key component in the development of the metabolic syndrome. Increased central adiposity, particularly in visceral region, leads to greater free fatty acid flux and inhibition of insulin action. Adipose tissue in obesity 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.

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 by fat cells. In obese subjects synthesis of several bioactive compounds – adipokines, by either adipocytes or adipose tissue infiltrated macrophages, is dysregulated, secretion of pro-inflammatory adipokines is elevated while that of anti-inflammatory is reduced.

All identified adipokines form a network linking adipose tissue with skeletal muscle, liver, adrenal cortex, brain and sympathetic nervous system. All these compounds participate in regulation of appetite and energy homeostasis, lipid metabolism, insulin sensitivity, immunity, angiogenesis, blood pressure and hemostasis.

Pro-inflammatory cytokines have been reported to induce insulin resistance in fat tissue and muscles. Prospective studies have shown that elevated levels of pro-inflammatory indices (like CRP) or diminished levels of protective anti-inflammatory marker (adiponectin) are important predictors of the development of type 2 diabetes.

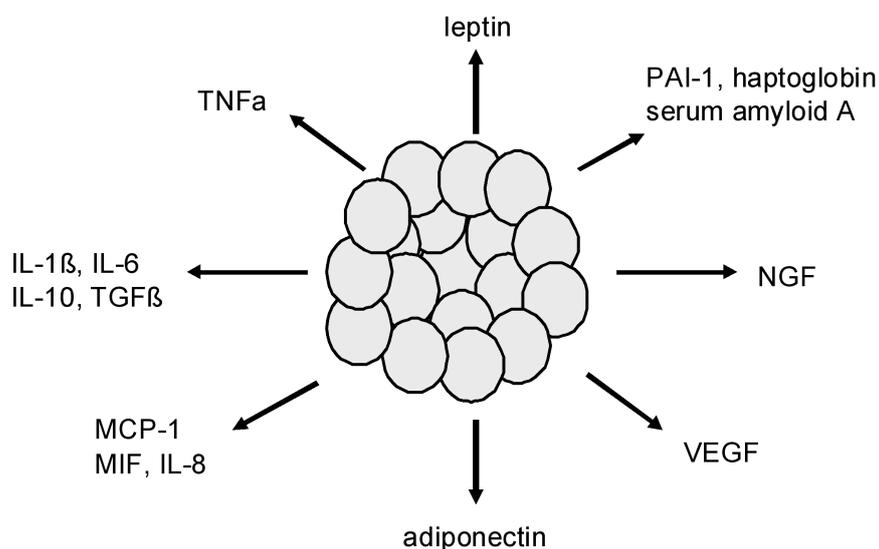
Thus low-grade inflammation constitutes the bridge linking atherosclerosis with metabolic syndrome and is associated with higher risk for acute cardiovascular syndromes.

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. The authors suggest that in growing adipose tissue mass, poorly vascularized, 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.

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. These adipokines play multiple roles in a wide range of physiological processes such as insulin sensitivity (adiponectin, resistin, visfatin), lipid metabolism (CETP, apoE, NEFA), hemostasis (PAI-1), blood pressure regulation (angiotensinogen) and angiogenesis (VEGF). Adipocytes release also: hormones (steroids, leptin) and prostaglandins, growth factors (TNF- α , TGF- β), cytokines (IL-1 β , IL-6, IL-10), chemokines (IL-8, MCP-1, MIF β – macrophage migration inhibitory factor) and some acute phase proteins (haptoglobin, SAA) (Figure 6.1.).

Figure 6.1. Adipokines linked to inflammation and the inflammatory response; According to (4).
NGF, nerve growth factor



Several, listed above, adipokines are associated to the immune system and inflammation. In obesity expression, synthesis and release of pro-inflammatory adipokines (TNF- α , IL-6, PAI-1, haptoglobin and leptin) is enhanced with concomitant decrease of protective adiponectin. It seems that in obese subjects the inflammation state reflects, at least partly, increased release of inflammatory peptides and proteins from adipose tissue as a major source. This is the case for PAI-1 but not for increased level of CRP in the blood. The latter is probably also expressed in adipocytes and released in low amounts but mostly derives from hepatocytes after IL-6 stimulation.

Adipokines play multiple roles in the inflammatory process. Leptin stimulates accumulation of cholesterol in macrophages, IL-6 stimulates liver production of CRP and through visfatin influence glucose tolerance, TNF- α increases expression of adhesion molecules (ICAM-1,

VCAM-1) enhancing monocyte adhesion to the vessel wall, and induces endothelium function changes, PAI-1 stimulates formation of thrombus after atherosclerotic plaque rupture.

1. Leptin

Leptin, is a hormone with divergent activities. This 16-kD cytokine, not exclusively (stomach, ovaries, placenta etc) produced by adipocytes remains a key hormone responsible for the regulation of appetite and energy balance by hypothalamus. Leptin, 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 affects vessel wall. Leptin may act as angiogenic factor and in vitro stimulates production of reactive oxygen species by activated monocytes. Also, it may contribute to arterial thrombosis through a platelet leptin receptor.

2. TNF- α

TNF- α is a multipotential cytokine with several immunologic functions. It is produced and released from adipocytes and its enhanced expression associated to induction of insulin resistance was reported in obese subjects. In adipose tissue TNF- α is also engaged in stimulation of lipolysis and apoptosis. Probably TNF- α activates transcription factor NF-kappa β that leads to increased production of cytokines and increases oxidative stress while adiponectin inhibits this factor.

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 (monocyte chemotactic protein), NGF, VEGF while adiponectin, adipisin, haptoglobin and leptin were decreased.

3. Interleukin-6

Interleukin-6 is a cytokine having multiple effects, secreted by immune cells, fibroblasts, endothelial cells, skeletal muscle and adipose tissue. This pro-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. 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. Induction of insulin resistance by IL-6 could be mediated by suppression of insulin receptor signal transduction in hepatocytes.

4. Adipsin

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

5. Resistin

Resistin was suggested to be a link between obesity and insulin resistance but until now its role is unclear. Resistin is poorly expressed in human fat cells. Since it is produced by blood monocytes its inflammatory activity and contribution to development of endothelial dysfunction has been suggested.

6. 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).

7. Adiponectin

Adiponectin has been considered as a key regulator of insulin sensitivity and tissue inflammation. This 30-kD protein synthesized exclusively by adipocytes (white and brown) is present at very high concentrations in the blood but its level inversely correlates with the amount of body fat. It means that adiponectin concentration is higher in non-obese than in obese people. 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.

Adiponectin may act as signaling molecule to regulate insulin action in the liver (improve hepatic insulin sensitivity) and skeletal muscle (increase 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 and enhances insulin sensitivity, reduces liver steatosis via increased PPAR- α . PPAR- α is a nuclear transcription factor that regulates expression of genes involved in FA beta-oxidation and regulates energy homeostasis.

Adiponectin antagonizes many effects of pro-inflammatory TNF- α , that in turn suppresses adiponectin production.

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

Protective role of adiponectin within the arteries 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.

It has been shown recently that in obese postmenopausal women visceral adipose tissue volume inversely correlated with leptin and tended to inversely correlate with adiponectin gene expression. 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.

Interestingly, the inflammation state in obese people can be partly reversed after weight loss. It has been shown that CRP levels decline with weight reduction.

Whether improving metabolic syndrome by weight loss and physical exercise is a consequence of changes in adipose tissue cytokine gene expression still needs explanation.

Recent studies indicate that regular physical activity improves insulin sensitivity and correlates inversely with leptin and mild inflammation (IL-6) in adolescents, independently of fat mass and localization. However, in this study beneficial effects of regular physical exercise on metabolic syndrome features were not totally explained by adipokines (adiponectin, TNF- α -receptor1).

Apart from impaired glucose tolerance and insulin resistance, dyslipidemia and hypertension a typical feature in metabolic syndrome is a prothrombotic 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.

The risk of thromboembolism is significantly increased in abdominal obesity that results from activation and changes of coagulation system. 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 (but 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: TF and factor VII and thus the risk of activation of coagulation cascade.

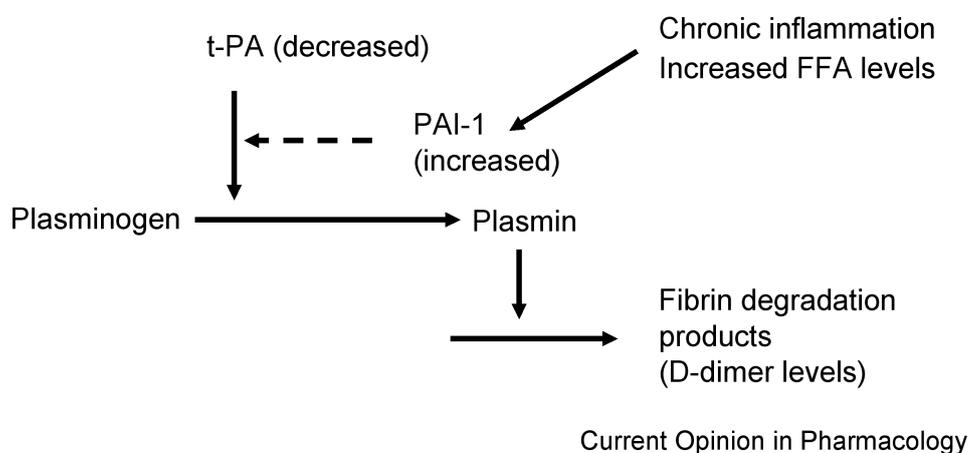
There are few studies in which interrelations between procoagulant factors and anticoagulant proteins were investigated in humans with wide range of body fat. Godsland et al (2005) have found that procoagulant factors VII and X, anticoagulant proteins C and S and PAI-1 correlated directly with total 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.

Fibrin degradation (fibrinolysis) is a process controlled by t-PA (tissue plasminogen activator) and PAI-1 balance. Decreased t-PA paralleled by increased plasma level of PAI-1 associated with insulin resistance are common in metabolic syndrome (Figure 6.2.).

Chronic inflammation and enhanced lipolysis in adipose tissue, leading to increased FFA, stimulate PAI-1 expression and synthesis, decrease conversion of plasminogen to plasmin and in consequence fibrin degradation being the important contributors of hypofibrinolysis.

Figure 6.2. Hemostatic risk factors and insulin sensitivity, regional body fat distribution and the metabolic syndrome. According to Godsland et al. *J Clin Endocrinol Metab* 2005, 90, 190-7.



The relationship between PAI-1 activity, adiponectin and CRP levels, insulin resistance and lipoproteins was studied in overweight and obese women. 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 DM. Excessive lipolysis resulting in chronic elevations of plasma FFA may induce endothelial dysfunction. This is reflected by high levels of markers such as thrombomodulin.

Insulin-resistance in obesity and dyslipidemia are associated with excessive platelet activation and aggregation. High levels of VLDL stimulate synthesis of thromboxane A₂ in platelets from FFA (through binding of CD 36 ligand to platelets). TxA₂ is known to enhance platelet aggregation.

In routine medical laboratory the easiest way to detect a proinflammatory state in a person without other detectable causes is measurement of hsCRP. If CRP level, measured twice within a few weeks, is above 3 mg/L a pro-inflammatory state is defined.

Then there is a need for lifestyle changes like weight reduction by diet or exercise. Therapies addressing the treatment of obesity related disorders should focus on modifying the inflammatory profile. When other risk factors are present together with elevated CRP, statins,

nicotinic acid, fibrates, ACE inhibitors or thiazolidinediones (glitazones) decreasing CRP level may be used for treatment.

8. Thiazolidinediones

Thiazolidinediones - peroxisome proliferator-activated receptor- γ agonists are known for their beneficial effect diminishing the risk of metabolic syndrome. PPAR- γ -peroxisome proliferator-activated receptor- γ , a nuclear receptor, is a ligand-activated transcriptional factor expressed in various types of cells but highly in human adipose tissue-derived cells.

They influence changes in secretion of adipokines linked with regulation of insulin sensitivity and other signalling molecules. It was shown that adiponectin and resistin genes are regulated by PPAR- γ . Moreover, TNF- α and leptin genes are regulated by PPAR- γ agonists.

The effect of short-term treatment with pioglitazone on lipoproteins, CRP and adipokines, adiponectin and resistin, in adult patients with metabolic syndrome without diabetes was studied by Szapary et al. It has been found that this drug significantly increased serum adiponectin (by 111%) while significantly decreased CRP (by 31%) and resistin level (by 10%). Also slight increase in HDL-C (by 15%) and favorable effect on LDL particle size was observed. Thiazolidinediones also significantly diminish plasma CRP levels and increase adiponectin in type 2 diabetics.

9. Fibrates

Fibrates known as lipid-lowering drugs (decrease TG) are the agonists of PPAR- α another transcription factor that regulates expression of genes involved in fatty acids beta-oxidation and regulates energy homeostasis. Fibrates also have anti-inflammatory and anti-thrombotic effects in the vessel wall in patients with metabolic syndrome.

10. Niacin (nicotinic acid)

The lipid-lowering drug niacin may modify inflammatory profile. It has been found however, that treatment with niacin significantly rises serum adiponectin (by 56% after 6 weeks) and leptin (by 27%) but does not change resistin, TNF-, IL-6 nor CRP thus fails to improve atheroprotective function attributed to adiponectin.

Assessment of prothrombotic state typically found in subjects with metabolic syndrome is not so easy in routine medical laboratory. The level of fibrinogen can be easily determined automatically, however, coagulation factors such as PAI-1 are generally not routinely measured.

Again changes in the lifestyle and regular physical exercise may increase production of fibrinolytic proteins thus exerting anti-coagulant action.

Treatment with thiazolidinediones may decrease the risk of thrombosis in metabolic syndrome. It has been reported that secretion of PAI-1 stimulated by insulin is suppressed by glitazones. PPAR- γ activated by glitazones suppress lipolysis (reduce FFA release) thus improve

endothelial dysfunction that is probably mediated by reduction of TNF- α level and action. PPAR- γ agonists tend to decrease CD 36 expression.

11. Aspirin

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

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