THE SECRET LIFE OF FAT: WHAT ARE FAT CELLS DOING FOR THE REGULATION OF METABOLISM

TAJNI ŽIVOT MASTI: ŠTA MASNE ĆELIJE ČINE ZA REGULACIJU METABOLIZMA

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Summary: Adipose tissue has long been regarded as an organ the sole purpose of which was to store excess energy as triglycerides, and release energy as free fatty acids, which itself is an essential self-defense system for survival during starvation. This point of view has now changed, fat tissue has emerged as an endocrine and secretory organ affecting more than one metabolic pathway. Its major endocrine function is secreting several hormones, notably leptin and adiponectin. Also, adipose tissue releases adipokines involved in inflammation and hemostasis: growth factors (TNFα, transforming growth factor-beta, nerve growth factor, VEGF), cytokines (IL-1β, IL-6, IL-10), chemokines (IL-8), acute-phase proteins (haptoglobin, serum amyloid A) and prothrombotic factor (plasminogen activator inhibitor-1). This review aims to present some of the recent topics of selected adipokine research that may be of particular importance.

Key words: adipocytes, adipokines, endocrine control

Kratak sadržaj: Adipozno tkivo se dugo smatralo organom čija je jedina svrha skladištenje viška energije u obliku trigliceri-da i otpuštanje energije u obliku slobodnih masnih kiselina, što samo po sebi predstavlja neophodni sistem samoodbrane u cilju preživljavanja tokom gladovanja. Takav stav je danas izmenjen, a masno tkivo se pokazalo kao endokrini i sekretorni organ koji utiče na nekoliko metaboličkih puteva. Njegova glavna endokrina funkcija je lučenje izvesnog broja hormona, pre svega leptina i adiponektina. Adipozno tkivo takođe otpušta adipokine uključene u inflamaciju i hemostazu: faktore rasta (TNFα, transmišући faktor rasta-beta, nervni faktor rasta, VEGF), citokine (IL-1γ, IL-6, IL-10), hemokine (IL-8), proteine akutne faze (haptoglobin, serumski amiloid A) i pro-trombotski faktor (plazminogen aktivator inhibitor-1). Cilj ove studije je da predstavi predmete odabranih skorašnjih istraživa-nja adipokina koji bi mogli imati poseban značaj.

Ključne reči: adipociti, adipokini, endokrina kontrola

Introduction

Adipose tissue plays essential metabolic roles, not only serving as a massive energy reservoir but also producing and releasing hormones and other biologically active molecules that regulate several metabolic activities. Adipocytes secrete a variety of factors, referred to as adipokines (1). As the master regulator of systemic lipid storage and through secretion of a number of these adipokines, adipose tissue has an influence on many processes, including energy metabolism, inflammation, and pathophysiological changes such as cancer and infectious disease (2). At the interface of energy metabolism and inflammation, adipose tissue also plays a key role in the development of the metabolic syndrome. As such, our views of adipose tissue have changed significantly over the past 20 years. Initially considered an inert storage compartment for triglycerides, pioneering work from the Spiegelman and Flier laboratories (3) in the mid-1980s highlighted for the first time that adipocytes are an abundant source of a specific secretory protein, called adipsin or complement factor D. In 1995, Jeffrey Friedman's group (4) identified leptin as a fat cell-specific secretory factor deficient in the *ob/ob* mouse that mediates the hormonal axis between fat and the brain.

Additional proteins have joined this exclusive club of adipocyte-specific secretory proteins since then, including adipokines such as resistin (5) and acylation-stimulating protein as well as the recently described visfatin (6) and retinol-binding protein-4 (7). Enzymes such as lipoprotein lipase are also abundantly produced and released from adipocytes. Finally, many proinflammatory cytokines and acute-phase reactants originate in the adipocyte. These include α1 acid glycoprotein, serum amyloid A, the

C-reactive protein homolog pentraxin-3, the lipocalin 24p3, and a host of cytokines (8). We are all painfully aware of the fact that adipose tissue is the only organ with unlimited growth potential at any stage of our life. These adipocytes can release protein and lipid derivatives that are highly proangiogenic and have an impact on the preexisting vasculature. Finally, the unique extracellular matrix environment of adipose tissue hosts a number of additional cells such as macrophages and offers unique growth potential for transformed cells such as breast cancer cells (9).

Using adipokines as one of the major communication tools, adipocytes affect a large number of other tissues, such as the liver, muscle, the brain, the reproductive system, pancreatic ß-cells, and, as mentioned above, the vasculature.

Differentiation of adipose tissue

The first hallmark of the adipogenesis process is the dramatic alteration in cell shape as the cells convert from fibroblastic to spherical shape. These morphological modifications are paralleled by changes in the level and type of extracellular matrix (ECM) components and the level of cytoskeletal components (10). Recent findings indicate that these events are key for regulating adipogenesis as they may promote expression of critical adipogenic transcription factors, including CCAAT/enhancer binding protein-α (C/EBP) and/or peroxisome proliferator-activated receptor-γ (PPARγ). Mediation of the proteolytic degradation of the stromal ECM of preadipocytes by the plasminogen cascade is required for cell-shape change, adipocyte-specific gene expression, and lipid accumulation (11). Plasma kallikreine or plasminogen deficiencies lead to inhibition of adipocyte differentiation *in vitro* and *in vivo*, probably through suppression of fibronectin degradation. Addition of exogenous fibronectin during adipocyte differentiation also inhibits adipogenesis, confirming the negative regulatory role of this ECM molecule (10, 11). ENC-1, a Drosophila kelch-related actin-binding protein may also play a regulatory role early in adipocyte differentiation by affecting cytoskeletal reorganization and cell-shape change. In preadipocytes, ENC-1 colocalizes with actin filaments, and its mRNA levels are transiently increased 8- to 12-fold early in adipocyte differentiation. ENC-1 induction precedes expression of PPARγ and C/EBPα, and decreasing endogenous ENC-1 levels effectively inhibit adipocyte differentiation (*Figure 1*) (10, 12).

During the terminal phase of differentiation, activation of the transcriptional cascade leads to increased activity, protein, and mRNA levels for enzymes involved in triacylglycerol synthesis and degradation. Glucose transporters, insulin receptor number, and insulin sensitivity also increase. Synthesis of adipocyte-secreted products including leptin, adipsin,
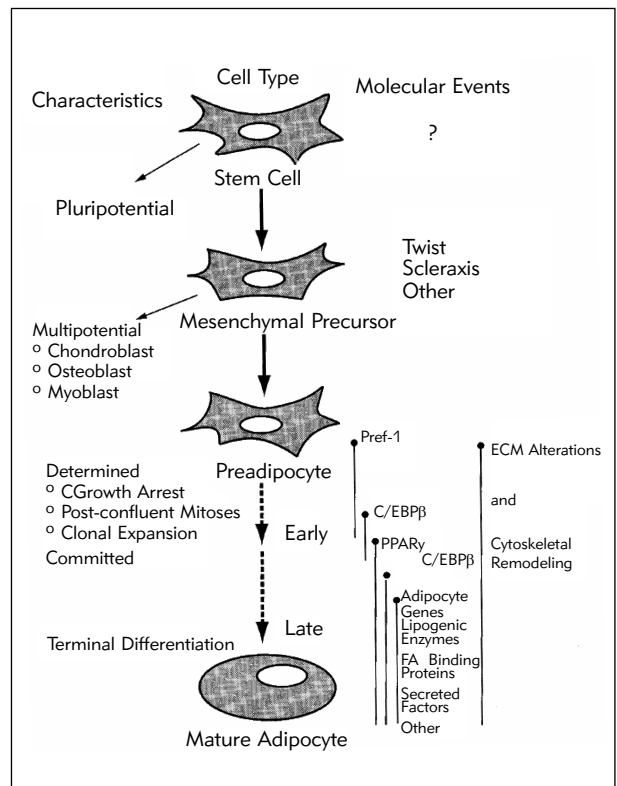

Figure 1   Differentiation of adipose tissue (Physiol Rev 1997; 78: 783–809).

resistin, and adipocyte-complement-related protein (Acrp30) begins, producing a highly specialized endocrine cell that will play key roles in various physiological processes. This complex series of events requires the cell to process a variety of combinatorial inputs during the decision to undergo differentiation. The identification of agents and molecules that modulate the adipogenesis process has provided insights into the signal transduction pathways involved. So far, a plethora of hormones, cytokines, and growth factors able to act as positive or negative adipogenic regulators have been identified. Their respective role on preadipocyte differentiation was recently reviewed in detail (10). Briefly, insulin, IGF-1, glucocorticoids, and agents that increase intracellular cAMP levels are generally recognized as positive effectors. Cytokines, growth factors belonging to the TGF-β family, and protein kinase C (PKC) inhibitors are viewed as negative regulators.

Mature adipocytes, the main cellular component of white adipose tissue (WAT), are uniquely equipped to function in energy storage and balance under tight hormonal control. However, with the realization that adipocytes secrete factors known to play a role in immunological responses, vascular diseases, and appetite regulation, a much more complex and dynamic role of WAT has emerged. In addition to proteins involved in lipid and lipoprotein metabolism, cytokines,

and growth factors, adipocytes also synthesize factors involved in the regulation of food intake and energy homeostasis. Adipocyte-derived factors include leptin, adipsin, acylation stimulation protein, agouti, angiotensin II, prostaglandins, Acrp30, resistin, TNF-β, macrophage migration inhibitory factor, secreted protein acidic and rich in cysteine (SPARC), and PPAR angiopoietin related (PGAR)/fasting-induced adipose factor (FIAF) (13).

Adiponectin

An adipokine that has been the focus of a large number of studies over the last couple of years is the protein adiponectin. Despite the fact that adiponectin is produced exclusively in adipocytes, its serum levels tend to be lower in patients with increased fat mass. Some of the best correlations can be seen with insulin sensitivity, whereby higher levels of serum adiponectin are associated with increased insulin sensitivity. Patients with cardiovascular disease and other states associated with increased inflammation tend to have decreased levels of adiponectin. Consistent with the improved insulin sensitivity generally observed in female compared with male subjects, adiponectin levels are higher in women than in men. Patients with type 2 diabetes and other diseases associated with reduced insulin sensitivity, such as generalized or HIV-induced lipodystrophies, have decreased levels. Frequently, decreased adiponectin levels are not only observed in association with type 2 diabetes and cardiovascular disease but also serve as powerful predictors for the future development of these syndromes even in the absence of any other manifestations of the disease.

Works in many laboratories, among them Takashi Kadowaki's and Yuji Matzusawa's group (14, 15), have studied the underlying molecular mechanisms for the antiatherosclerotic effects of adiponectin, which has antiinflammatory properties as well as effects on smooth muscle cell proliferation and the suppression of the conversion of macrophages to foam cells. The absence of adiponectin leads to increased neointimal proliferation in response to vascular cuff injury in the absence of adiponectin.

Adiponectin circulates in several different size complexes in serum. Its basic unit is a homotrimer. These homotrimers can assemble into higher-order structures, such as a hexamer, and several of these hexamers can assemble into a high-molecular weight (HMW) complex. All three forms can be found in serum. These structural variations have important biological implications. For instance, the higher levels of adiponectin in female subjects are primarily due to increased levels of the HMW complex. A metabolic challenge, such as a glucose or insulin infusion, results in a selective and transient reduction of the HMW form in circulation. The lower-molecular weight hexamer, in contrast, is not affected under those conditions. Very little information about the physiological changes related to the trimer is available at this stage (15).

Since lipid accumulation in tissues such as liver and muscle has a dramatic negative impact on the insulin sensitivity of these tissues, during times of caloric excess, depositing lipids into adipocytes is far more desirable than depositing lipids into these other tissues. In this context, we could view adiponectin as a starvation signal produced and released from the adipocyte, mediating a redistribution of lipid deposition away from tissues such as liver and muscle into adipose tissue where these triglycerides can be stored in a more inert fashion. As a result, decreased lipid levels in muscle, and particularly in the liver, cause improvements in insulin sensitivity. Overall, the adiponectin-mediated redistribution of triglycerides is remarkably similar to the actions of PPAR-α agonists. The proposed mechanism of action of these insulin-sensitizing compounds relies to a large extent on the ability to redistribute triglycerides to adipose tissue and to partition triglycerides within the adipose tissue into an increased number of smaller adipocytes.

Adiponectin seems to be the most interesting and promising biologically active molecule released from fat cells since it has profound protective actions in the pathogenesis of diabetes mellitus and cardiovascular disease. This protein is also called ADIPOQ, gelatin-binding protein 28, Acrp30. It was discovered in 1995, at about the same time as leptin, as a product of the adipose tissue most abundant gene transcript (16). Adiponectin, a protein synthesized almost exclusively by fat cells, plays an important role in the regulation of whole body energy homeostasis, glucose and lipid metabolism and antiinflammatory responses in the vascular system (17). Human plasma adiponectin concentration is about 1000 times higher than that of any other hormone and is higher in women than in men. Adiponectin levels are decreased in obesity, subjects with insulin resistance, type 2 diabetes and dyslipidemia and are particularly low in subjects with coronary artery disease. Adiponectin increases insulin sensitivity in various models of insulin resistance and in vitro increases the ability of subphysiologic levels of insulin to suppress glucose production in isolated hepatocytes. This protein intensifies peripheral tissue sensitivity to insulin and its deficiency can contribute to the development of insulin resistance in type 2 diabetes and obesity (17). The most important feature of adiponectin is its lower expression in the adipose tissue and lower concentration in plasma in overweight, obese and diabetic patients. Plasma adiponectin is negatively correlated with the BMI, visceral fat volume, waist/hip ratio, fasting plasma insulin, plasma glucose and triglyceride concentrations (18). Furthermore, it positively correlates with HDL-cholesterol level.

Adiponectin is also involved in the regulation of energy balance and body weight and it reduces weight gain. Weight loss leads to increased levels of that adipokine in plasma. Obese patients who received gastric partition surgery (gastric stapling) showed a 21% reduction in mean BMI that was accompanied by a 46%, in average, increase in plasma adiponectin values.

Circulating concentrations of most hormones produced by the adipose tissue, including leptin, tumor necrosis factor (TNF-α), plasma activator inhibitor 1, and ASP, are positively related to body adiposity. In contrast, circulating adiponectin concentrations are reduced in obese animals and humans (19, 20). One explanation is that adiponectin is primarily produced by visceral adipose tissue, but that large triglyceride-filled visceral adipocytes produce less adiponectin. It has been reported that omental adipocytes secrete more adiponectin that adipocytes isolated from subcutaneous fat. The known insulin-sensitizing actions of adiponectin suggest that reduced adiponectin production may contribute to the well-known relationship between visceral fat deposition and insulin resistance. Humans with severe insulin-resistant diabetes due to dominant-negative mutations that inactivate peroxisome proliferator-activated receptor-γ 128 have very low circulating adiponectin levels (21).

Adiponectin plays an important role in the regulation of lipid metabolism (increases fuel oxidation) and carbohydrate metabolism (improves hepatic insulin sensitivity). Adiponectin has been shown to exert antiinflammatory and antiatherogenic properties within the arteries and thus may negatively modulate the process of atherogenesis (17, 18). The importance of adipokines, especially focusing on adiponectin, is discussed with respect to cardiovascular disease. High concentrations of adiponectin are found in the bloodstream inside the vascular walls. Matsuzawa (13) have shown that adiponectin can enter the vascular walls, bind specifically to collagen types I, III, V and VIII present in the vascular intima and selectively accumulate in injured vessel walls, indicating that it may be involved in the repair process of damaged vasculature. Recent studies suggest that adiponectin may play a role in the modulation of inflammatory vascular response by suppressing the expression of adhesion molecules on endothelial cells, inhibiting endothelial cell NF- B signaling and suppressing macrophage function (foam cell formation). In doing so, adiponectin inhibits the development of atherosclerotic plaques (19). It was also suggested that adiponectin gene variations are associated with the risk of myocardial infarction and ischemic stroke; in particular selected gene variants were found to be associated with diminished cardiovascular risk in subjects with or without diabetes (22).

Resistin

Resistin is an adipocyte-derived secreted product that potentially links obesity to diabetes (5). It belongs to a family of tissue-specific secreted proteins that include resistin-like molecules α and ß (RELM-α and RELM-ß). Resistin is exclusively expressed in the adipose tissue; RELM-α has a restricted tissue distribution with α highest levels in adipose tissue and RELM-ß is expressed only in the gastrointestinal tract (23). Resistin is a thiazolidinedione-regulated protein as evidenced by the finding that thiazolidinedione treatment decreases resistin mRNA in 3T3-L1 adipocytes. Resistin mRNA is markedly induced during adipocyte differentiation as well as in diet-induced and genetic forms of obesity. Its expression is under nutritional and hormonal regulation, with resistin message being very low during fasting and in WAT derived from streptozotocin-treated (i.e., insulin-deficient) rats, and increasing upon refeeding and insulin administration, respectively (5). Immunoneutralization of circulating resistin improves blood glucose levels and insulin action in high fat-fed mice, suggesting that elevated resistin levels may result in decreased insulin sensitivity in obese rodents (5). Interestingly, resistin also exerts an inhibitory effect on adipocyte differentiation; i.e., treatment of 3T3-L1 preadipocytes with conditioned medium from COS cells transfected with murine resistin markedly decreases expression of adipocyte markers. This indicates that resistin may function as a feedback signal to restrict adipocyte formation (13). Such a finding implies that adipocytes express resistin receptors and that resistin is likely to have autocrine, paracrine, and endocrine functions. However, further investigations are required to determine the precise role played by resistin *in vivo*.

Leptin

Leptin, the obese (ob) gene product, is a hormone that is primarily made and secreted by mature adipocytes and that plays a crucial role in the regulation of energy balance. Current knowledge on leptin production, regulation, and action has been recently reviewed (24, 25). The functions of agouti, acylation stimulation protein, angiotensin II, and prostaglandins in the regulation of energy balance and whole-body homeostasis have also been recently described.

It is now apparent that the primary importance of leptin in the regulation of energy homeostasis is for reduced leptin production to function as a signal of negative energy balance and low energy reserves, rather than as an indicator of positive energy balance and increased energy reserves in the prevention of obesity. Accordingly, the physiological effects of decreased leptin concentrations are notably more pronounced than when leptin levels are increased above the normal physiological range. Thus, the dose

response to increasing leptin concentrations appears to be near maximal at physiological levels. As in rodents, genetic mutations in the leptin gene (26, 27) or defects in the leptin receptor (28) in humans result in extreme hyperphagia and obesity. Treatment with recombinant leptin reduces the marked hyperphagia and produces weight loss in leptin-deficient subjects (29). Leptin administration corrects many of the neuroendocrine, reproductive, metabolic, and immune system deficits associated with leptin deficiency (30).

Heterozygous mutations of the leptin gene result in a partial deficiency syndrome characterized by increased body adiposity. Physiological leptin replacement prevents the onset of hyperphagia in untreated insulin-deficient diabetes and the increase of food-seeking behavior in energy-restricted rats. Increased sensations of hunger during dieting are related to the magnitude of decreases of leptin (31, 32), and in one study, reduced appetite was reported in humans treated with leptin. In addition, it was recently demonstrated that the normal compensatory decreases of energy expenditure and thyroid axis function in response to consuming an energy-restricted diet in humans were prevented by low-dose leptin replacement. Together, these data suggest that decreases of leptin during weight loss could contribute to hunger, a lowered metabolic rate, and weight regain. New studies are needed to determine whether leptin replacement, or the use of strategies to increase endo-genous leptin production to prevent the fall of leptin during dieting and weight loss, will help prevent weight regain in weight-reduced subjects.

Leptin acts within the CNS to inhibit food intake and increase energy expenditure, perhaps via its effects to activate the sympathetic nervous system. Leptin also influences the reproductive and neuroendocrine function. Leptin can increase insulin sensitivity, and this action appears to be mediated by direct and indirect (CNS) effects to activate AMP kinase (AMP-K) and increase muscle fatty acid oxidation (FAOx), leading to decreased intramyocellular lipid (IMCL) content. In addition to the CNS, leptin receptors are also found in numerous peripheral tissues where the hormone exerts diverse effects. Leptin secretion is primarily mediated by changes in adipocyte glucose metabolism driven by increases or decreases of meal-induced insulin secretion. Catecholamines and TZDs have been reported to inhibit leptin production; however, the physiological role of these mechanisms has not been definitively established. ASP has anabolic effects to increase triglyceride (TG) synthesis by increasing adipocyte glucose uptake, activating DGAT, and inhibiting hormone-sensitive lipase (HSL). ASP has recently been shown to stimulate insulin secretion. ASP deficiency results in obesity resistance and increased insulin sensitivity. ASP production is stimulated by insulin and by the presence of chylomicrons/VLDL after meals. Adipo-
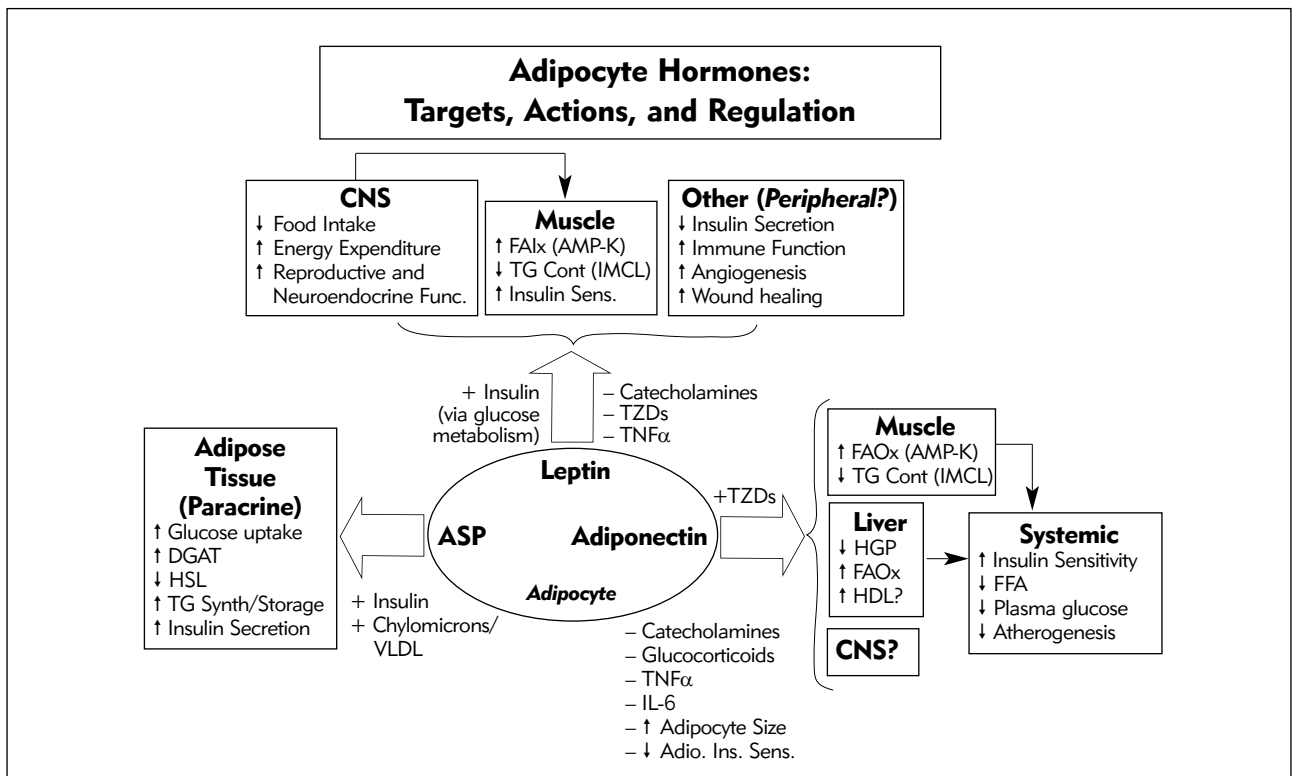

Figure 1 The function of adipocyte (21).

nectin increases insulin sensitivity, decreases hepatic glucose production (HGP), and lowers glucose plasma levels. The insulin-sensitizing effects of adiponectin appear to be mediated by activation of AMP-K, resulting in increased FAOx, and a lowering of hepatic triglyceride and IMCL content. TZD agonists of via peroxisome proliferator-activated receptor (PPAR-γ) may increase insulin sensitivity by stimulating adiponectin production. Adiponectin expression and secretion are inhibited by catecholamines, glucocorticoids, TNF-γ interleukin-6 (IL-6), increased adipocyte size, and possibly decreased adipocyte insulin sensitivity (21) (*Figure 2*).

Acylation stimulating protein

The acylation stimulating protein (ASP) is a unique hormone produced from complement factor C via an interaction requiring factor B and adipsin (factor D), resulting in the formation of the C3, C3a-des-Arg, which is also known as ASP. ASP acts locally in adipose tissue, where it stimulates glucose uptake, increases the activity of diacylglycerol acyltransferase, and inhibits hormone-sensitive lipase activity. (*Figure 2*). These actions of ASP increase the efficiency of triglyceride synthesis and storage in adipocytes. Results from the genetic study demonstrating that plasma ASP levels are related to genes controlling total cholesterol, LDL and triglyceride levels support a role for ASP in the regulation of lipid metabolism in humans (21). ASP action is also a determinant of energy homeostasis and insulin action. Adiposity is an important determinant of circulating ASP levels, which are elevated in obese subjects in proportion to body adiposity.

Visfatin

In 2004, Fukuhara et al. identified a molecule that is expressed at much higher levels in visceral fat than in subcutaneous fat which was named visfatin (33). This adipokine is highly expressed in the visceral adipose tissue of both humans and rodents. Visfatin was found to be identical to a cytokine expressed by lymphocytes – the pre-B cell colony-enhancing factor (PBEF). Visfatin binds to the insulin receptor at a site distinct from insulin and exerts a hypoglycemic effect by reducing glucose release from hepatocytes and stimulating glucose utilization in peripheral tissues. Since insulin-mimetic actions of visfatin may be part of the feedback regulation of glucose homeostasis, a hypothesis may be raised that visfatin concentrations are influenced by glucose or insulin blood levels in humans. This possibility offers new therapeutic options for diabetics (16, 33).

Brendt et al. (35) examined whether the visfatin plasma concentration and mRNA expression in visceral and subcutaneous fat correlates with anthropometric and metabolic parameters in subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance (34). They have found correlations between visfatin plasma concentrations and visceral visfatin mRNA expression and the measures of obesity but not with visceral fat mass or waist-to-hip ratio. Surprisingly, they did not find any differences in visfatin mRNA expression between visceral and subcutaneous adipose tissue (34). Further study of visfatin's physiological role may lead to new insights into glucose homeostasis and its dysregulation in obesity-related diseases, such as diabetes mellitus and cardiovascular disease.

Apelin

A novel adipokine apelin, produced and secreted from fat cells, was discovered recently. This bioactive peptide is the endogenous ligand of the orphan G protein-coupled receptor, APJ. Apelin may act as a potent vasodilator, thus lowering blood pressure, and exerting positive inotropic effects in rats and humans. Furthermore, the apelin system may modulate pituitary hormone release and food and water intake, regulate insulin sensitivity, play a role in stress activation (30). This neuropeptide is involved in the regulation of body fluid homeostasis and cardiovascular functions. Moreover a recent study showed that apelin acts as an angiogenic factor for endothelial cells and exerts potent diuretic effects through inhibition of arginine vasopressin (AVP) neuron activity and AVP release (16, 35)

Eotaxin

Eotaxin is a chemokine produced by fat tissue. The eotaxin family comprises three distinct peptides (eotaxin, eotaxin-2 and eotaxin-3) which have been implicated in eosinophilic inflammation. Eotaxin binds with high affinity and specificity to the chemokine receptor CCR3 and plays an important role in the pathogenesis of allergic disease. Eotaxin belongs to CC chemokines with selective activity for eosinophils and basophils and it is important in extrinsic asthma, an inflammatory disorder. Asthma is often more severe in the obese subjects. Eotaxin and cytokines produced by adipose tissue may possibly directly influence airways hyperresponsiveness, leading to an increased prevalence and severity of asthma symptoms in obese individuals. Circulating eotaxin levels are increased in diet-induced obesity in both mice and humans, and eotaxin mRNA levels were high in visceral adipose tissue in both species. Diet-induced weight loss in humans led to a reduction in plasma eotaxin levels (36).

An other study showed that a reduced level of circulating eotaxin-3 may represent a potentially powerful biochemical marker for predicting future adverse cardiac events in patients with coronary artery disease

(CAD). *In vitro* and clinical studies suggest that eotaxins could play a role in vascular inflammation, but no data are available on their prognostic significance in patients with angiographically documented coronary artery disease (37).

Conclusion

We aimed to provide a concise summary of actual knowledge on the important adipokines, and to give an update on the latest findings and current fields of adipokine research in association to obesity

and obesity-related diseases. The molecular effects of adipokines are a challenging area of research and their in-depth understanding will undoubtedly lead to the discovery of effective therapeutic interventions. The disturbances in expression, synthesis and release, function and balance of adiponectin, visfatin, resistin and eotaxin may be considered not only as a link between visceral adiposity and cardiovascular risk but also as independent risk factors for coronary heart disease. Elucidation of the mechanisms linking obesity, diabetes and atherosclerosis is fundamental for developing new therapeutic interventions.

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