



CONTENTS

Multiple facets of
metabolic syndrome

Complex milieu of the white
adipose tissue: secretion of
pro-inflammatory
adipokines & cytokines

Adipokines

Melanocortins

Expert commentary
& five-year view

Key issues

References

Affiliations

[†] Author for correspondence
Inova Fairfax Hospital, Center for
Liver Diseases, Department of
Medicine, 3300 Gallows Road,
Falls Church, VA22042, USA
Tel.: +1 703 698 3182;
+1 703 208 6650
Fax: +1 703 698 3482;
+1 703 208 6655
zobair.younossi@inova.com

KEYWORDS:
adiponectin, α -MSH, insulin
resistance, leptin, metabolic
syndrome, nonalcoholic fatty
liver disease, nonalcoholic
steatohepatitis, TNF- α ,
visceral adipose

Adipokines and melanocortins in the hepatic manifestation of metabolic syndrome: nonalcoholic fatty liver disease

Ancha Baranova, Manpreet Randhawa, Mohammed Jarrar
and Zobair M Younossi[†]

Metabolic syndrome is associated with nonalcoholic fatty liver disease and its more aggressive form, nonalcoholic steatohepatitis. Adipokines produced by white adipose tissue possess broad physiological activity and play an important autocrine role in obesity-associated complications, including metabolic syndrome, nonalcoholic fatty liver disease and cardiovascular disease. Various adipokines may have beneficial or harmful effects. Other tissues, particularly stomach and intestine, produce active molecules that can influence the function of adipocytes and, possibly, the levels of adipokine secretion. In some cases, the production sites of these molecules remain unknown. The review focuses on our current understanding of the disease-related effects of the adipokines and the melanocortins on various peripheral tissues, and discusses some of their potential interactions with each other. Potential therapeutic applications are also considered.

Expert Rev. Mol. Diagn. 7(2), 195–205 (2007)

Multiple facets of metabolic syndrome

Metabolic syndrome (MS) was first introduced as a clinical entity by Reaven in 1988 [1]. MS represents a cluster of risk factors commonly associated with central obesity, insulin-resistance, hypertension and elevated triglycerides, as well as decreased high-density lipoprotein cholesterol. In turn, MS is associated with an increased risk of cardiovascular disease and is a common early abnormality in the development of Type 2 diabetes. Additionally, MS plays a well-recognized role in the development of the obstructive sleep apnea, erectile dysfunction, polycystic ovary syndrome and malignant tumors. It is noteworthy that, despite tremendous clinical impact of MS, there is no clear consensus regarding the diagnostic criteria for this important nosological entity. To date, different criteria have been proposed by the WHO, by the third report of the National Cholesterol Education Program Adult Treatment Panel III (ATPIII) on Detection, Evaluation, and Treatment of High

Blood Cholesterol in Adults, International Diabetes Federation, European Group for the Study of Insulin Resistance, and American College of Endocrinology. The problem of multiple MS definitions was recently addressed in a joint statement of The American Diabetes Association and the European Association for the Study of Diabetes [2]. The statement and other publications stress the necessity of introduction of additional criteria for MS including concentrations of the pro- and anti-inflammatory proteins in the serum. It is most likely that the list of the etiological factors driving the development of MS includes abnormalities in visceral adipose tissue or an altered inflammatory background that, in turn, stimulate the development of the secondary complications of MS.

Recently, nonalcoholic fatty liver disease (NAFLD) and its more aggressive form, non-alcoholic steatohepatitis (NASH), have come to be regarded as the hepatic manifestation of MS. In this context, insulin resistance (IR) is

the key event linking NAFLD to the MS [3]. The epidemiology, pathogenesis and approach to treatment of NAFLD follow the same trends as all other metabolic disorders [4,5]. NAFLD represents a spectrum of clinicopathologic disorders. At one end of the NAFLD spectrum is simple steatosis, and at the other end is NASH, characterized by hepatic steatosis, hepatocyte ballooning degeneration, lobular inflammation with or without Mallory hyalines, or sinusoidal fibrosis [6]. To date, no treatment has been proven effective for NASH. Treatment strategies mostly pursue modification of underlying metabolic abnormalities such as Type 2 diabetes mellitus, hyperlipidemia and obesity [7].

The pathogenesis of NASH is multifactorial and is the subject of intense research. Potential theories include influences of the abnormalities of lipid metabolism and the production of reactive oxygen species, leading to increased hepatic lipid peroxidation, activated fibrocytes and abnormal patterns of cytokine production. These events can lead to multiple hits responsible for NASH-related liver cell injury and fibrosis [3]. In the multihit hypothesis of NASH, the first hit appears to be the accumulation of excessive fat in the hepatic parenchyma [7,8]. This first hit has been linked to IR, which has been consistently observed in patients with NASH [3]. The second hit involves oxidative stress, resulting from an imbalance between pro- and antioxidant processes [3,8]. Both the first and the second hits, which are considered important for development of NAFLD and its progression, are dependent on local and circulating levels of various pro- and anti-inflammatory cytokines, including adipokines.

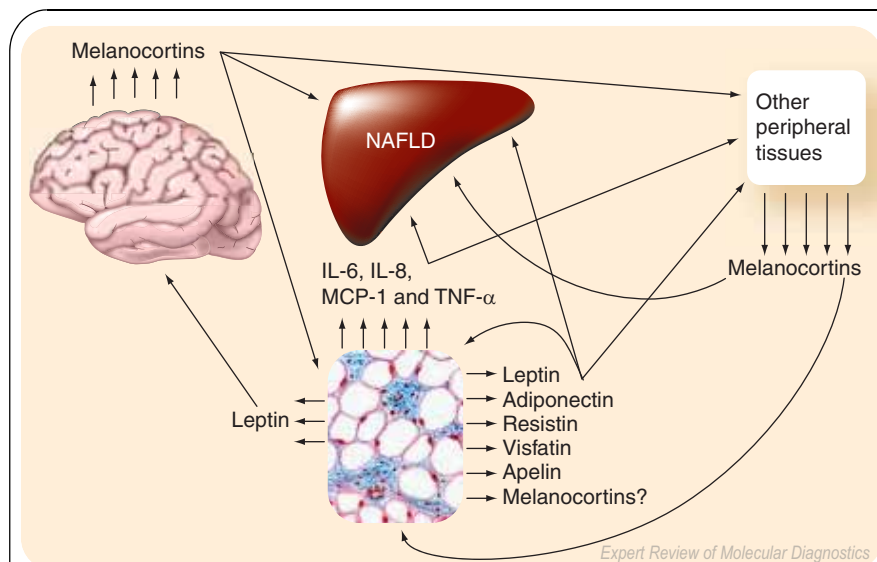
Complex milieu of the white adipose tissue: secretion of proinflammatory adipokines & cytokines

White adipose tissue (WAT) is increasingly recognized as an endocrine organ producing numerous proteins collectively referred to as adipokines. A partial list of these proteins

includes: adiponectin, resistin, leptin, visfatin, apelin, vaspin and other bioactive molecules. These proteins possess broad physiological activity and play an important autocrine role in obesity-associated complications, including MS, NAFLD and cardiovascular diseases [9].

In addition to adipokines in a *sensu stricto*, adipose tissue produces a number of traditional cytokines, such as tumor-necrosis factor (TNF)- α , monocyte chemoattractant protein (MCP) 1 and interleukin (IL)-6, thus adding pro-inflammatory pressure to other peripheral tissues of the human body. These traditional cytokines are mostly produced by macrophages embedded in the adipose stroma [10]. In addition to the elevation of the traditional cytokines, excessive adipose tissue also produces abnormal amounts of adipokines. In general, adipokines modulate many physiological processes, including energy homeostasis, lipid metabolism, blood-pressure regulation, insulin sensitivity and angiogenesis [11]. One example of the adipocyte cross-talk with other peripheral tissues has been discovered in the co-culture experiments of human fat and skeletal muscle cells [12]. It is now clear that the imbalance in the synthesis and secretion of adipokines negatively impacts skeletal muscles and promotes the vicious cycle of IR [13,14].

Systematic studies of cytokine and adipokine levels in patients with chronic diseases usually evaluate protein content in serum samples reflecting the total volume of the production of a particular molecule by all the tissues. Even the classical adipokines, including resistin and adiponectin, could be produced somewhere else in the body in addition to adipose depots, as was demonstrated both at the protein and mRNA level. Nevertheless, WAT remains the most important source of these molecules, especially in obese individuals. A net elevation in the WAT secretion of pro-inflammatory molecules comes from multiple sources, including overall increases in body fat volume and an increase in



the number of infiltrating macrophages per gram of fat that parallels increase in the fat mass during the progression of obesity [10,15]. Visceral adipose tissue releases more IL-6, transforming growth factor (TGF)- β , IL-8, IL-10 and some other cytokines per gram of tissue than abdominal subcutaneous adipose tissue [10]. Therefore, preferential accumulation of the visceral adipose in the central obesity of patients with so-called apple-shaped obesity provides the inflammatory background that predisposes these patients to the development of the MS and its complications.

It is important to emphasize that different adipokines may have beneficial or harmful effects. Furthermore, these effects can be tissue specific, leading to the development of a particular complication of obesity. Some adipokines contribute to the pro-inflammatory effects of the adipose accumulation, whereas others counteract

Figure 1. Complexity of interplay between cytokines and adipokines possibly involved in the development of insulin resistance, NAFLD and nonalcoholic steatohepatitis.

IL: Interleukin; MCP: Monocyte chemoattractant protein; NAFLD: Nonalcoholic fatty liver disease; TNF: Tumor necrosis factor.

IR or alleviate hepatic steatosis and fibrosis. However, specific signaling pathways evoked by adipokine binding to their specific receptors and subsequent molecular consequences of this binding are unknown in most cases. In addition to their paracrine effects in the distant peripheral tissues, adipokines could signal locally through receptors displayed at the membranes of the adjacent adipocytes. These signaling events may reciprocally modulate production of other adipokines and cytokines or cross-talk with other molecular cascades (FIGURE 1). In addition, other tissues, particularly stomach and intestine, produce active molecules that can influence the function of adipocytes and, possibly, the levels of adipokine secretion [16]. In some cases, the production sites of these molecules remain unknown. For example, melanocortins previously implicated in energy homeostasis in the hypothalamus circulate in the blood and exert numerous peripheral effects.

The remainder of this review focuses on our current understanding of the disease-related effects of the adipokines and the melanocortins on various peripheral tissues, and describes some of their potential interactions. Therapeutic implications are also discussed.

Adipokines

Adiponectin

Adiponectin, which is also known as AdipoQ and Acrp30 possesses structural homology to collagens and to complement factor C1q, and is downregulated in obese individuals. Many experimental studies have suggested that adiponectin plays a protective role in the development of IR, atherosclerosis and other inflammatory processes [17]. There is the possibility of some direct link between insulin and adiponectin signaling, as adiponectin stimulates the interaction between a small GTPase Rab5 and amyloid precursor protein-like protein (APPL)1, leading to increased glucose transporter (GLUT)4 membrane translocation [18]. Other pharmacological effects of adiponectin in reducing IR are related to a decrease in plasma fatty acid levels and in triglyceride content in muscle and liver, possibly by induction of acyl-CoA oxidase and uncoupling protein-2 [19]. Adiponectin activates adenosine monophosphate-activated protein kinase (AMPK) α 1 and α 2 by increasing Thr172 phosphorylation, an effect associated with increased acetyl-coenzyme A carboxylase (ACC)- β , Ser221 phosphorylation and enhanced rates of fatty acid oxidation [20]. Adiponectin-associated AMPK activation may result in a net decrease in hepatocyte triglyceride storage either by inhibiting genes required for *de novo* free fatty acid synthesis, or by increasing the activity of peroxisome proliferator-activated receptors (PPARs) resulting

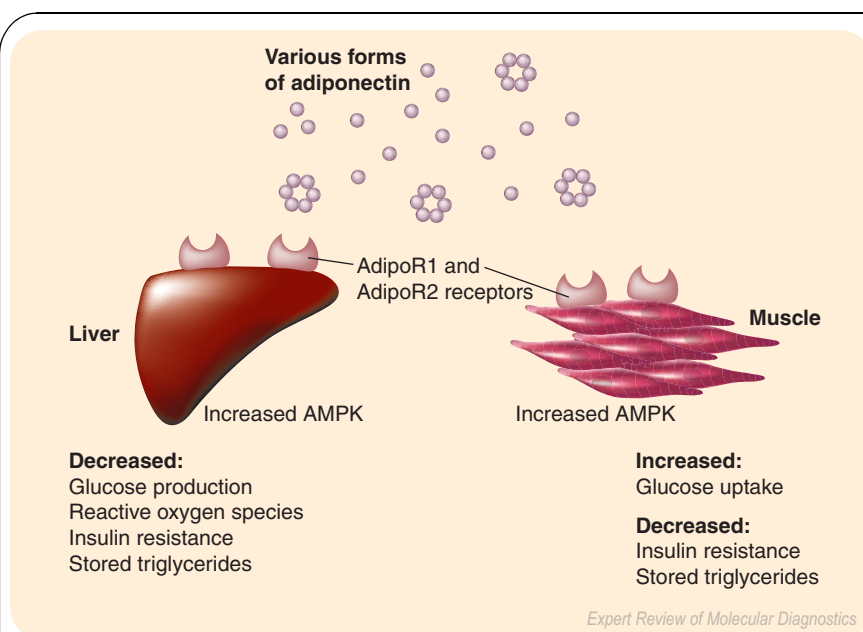


Figure 2. Physiological effects of adiponectin in liver and muscle tissue.

AdipoR: Adiponectin receptor; AMPK: Adenosine monophosphate-activated protein kinase.

in increased β -oxidation of free fatty acids. In addition, adiponectin regulates energy balance at the mitochondrial level through augmentation of the mitochondrial biogenesis, palmitate oxidation and citrate synthase activity, and the decrease of the reactive oxygen species levels [21]. Adiponectin also inhibits hypertrophic signaling in the myocardium, thus exerting beneficial actions on the heart after pressure overload and ischemia-reperfusion injury [22], as well as preventing vascular inflammation through selective increase of the tissue inhibitor of metalloproteinase (TIMP)-1 expression in human macrophages. IR-related peripheral effects of adiponectin are summarized in FIGURE 2.

The autocrine signaling mechanisms of adiponectin that act upon neighboring adipocytes are more poorly understood than its paracrine effects. In WAT, adiponectin acts as a key regulator of adipocyte secretory function [23] and differentiation [24]. In particular, adiponectin reduces the release of fat cell secretory products, including IL-6, IL-8, growth-regulated oncogene (GRO)- α , MCP-1, macrophage inflammatory protein (MIP)-1 α and -1 β , as well as TIMP-1 and -2 [23]. This function of adiponectin is consistent with the hypothesis that its obesity-related decrease profoundly perturbs the entire adipokine and cytokine milieu of the human body, providing a pro-inflammatory background that predisposes individuals to various comorbidities. Both adiponectin receptor (AdipoR)1 and 2 are expressed in adipocytes. AdipoR1 expression in adipose tissue is reduced in obese individuals and is increased after weight loss [25]. This may lead to a reduction in the biological function of the adiponectin in WAT, further aggravating the negative metabolic effect of low adiponectin levels.

Accumulating evidence suggests that hypoadiponectinemia may predispose morbidly obese patients to the secondary cardiovascular and hepatic complications. Plasma adiponectin levels

are significantly lower in NAFLD patients than in their matched controls [26,27]. This is also true for the patients with coronary artery disease [28]. Moreover, researchers report a similar relationship between low adiponectin production and other obesity-associated morbidities, including major depression [29], decreased ovarian function [30] and endometrial cancer [31]. These observations suggest that hypoadiponectinemia may be responsible for obesity-related health deterioration rather than being the molecular trigger for particular complications of MS.

The role of adiponectin in the pathogenesis of NAFLD and NASH is under investigation. Despite its clear involvement in NAFLD, adiponectin alone does not clearly distinguish patients with NASH from those whose liver biopsies demonstrate simple steatosis [27]. One study demonstrated that reductions in the levels of circulating adiponectin in NAFLD are related to hepatic insulin sensitivity and the amount of hepatic fat, but not to the severity of necroinflammation and fibrosis [32]. In contrast, some studies have reported that hypoadiponectinemia is associated with hepatic necroinflammation and fibrosis, independent of IR [33,34]. Lower levels of adiponectin in patients with NASH compared with those with simple steatosis, are accompanied by a decrease in AdipoR2 mRNA expression in NASH livers [35]. However, this correlation between adiponectin and AdipoR2 reduction is not uniform across the cell types, which is indicative of the complex regulation of adiponectin signaling within the liver [35].

In summary, despite the recent excitement about the role of adiponectin in the pathogenesis of MS-related complications (e.g., coronary artery disease [CAD], IR and NAFLD) and its potential therapeutic roles, additional studies are needed to further elucidate and clarify this potential role.

Leptin

Leptin released by human adipocytes negatively regulates food intake and fatty acid metabolism through its actions on specific hypothalamic nuclei. Recent work shows that receptors to leptin, encoded by the gene *LEPR*, are expressed both centrally and peripherally. High mRNA levels of both short and long leptin receptor (LEPR) isoforms have been identified in the stomach, intestine, liver and pancreas [36].

Whether serum leptin elevation contributes a metabolic effect to the development of NAFLD is unclear. Higher than normal leptin concentrations are found in various types of NAFLD, including NASH [37,38]. Observations on serum leptin concentrations in 122 Japanese workers indicated a correlation with serum activity of alanine aminotransferase (ALT) [39]. Because patients with hepatic viral infection, autoimmune liver diseases, liver cirrhosis and drinking problems were excluded from this study, the NAFLD type of the metabolic disorders was expected to be a major cause of increased transaminase levels in the studied cohort. The absence of significant associations between ethanol consumption or lifestyle parameters and serum leptin levels support the assumption that leptin directly promotes fat accumulation in the liver [39].

Leptin can contribute to the development of the NASH in two ways. First, a dysfunctional leptin system promotes IR and alters insulin signaling in hepatocytes to increase fatty acids influx and induce lipotoxicity. Recent experiments modulating hepatic triglyceride (TG) stores in a genetically leptin-resistant rat with removed WAT confirmed that resistance to leptin is the major physiological mechanism for hepatic steatosis [41]. This view is also supported by the observation that leptin levels correlate directly with the severity of hepatic steatosis, but not with inflammation or fibrosis [42].

Also, in a later stage, leptin may contribute to the progression of steatosis to steatohepatitis by enhancing the systemic low-grade inflammation that could be evaluated by monitoring the serum concentrations of C-reactive protein [43]. In particular, activation of the LEPR in hepatic stellate cells leads to increased expression of proinflammatory cytokine MCP-1 and proangiogenic vascular endothelial growth factor (VEGF) and angiopoietin-1 [44]. It was also postulated that leptin acts as a pro-fibrogenic cytokine in sinusoidal microenvironment by acting both on endothelial cells and Kupffer cells [45], as it participates in the development of the liver fibrosis associated with chronic viral infections and with primary biliary cirrhosis [46].

The involvement of the leptin in the development of the fibrosis in NAFLD is less obvious; a longitudinal study of NAFLD patients revealed no differences in leptin levels in patients with fibrosis progression and those who did not progress [47]. On the other hand, an increase in expression was found both for LEPR mRNA and its protein in patients with NASH, especially those with fibrosis [48]. There was a strong correlation between the expression of the LEPR and profibrogenic factor TGF- β [48]. It is possible that leptin directly stimulates TGF- β production in the liver, as it does in peritoneal mesothelial cells [49]. Another mechanism of the fibrogenic action of the leptin is its direct stimulation of Collagen I and III mRNA transcription, which is pronounced at concentrations observed in obesity (30–50 ng/ml), but not concentrations observed in nonobese individuals (<10 ng/ml) [50].

Resistin & resistin-like molecules

Resistin and resistin-like molecules (RELM) comprise a family of proteins related to IR and inflammation. Resistin, also known as FIZZ3, is increasingly recognized as a factor that directly causes IR in animal models [51]. Treatment of skeletal muscle cells with recombinant resistin reduces the function of insulin receptor substrate (IRS)-1 and serine/threonine kinase Akt1 and decreases the translocation of GLUT4 and glucose uptake in response to insulin [52]. Resistin also decreases fatty acid oxidation in skeletal muscle while increasing intracellular lipid accumulation [52].

Resistin plays several roles in adipose tissue. In 3T3-L1 adipocytes, resistin impairs insulin action at multiple steps in the signaling cascade, leading to GLUT4 inhibition and the reduction of the glucose uptake by 30% [53]. Recent findings also suggest that resistin inhibits adipocyte differentiation [53]. Differentiated 3T3-L1 adipocytes that overexpress resistin produce

much larger quantities of the proinflammatory cytokines, particularly $\text{TNF}\alpha$, IL-6 and MCP-1, which play important roles in IR and in glucose and lipid metabolism during adipogenesis [53]. Resistin may also influence the proliferation of muscle cells and the fibrotic process. The introduction of RELM- α (a homologue of the resistin) into fibroblasts boosts the deposition of collagen and stimulates myofibroblast differentiation [54]. RELM- α also possesses angiogenic and vasoconstrictive properties that exceed those of endothelin-1 or angiotensin II [55], and plays a role in bone metabolism [56].

It should be noted that these findings are based on mouse models and rodent cell lines, and that human resistin signaling may be different (there are substantial differences in the resistin encoding genes in rodents and humans). For example, resistin mRNA expression levels in adipose tissue and serum levels of resistin are extremely low in humans (one of 250 of that seen in the mouse). The spectrum of RELMs in humans and mice are also different. In fact, the resistin- α -encoding gene is absent in humans. These differences suggest that the family of resistin-encoding genes is not been well conserved in evolution and it functions differently in different species [57]. For example, adipocytes treated with human resistin demonstrate an intact insulin response [58]. Together, these findings suggest that researchers should exercise caution when interpreting resistin-related results obtained in rodent models and generalizing to humans.

Clinical studies using enzyme-linked immunosorbent assay (ELISA)-based measurements of resistin point to its potential role in the pathogenesis of obesity-mediated IR and Type 2 diabetes mellitus [59]. However, other clinical studies have failed to establish a direct connection between resistin, IR and obesity [60,61]. The role of resistin in the pathogenesis of NAFLD is under investigation. Due to its pro-inflammatory properties, resistin may play a role in the development of NASH in individuals with fatty liver. However, the relationship between serum levels of resistin and NAFLD pathologies remains elusive. One study demonstrated that plasma resistin concentrations correlate positively with hepatic fat content [62], but another study demonstrated that these correlations were negative [63]. Furthermore, quantification of the resistin mRNA in subcutaneous adipose tissue led to the conclusion that, in NAFLD, hyper-resistinemia is related to the histological severity of liver disease, but not to IR or body mass index (BMI) [64]. Other researchers report that plasma resistin concentrations are similar in NASH and BMI-matched non-NASH controls [34,65]. These contradictory data reflect our current lack of understanding regarding the role of resistin in the pathogenesis of NASH. Continued investigations into the molecular, physiological and clinical studies are needed to determine the role of resistin in the pathogenesis of NAFLD.

Visfatin & apelin

Apelin and visfatin are newly discovered hormones secreted by adipose tissue that undergo compensatory upregulation with obesity [66,67]. These hormones could theoretically exert beneficial effects through partial alleviation of some of the obesity-related

complications [68]. It is important to note that, in addition to visceral and subcutaneous adipose, apelin and visfatin are synthesized elsewhere, particularly skeletal muscle, liver and osteoblasts [66,68]. Therefore, these proteins can not be called adipokines in a *sensu stricto*.

Visfatin is capable of lowering plasma glucose levels, mimicking the effects of insulin on various tissues [69]. For example, visfatin binds to and activates the insulin receptor and stimulates phosphorylation of both IRS-1 and -2, leading to the activation of the downstream Akt and mitogen-activated protein kinases (MAPKs) [68,69]. In addition to insulin-like action, visfatin also possesses an enzymatic function that provides nicotinamide mononucleotide (NMN) for a NAD^+ biosynthetic pathway [70]. Enzymatic function of visfatin as the rate-limiting component in the NAD biosynthesis regulates the transcriptional function of SIRT1, NAD^+ -dependent deacetylase, which modulates gene silencing, aging and controls the metabolism of WAT. In turn, activated SIRT1, suppresses PPAR- γ , the nuclear receptor that promotes adipogenesis; the effect is lipolysis and loss of fat [71]. Therefore, visfatin activity should be antagonistic to thiazolidinediones activity, antidiabetic drugs known to stimulate the adipose mass resulting in weight gain.

It is not known whether increased visfatin production is a simple reflection of the visfatin resistance that could parallel the IR often accompanying morbid obesity, or whether it represents an important compensatory pathway. The latter hypothesis is more plausible, as common polymorphisms in the promoter of the visfatin gene pre-B-cell colony-enhancing factor (*PBEF*) 1 influence plasma insulin and glucose levels [72]. The fact that circulating visfatin levels increase along with the deterioration of the pancreatic β -cells also corroborates the compensatory hypothesis [73]. Nevertheless, repeated attempts to correlate serum levels of the visfatin with various clinical parameters of MS have produced contradictory results. Relationships between visfatin levels and diseases of the NAFLD spectrum are unknown.

Apelin, another adipokine, has been associated with peripheral IR because it inhibits glucose-stimulated insulin secretion through its receptor apelin-angiotension (APJ), which is expressed in β -cells of pancreatic islands [74]. Apelin expression in adipocytes is strongly inhibited by fasting and is recovered after re-feeding, and is directly enhanced by insulin [67]. In addition to its pancreatic action, apelin reduces blood pressure by inducing nitric oxide (NO)-mediated vasorelaxation and inhibiting water intake [68]. In muscle cells, apelin exerts a selective positive inotropic action and induces contraction [75]. Apelin is also expressed in human osteoblasts and stimulates their proliferation [76].

In mice, apelin expression in fat cells and plasma apelin levels are largely increased in all the hyperinsulinemia-associated obese states, independent of diet composition [67]. Plasma apelin levels are also significantly higher in obese individuals than in normal controls [67,77]. Interestingly, apelin is upregulated by $\text{TNF}\alpha$ in human adipocytes, suggesting another possible link between IR and inflammation [78].

Melanocortins

MSH- α & MCH

Historically, the melanocortin system has been linked to the control of skin and hair pigmentation. Recently, this system has been implicated in the regulation of energy homeostasis and the control of metabolism. A major component of the melanocortin system, α -melanocyte-stimulating hormone (MSH), is produced proteolytically from the pro-opiomelanocortin precursor peptide and serves as a feeding suppressor. Genetic defects inactivating the receptors for α -MSH have lead to obesity in experimental animals and humans [79]. Melanin-concentrating hormone (MCH), an antagonist of α -MSH, is an appetite-stimulating cyclic neuropeptide encoded by the gene *PMCH*. Both peptides are produced in the hypothalamus and act centrally. In addition, α -MSH and MCH peptides are produced in various peripheral tissues, including adipose and exert profound autocrine and paracrine effects. It is likely that the peripheral action of α -MSH/MCH participates in the development of IR and its hepatic manifestation: NAFLD.

Little is known about the signaling events following activation of the MCH receptors (MCH-R1 or -R2). MCH-R1 appears to couple to three different $G\alpha$ subunits, $G\alpha i$, $G\alpha o$ and $G\alpha q$, demonstrating the potential for this receptor to activate multiple signaling cascades [80]. Downstream of $G\alpha$, MCH induces activation of p44/42 (extracellular signal-regulated kinase [ERK]1/ERK2) MAPK and pp70 S6 kinase activities [81]. Interest in α -MSH has been enhanced by the finding that this peptide and its synthetic analogues protect against ischemia/reperfusion injury in various tissues. In myocytes, α -MSH induces the expression of cytoskeleton proteins and represses immune, inflammatory, cell cycle and protein turnover mediators [82]. Another interesting consequence of α -MSH signaling is the suppression of the collagen synthesis and deposition [83]. Furthermore, in fibrotic tissues, α -MSH treatment modulates the balance between matrix metalloproteinase (MMP)-1, MMP-8 and their inhibitors (TIMPs) [84].

In adipose tissue, α -MSH inhibits leptin secretion in differentiated rat adipocytes cultured *in vitro*. In turn, leptin administered to ob/ob mice increases the release of α -MSH into the circulation, suggesting a possible feedback loop between the sites of α -MSH release and the release of leptin from the adipose tissue [85,86]. The physiological significance of this putative feedback loop probably depends upon the underlying state of energy balance, because low plasma levels of α -MSH in fasting animals are paralleled by low plasma leptin [85]. Among other α -MSH-dependent peripheral effects relevant to MS is the decrease in insulin-secretion by the pancreatic β -cell [87] and stimulation of lipolysis [88].

Recent studies have focused on the immunosuppressive and anticytokine functions of α -MSH [89]. Both peripheral blood cells and macrophages residing in human tissues are responsive to α -MSH. In particular, α -MSH downregulates the endotoxin receptor CD14 present on the macrophages, induces neutrophil

elastase, decreases the production of interferon- γ by human T cells and modulates immunoglobulin E synthesis by human B cells [89]. In many cell types, α -MSH specifically down-regulates pro-inflammatory transcription factor nuclear factor (NF)- κ B. Furthermore, α -MSH inhibits systemic production of NO and the chemoattractant chemokines, thus modulating inflammatory cell infiltration. Because the same type of the chemokines (e.g., CCL2/MCP-1, CCL19, IL-6 and IL-8) have been attributed to the progression of simple steatosis to NASH, it is reasonable to hypothesize that α -MSH may play a role in counteracting this progression [90,91]. In addition, broad anticytokine effects of α -MSH suggest that this peptide may also regulate adipokine release, thus producing secondary effects on NAFLD.

Accumulating evidence suggests that α -MSH mediates liver injury modulation. α -MSH reduces endotoxin-induced liver inflammation in the mouse model [92], protects against thioacetamide-induced acute liver failure [84] and attenuates carbon tetrachloride (CCl_4)-induced liver fibrosis [93]. The most likely mechanisms for this protection include α -MSH-dependent inhibition of cell adhesion molecules and cyclooxygenase (COX)-2 expression, as well as the collagenolytic effects exerted through MMP and TIMP modulation [84]. Since hepatic fibrosis may progress in patients with NASH (as with patients with other chronic liver diseases), the anticollagenic effects of α -MSH may be beneficial. In fact, daily or twice-daily administration of α -MSH reduces the symptoms of several other inflammatory diseases in animal models [94]. Additionally, α -MSH may prolong allograft survival of the experimental heart transplants [82]. Finally, the beneficial effects of α -MSH have also been demonstrated in several ischemia/reperfusion models [92,95,96]. Together, these findings suggest that α -MSH has a potential hepatoprotective effect.

Studies of serum α -MSH levels in humans are scarce. One study demonstrates that plasma levels of α -MSH are significantly elevated in obese humans, and that this elevation correlates closely with fat mass ($r = 0.586$; $p < 0.001$) and with leptin levels ($r = 0.41$; $p < 0.05$) [85]. Another study involving obese and lean females did not reveal any obesity-associated changes in cerebrospinal fluid or plasma α -MSH levels [97]. Intranasal administration of α -MSH to lean subjects results in distinct reductions of body weight and body fat, which are accompanied by significant decreases in leptin and insulin plasma concentrations [98]. It has been suggested that, unlike normal-weight humans, overweight subjects are not sensitive to the effects of α -MSH, due to central or peripheral resistance to this peptide [99].

The relationship between plasma levels of α -MSH and the presence or the progression of other chronic diseases is also unclear. For example, α -MSH plasma levels are elevated in patients with congestive heart disease compared with controls [100], whereas α -MSH levels are decreased in patients with vitiligo [101]. Plasma α -MSH levels are low in samples collected from patients with septic shock at the beginning of septicemia, and gradually increase in patients who recover, but not in those

who die [102]. It will be interesting to know whether α -MSH levels change in NAFLD and NASH, the direction of these changes, and how they reflect the prognosis.

Expert commentary & five-year view

The importance of NAFLD as the hepatic manifestation of MS and IR has been increasingly recognized in the past decade. The development of its potentially progressive subtype, NASH, is a complex process that involves multiple mechanisms and is hastened by disturbances of adipocytic secretions (adipokines). The secretion of each adipokine that contributes to NASH depends on both circulating and local concentrations of other bioactive molecules produced in adipose tissue, as well as the macrophage infiltration of adipose tissue. For example, TNF- α potentially reduces the secretion of adiponectin and enhances the expression mRNAs encoding pro-inflammatory adipocytokines IL-6 and -8 [103]. Additionally, TNF- α contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes [104]. In turn, leptin enhances TNF- α production in lipopolysaccharide (LPS)-stimulated Kupffer cells [105]. TNF- α suppresses production of visfatin and stimulates apelin production [78,106]. These and other effects of TNF- α on the accumulation of the fat in the liver and its subsequent inflammation have been thoroughly reviewed [107].

Bioactive molecules released by adipocytes circulate in the serum and interact with corresponding receptors in liver cells and macrophages. These mutually regulating circuits contribute to the vicious pro-inflammatory circle favoring various complications of MS. Larger shifts in the serum content of certain molecules may preferentially drive the development of the particular comorbidities, such as NAFLD.

It is important to note that many of the peripheral signals mentioned in this review are beneficial for homeostasis of the peripheral tissues. Important examples are adiponectin and α -MSH, which may be capable of alleviating some of the complications of MS. Additionally, α -MSH receptors may represent potential targets for therapeutic intervention in NAFLD. Synthetic agonists of these receptors, melanotans I and II, have already been demonstrated as safe in human trials for therapeutic tanning [108]. A new MTII analogue, PT-141 (Palatin

Technologies, Inc.), which may improve sexual function in both males and females, is scheduled to enter pivotal Stage III clinical trials leading to commercialization [108]. The major difference between these trials and those aimed at the prevention or treatment of NASH is the necessity for long-term administration of these therapeutic compounds. The consequences of the long-term administration of α -MSH analogues have not been examined. The potential side effects of prolonged exposure to α -MSH include the possibility of developing resistance to its action [99] and increases in bone turnover, which may lead to a net loss of trabecular bone [109]. Both these side effects appear to be relatively mild, so controlled α -MSH application in patients with diseases within the NASH spectrum may be worth a try.

Adiponectin replenishment is another approach with potential therapeutic application for NASH. However, implementations of these clinical trials are more difficult because adiponectin is a relatively large protein. Furthermore, it has been shown that adiponectin circulates in serum both as a lower molecular weight hexamer and as larger multimeric structures of high molecular weight, which represents its active form [110]. As the formation of high-molecular-weight adiponectin complexes depends on its post-translational hydroxylation and glycosylation [111], it is unlikely that molecular drugs based on recombinant adiponectin will be created anytime soon.

Another breakthrough expected in the field of the adipokine research is the development of novel diagnostic methods based on the multiplexed evaluation of the adipokine and cytokine content of blood serum. Such diagnostics could provide a snapshot of the bioactive serum proteome and reveal the predisposition of an individual to secondary comorbidities within the metabolic spectrum. Early assessment of these predispositions could help clinicians provide patients with individualized recommendation for treatment and necessary lifestyle modifications.

The list of the adipokines and other bioactive molecules influencing the development of metabolic comorbidities is far from complete. A number of interesting molecules with unknown therapeutic potential have recently emerged, including vaspin [112], omentin [113] and others. The interplay between the signaling events produced by bioactive molecules, resistance to

Key issues

- Metabolic syndrome (MS) is often associated with nonalcoholic fatty liver disease (NAFLD) and its more aggressive form, nonalcoholic steatohepatitis (NASH).
- Adipokines produced by white adipose tissue possess broad physiological activity and play an important autocrine role in obesity-associated complications, including MS, NAFLD and cardiovascular disease.
- Adiponectin and leptin might influence the development of NAFLD and NASH directly.
- Among a plethora of novel soluble molecules regulating or related to fat metabolism, particular attention must be paid to melanocortins. α -melanocyte-stimulating hormone and similar anti-inflammatory peptides are safe and have a relatively simple structure, which makes them attractive candidates for future clinical trials.
- A number of well-known (e.g., tumor necrosis factor- α) and recently discovered (e.g., visfatin and omentin) molecules, are produced by omental adipose. These molecules warrant further investigation as possible participants in the MS milieu.

insulin and disturbance of lipid metabolism characteristic for morbid obesity, represent an important avenue for future studies on the pathogenesis, diagnosis and treatment of NAFLD and NASH.

Acknowledgement

Research on melanocortins was partially supported by a research grant from Thomas F and Kate Miller Jeffress Foundation (Ancha Baranova and Manpreet Randhawa).

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Reaven GM. Banting lecture. Role of IR in human disease. *Diabetes* 37, 1595–1607 (1988).
- **Systematic description of the metabolic syndrome.**
- 2 Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal. Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia* 48(9), 1684–1699 (2005).
- 3 Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin. Liver Dis.* 21(1), 27–41 (2001).
- **Summary of the multihit hypothesis of nonalcoholic steatohepatitis.**
- 4 Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr. Opin. Lipidol.* 16(4), 421–427 (2005).
- 5 Collantes RS, Ong JP, Younossi ZM. The metabolic syndrome and nonalcoholic fatty liver disease. *Panminerva Med.* 48(1), 41–48 (2006).
- 6 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 116(6), 1413–1419 (1999).
- 7 Younossi ZM, Diehl AM, Ong JP. Nonalcoholic fatty liver disease: an agenda for clinical research. *Hepatology* 35(4), 746–752 (2002).
- 8 Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology* 114(4), 842–845 (1998).
- 9 Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ. Res.* 96(9), 939–949 (2005).
- 10 Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam. Horm.* 74, 443–477 (2006).
- **Comparative study of the adipokine and cytokine release by various components of adipose. See also other experimental papers of the same author.**
- 11 Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem. Soc. Trans.* 33, 1078–1081 (2005).
- 12 Dietze D, Koenen M, Rohrig K, Horikoshi H, Hauner H, Eckel J. Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes. *Diabetes* 51, 2369–2376 (2002).
- 13 Sell H, Dietze-Schroeder D, Eckel J. The adipocyte–myocyte axis in insulin resistance. *Trends Endocrinol. Metab.* 17, 416–422 (2006).
- 14 Sell H, Eckel J, Dietze-Schroeder D. Pathways leading to muscle insulin resistance – the muscle–fat connection. *Arch. Physiol. Biochem.* 112, 105–113 (2006).
- 15 Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808 (2003).
- 16 Broglio F, Prodam F, Riganti F, Muccioli G, Ghigo E. Ghrelin: from somatotrope secretion to new perspectives in the regulation of peripheral metabolic functions. *Front. Horm. Res.* 35, 102–108 (2006).
- **Example of the adipocyte cross-talk between the signaling events originating on different bioactive molecules.**
- 17 Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. *Curr. Opin. Lipidol.* 14(6), 561–566 (2003).
- 18 Mao X, Kikani CK, Riojas RA *et al.* APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nat. Cell. Biol.* 8(5), 516–523 (2006).
- 19 Yamauchi T, Kamon J, Minokoshi Y *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.* 8(11), 1288–1295 (2002).
- 20 Chen MB, McAinch AJ, Macaulay SL *et al.* Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese Type 2 diabetics. *J. Clin. Endocrinol. Metab.* 90(6), 3665–3672 (2005).
- 21 Civitarese AE, Ukropcova B, Carling S *et al.* Role of adiponectin in human skeletal muscle bioenergetics. *Cell Metab.* 4(1), 75–87 (2006).
- 22 Ouchi N, Shibata R, Walsh K. Cardioprotection by adiponectin. *Trends Cardiovasc. Med.* 16(5), 141–146 (2006).
- 23 Dietze-Schroeder D, Sell H, Uhlig M, Koenen M, Eckel J. Autocrine action of adiponectin on human fat cells prevents the release of insulin resistance-inducing factors. *Diabetes* 54(7), 2003–2011 (2005).
- **Excellent experiments indicating that autocrine action of adiponectin prevent the induction of insulin resistance in peripheral tissues.**
- 24 Fu Y, Luo N, Klein RL, Garvey WT. Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. *J. Lipid Res.* 46(7), 1369–1379 (2005).
- 25 Rasmussen MS, Lihn AS, Pedersen SB, Bruun JM, Rasmussen M, Richelsen B. Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. *Obesity (Silver Spring)* 14(1), 28–35 (2006).
- 26 Mendez-Sanchez N, Chavez-Tapia NC, Villa AR *et al.* Adiponectin as a protective factor in hepatic steatosis. *World J. Gastroenterol.* 11(12), 1737–1741 (2005).
- 27 Pagano C, Soardo G, Esposito W *et al.* Plasma adiponectin is decreased in nonalcoholic fatty liver disease. *Eur. J. Endocrinol.* 152(1), 113–118 (2005).
- 28 Cesari M, Pessina AC, Zanchetta M *et al.* Low plasma adiponectin is associated with coronary artery disease but not with hypertension in high-risk nondiabetic patients. *J. Intern. Med.* 260(5), 474–483 (2006).
- 29 Leo R, Di Lorenzo G, Tesaro M *et al.* Decreased plasma adiponectin concentration in major depression. *Neurosci. Lett.* 407(3), 211–213 (2006).
- 30 Bersinger NA, Birkhauser MH, Wunder DM. Adiponectin as a marker of success in intracytoplasmic sperm injection/embryo transfer cycles. *Gynecol. Endocrinol.* 22(9), 479–483 (2006).
- 31 Cust AE, Kaaks R, Friedenreich C *et al.* Plasma adiponectin levels and endometrial cancer risk in pre- and post-menopausal women. *J. Clin. Endocrinol. Metab.* 92(1), 255–263 (2006).

- 32 Bugianesi E, Pagotto U, Manini R *et al.* Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J. Clin. Endocrinol. Metab.* 90, 3498–3504 (2005).
- 33 Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF- α or adiponectin? *Hepatology* 40, 46–54 (2004).
- 34 Musso G, Gambino R, Biroli G *et al.* Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic β -cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. *Am. J. Gastroenterol.* 100, 2438–2446 (2005).
- 35 Kaser S, Moschen A, Cayon A *et al.* Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 54, 117–121 (2005).
- 36 Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. *Recent Prog. Horm. Res.* 59, 305–331 (2004).
- **Excellent overview of the leptin signaling.**
- 37 Nobili V, Manco M, Ciampalini P *et al.* Leptin, free leptin index, insulin resistance and liver fibrosis in children with non-alcoholic fatty liver disease. *Eur. J. Endocrinol.* 155(5), 735–743 (2006).
- 38 Uygun A, Kadayifci A, Yesilova Z *et al.* Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am. J. Gastroenterol.* 95(12), 3584–3589 (2000).
- 39 Yokoyama H, Hirose H, Ohgo H, Saito I. Associations between serum leptin levels and transaminase activities and the status of lifestyle in Japanese workers. *Alcohol Clin. Exp. Res.* 28(8 Suppl. Proceedings), 159S–163S (2004).
- 40 Muoio DM, Lynis Dohm G. Peripheral metabolic actions of leptin. *Best Pract. Res. Clin. Endocrinol. Metab.* 16(4), 653–666 (2002).
- 41 Fishman S, Muzumdar RH, Atzmon G *et al.* Resistance to leptin action is the major determinant of hepatic triglyceride accumulation *in vivo*. *FASEB J.* 21(1), 53–60 (2006).
- 42 Chitturi S, Farrell G, Frost L *et al.* Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 36(2), 403–409 (2002).
- 43 Shamsuzzaman AS, Winnicki M, Wolk R *et al.* Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation* 109(18), 2181–2185 (2004).
- 44 Aleffi S, Petrai I, Bertolani C *et al.* Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. *Hepatology* 42(6), 1339–1348 (2005).
- 45 Ikejima K, Okumura K, Lang T *et al.* The role of leptin in progression of non-alcoholic fatty liver disease. *Hepatol. Res.* 33(2), 151–154 (2005).
- 46 Bethanis SK, Theocharis SE. Leptin in the field of hepatic fibrosis: a pivotal or an incidental player? *Dig. Dis. Sci.* 51(10), 1685–1696 (2006).
- 47 Angulo P, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. *J. Hepatol.* 41(6), 943–949 (2004).
- 48 Cayon A, Crespo J, Mayorga M, Guerra A, Pons-Romero F. Increased expression of Ob-Rb and its relationship with the overexpression of TGF- β 1 and the stage of fibrosis in patients with nonalcoholic steatohepatitis. *Liver Int.* 26(9), 1065–1071 (2006).
- 49 Leung JC, Chan LY, Tang SC, Chu KM, Lai KN. Leptin induces TGF- β synthesis through functional leptin receptor expressed by human peritoneal mesothelial cell. *Kidney Int.* 69(11), 2078–2086 (2006).
- 50 Choudhury J, Mirshahi F, Murthy KS, Yager DR, Sanyal AJ. Physiologic concentrations of leptin increase collagen production by non-immortalized human hepatic stellate cells. *Metabolism* 55(10), 1317–1322 (2006).
- 51 Wolf G. Insulin resistance and obesity: resistin, a hormone secreted by adipose tissue. *Nutr. Rev.* 62(10), 389–394 (2004).
- 52 Palanivel R, Maida A, Liu Y, Sweeney G. Regulation of insulin signalling, glucose uptake and metabolism in rat skeletal muscle cells upon prolonged exposure to resistin. *Diabetologia* 49(1), 183–190 (2006).
- 53 Fu Y, Luo L, Luo N, Garvey WT. Proinflammatory cytokine production and insulin sensitivity regulated by overexpression of resistin in 3T3-L1 adipocytes. *Nutr. Metab. (Lond.)* 3, 28 (2006).
- **See also other papers by the same group of authors.**
- 54 Liu T, Dhanasekaran SM, Jin H *et al.* FIZZ1 stimulation of myofibroblast differentiation. *Am. J. Pathol.* 164(4), 1315–1326 (2004).
- 55 Teng X, Li D, Champion HC, Johns RA. FIZZ1/RELMA, a novel hypoxia-induced mitogenic factor in lung with vasoconstrictive and angiogenic properties. *Circ. Res.* 92(10), 1065–1067 (2003).
- 56 Thommesen L, Stunes AK, Monjo M *et al.* Expression and regulation of resistin in osteoblasts and osteoclasts indicate a role in bone metabolism. *J. Cell. Biochem.* 99(3), 824–834 (2006).
- 57 Yang RZ, Huang Q, Xu A *et al.* Comparative studies of resistin expression and phylogenomics in human and mouse. *Biochem. Biophys. Res. Commun.* 310(3), 927–935 (2003).
- **Review of the human and rodent resistin families with emphasis on their differences.**
- 58 Ort T, Arjona AA, MacDougall JR *et al.* Recombinant human FIZZ3/resistin stimulates lipolysis in cultured human adipocytes, mouse adipose explants, and normal mice. *Endocrinology* 146(5), 2200–2209 (2005).
- **Experimental proofs that human and murine resistins act upon adipocytes differently.**
- 59 Kusminski CM, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. *Clin. Sci. (Lond.)* 109(3), 243–256 (2005).
- 60 Fehmann HC, Heyn J. Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. *Horm. Metab. Res.* 34(11–12), 671–373 (2002).
- 61 Lee JH, Chan JL, Yiannakouris N *et al.* Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J. Clin. Endocrinol. Metab.* 88(10), 4848–4856 (2003).
- 62 Bajaj M, Suraamornkul S, Hardies LJ, Pratipanawatr T, DeFronzo RA. Plasma resistin concentration, hepatic fat content, and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. *Int. J. Obes. Relat. Metab. Disord.* 28(6), 783–789 (2004).
- 63 Perseghin G, Lattuada G, De Cobelli F *et al.* Serum resistin and intra-hepatic fat content in non-diabetic individuals. *J. Clin. Endocrinol. Metab.* (2006).
- 64 Pagano C, Soardo G, Pilon C *et al.* Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. *J. Clin. Endocrinol. Metab.* 91(3), 1081–1086 (2006).

- 65 Baranova A, Gowder SJ, Schlauch K *et al*. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. *Obes. Surg.* 16(9), 1118–1125 (2006).
- 66 Berndt J, Kloting N, Kralisch S *et al*. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 54(10), 2911–2916 (2005).
- 67 Boucher J, Masri B, Daviaud D *et al*. Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* 146(4), 1764–1771 (2005).
- 68 Beltowski J. Apelin and visfatin: unique “beneficial” adipokines upregulated in obesity? *Med. Sci. Monit.* 12(6), RA112–RE119 (2006).
- 69 Fukuhara A, Matsuda M, Nishizawa M *et al*. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307(5708), 426–30(2005).
- **Study of the insulin-mimetic effects of visfatin.**
- 70 Wang T, Zhang X, Bheda P, Revollo JR, Imai S, Wolberger C. Structure of Namp1/PBEF/visfatin, a mammalian NAD⁺ biosynthetic enzyme. *Nat. Struct. Mol. Biol.* 13(7), 661–662 (2006).
- 71 Wolf G. Calorie restriction increases life span: a molecular mechanism. *Nutr. Rev.* 64(2 Pt 1), 89–92 (2006).
- 72 Bailey SD, Loreda-Osti JC, Lepage P *et al*. Common polymorphisms in the promoter of the visfatin gene (*PBEF1*) influence plasma insulin levels in a French-Canadian population. *Diabetes* 55(10), 2896–2902 (2006).
- 73 Lopez-Bermejo A, Chico-Julia B, Fernandez-Balsells M *et al*. Serum visfatin increases with progressive β -cell deterioration. *Diabetes* 55(10), 2871–2875 (2006).
- 74 Sorhede Winzell M, Magnusson C, Ahren B. The APJ receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. *Regul. Pept.* 131(1–3), 12–17 (2005).
- 75 Szokodi I, Tavi P, Foldes G *et al*. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ. Res.* 91(5), 434–440 (2002).
- 76 Xie H, Tang SY, Cui RR *et al*. Apelin and its receptor are expressed in human osteoblasts. *Regul. Pept.* 134(2–3), 118–125 (2006).
- 77 Heinonen MV, Purhonen AK, Miettinen P *et al*. Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul. Pept.* 130(1–2), 7–13 (2005).
- 78 Daviaud D, Boucher J, Gesta S *et al*. TNF α up-regulates apelin expression in human and mouse adipose tissue. *FASEB J.* 20(9), 1528–1530 (2006).
- 79 Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O’Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N. Engl. J. Med.* 348(12), 1085–1095 (2003).
- 80 Eberle AN, Mild G, Schlumberger S, Drozd R, Hintermann E, Zumsteg U. Expression and characterization of melanin-concentrating hormone receptors on mammalian cell lines. *Peptides* 25(10), 1585–1595 (2004).
- 81 Bradley RL, Mansfield JP, Maratos-Flier E, Cheatham B. Melanin-concentrating hormone activates signaling pathways in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metab.* 283(3), E584–E592 (2002).
- 82 Colombo G, Gatti S, Turcatti F *et al*. Gene expression profiling reveals multiple protective influences of the peptide α melanocyte-stimulating hormone in experimental heart transplantation. *J. Immunol.* 175, 3391–3401 (2005).
- 83 Bohm M, Raghunath M, Sunderkotter C *et al*. Collagen metabolism is a novel target of the neuropeptide α -melanocyte-stimulating hormone. *J. Biol. Chem.* 279(8), 6959–6966 (2004).
- 84 Wang CH, Jawan B, Lee TH *et al*. Single injection of naked plasmid encoding α -melanocyte-stimulating hormone protects against thioacetamide-induced acute liver failure in mice. *Biochem. Biophys. Res. Commun.* 322, 153–161 (2004).
- 85 Hoggard N, Hunter L, Duncan JS, Rayner DV. Regulation of adipose tissue leptin secretion by α -melanocyte-stimulating hormone and agouti-related protein: further evidence of an interaction between leptin and the melanocortin signalling system. *J. Mol. Endocrinol.* 32(1), 145–153 (2004).
- 86 Norman D, Isidori AM, Frajese V *et al*. ACTH and α -MSH inhibit leptin expression and secretion in 3T3-L1 adipocytes: model for a central-peripheral melanocortin–leptin pathway. *Mol. Cell Endocrinol.* 200(1–2), 99–109 (2003).
- 87 Shimizu H, Tanaka Y, Sato N, Mori M. α -melanocyte-stimulating hormone (MSH) inhibits insulin secretion in HIT-T 15 cells. *Peptides* 16(4), 605–608 (1995).
- 88 Cho KJ, Shim JH, Cho MC *et al*. Signaling pathways implicated in α -melanocyte stimulating hormone-induced lipolysis in 3T3-L1 adipocytes. *J. Cell Biochem.* 96(4), 869–878 (2005).
- 89 Maaser C, Kannengiesser K, Kucharzik T. Role of the melanocortin system in inflammation. *Ann. NY Acad. Sci.* 1072, 123–134 (2006).
- 90 Haukeland JW, Damas JK, Konopski Z *et al*. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. *J. Hepatol.* 44(6), 1167–1174 (2006).
- 91 Bahcecioglu IH, Yalniz M, Ataseven H *et al*. Levels of serum hyaluronic acid, TNF- α and IL-8 in patients with nonalcoholic steatohepatitis. *Hepatogastroenterology* 52(65), 1549–1553 (2005).
- 92 Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. α -melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J. Clin. Invest.* 99(6), 1165–1172 (1997).
- 93 Lee TH, Jawan B, Chou WY *et al*. α -melanocyte-stimulating hormone gene therapy reverses carbon tetrachloride induced liver fibrosis in mice. *J. Gene Med.* 8(6), 764–772 (2006).
- 94 Rajora N, Boccoli G, Catania A, Lipton JM. α -MSH modulates experimental inflammatory bowel disease. *Peptides* 18(3), 381–385 (1997).
- 95 Huang Q, Tatro JB. α -melanocyte stimulating hormone suppresses intracerebral tumor necrosis factor- α and interleukin-1 β gene expression following transient cerebral ischemia in mice. *Neurosci. Lett.* 16, 334(3), 186–190 (2002).
- 96 Deng J, Hu X, Yuen PS, Star RA. α -melanocyte-stimulating hormone inhibits lung injury after renal ischemia/reperfusion. *Am. J. Respir. Crit. Care Med.* 169(6), 749–756 (2004).
- 97 Nam SY, Kratzsch J, Kim KW, Kim KR, Lim SK, Marcus C. Cerebrospinal fluid and plasma concentrations of leptin, NPY, and α -MSH in obese women and their relationship to negative energy balance. *J. Clin. Endocrinol. Metab.* 86(10), 4849–4853 (2001).
- 98 Fehm HL, Smolnik R, Kern W, McGregor GP, Bickel U, Born J. The melanocortin melanocyte-stimulating hormone/adrenocorticotropin (4–10) decreases body fat in humans. *J. Clin. Endocrinol. Metab.* 86(3), 1144–1148 (2001).
- 99 Hallschmid M, Smolnik R, McGregor G, Born J, Fehm HL. Overweight humans are resistant to the weight-reducing effects of melanocortin4–10. *J. Clin. Endocrinol. Metab.* 91(2), 522–525 (2006).

- 100 Yamaoka-Tojo M, Tojo T, Shioi T, Masuda T, Inomata T, Izumi T. Central neurotensin, α -melanocyte-stimulating hormone (α -MSH) is upregulated in patients with congestive heart failure. *Intern. Med.* 45(7), 429–434 (2006).
- 101 Pichler R, Sfetsos K, Badics B, Gutenbrunner S, Aubock J. Vitiligo patients present lower plasma levels of α -melanotropin immunoreactivities. *NeuroPeptides* 40(3), 177–183 (2006).
- 102 Catania A, Cutuli M, Garofalo L *et al.* Plasma concentrations and anti-L-cytokine effects of α -melanocyte stimulating hormone in septic patients. *Crit. Care Med.* 28(5), 1403–1407 (2000).
- See also other publications of the same author.
- 103 Kim KY, Kim JK, Jeon JH, Yoon SR, Choi I, Yang Y. c-Jun N-terminal kinase is involved in the suppression of adiponectin expression by TNF- α in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 327(2), 460–467 (2005).
- 104 Kirchgessner TG, Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Tumor necrosis factor- α contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J. Clin. Invest.* 100(11), 2777–2782 (1997).
- 105 Shen J, Sakaida I, Uchida K, Terai S, Okita K. Leptin enhances TNF- α production via p38 and JNK MAPK in LPS-stimulated Kupffer cells. *Life Sci.* 77(13), 1502–1515 (2005).
- 106 Kralisch S, Klein J, Lossner U *et al.* Hormonal regulation of the novel adipocytokine visfatin in 3T3-L1 adipocytes. *J. Endocrinol.* 85, R1–R8 (2005).
- 107 Diehl AM. Tumor necrosis factor and its potential role in insulin resistance and nonalcoholic fatty liver disease. *Clin. Liver Dis.* 8(3), 619–38 (2004).
- 108 Hadley ME, Dorr RT. Melanocortin peptide therapeutics: historical milestones, clinical studies and commercialization. *Peptides* 27(4), 921–930 (2006).
- 109 Cornish J, Callon KE, Mountjoy KG *et al.* α -melanocyte-stimulating hormone is a novel regulator of bone. *Am. J. Physiol. Endocrinol. Metab.* 284(6), E1181–E1190 (2003).
- 110 Fisher FF, Trujillo ME, Hanif W *et al.* Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. *Diabetologia* 48(6), 1084–1087 (2005).
- 111 Wang Y, Lam KS, Chan L *et al.* Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. *J. Biol. Chem.* 281(24), 16391–16400 (2006).
- 112 Hida K, Wada J, Eguchi J *et al.* Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc. Natl Acad. Sci. USA* 102(30), 10610–10615 (2005).
- 113 Yang RZ, Lee MJ, Hu H *et al.* Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am. J. Physiol. Endocrinol. Metab.* 290(6), E1253–E1261 (2006).

Affiliations

- Ancha Baranova, PhD, DSci
Center for Liver Diseases, Inova Fairfax Hospital, VA, USA
Tel.: +1 703 993 42 92
Fax: +1 703 993 43 93
abarano@gmu.edu
- Manpreet Randhawa, MS
George Mason University, Center for the Study of Genomics in Liver Diseases, Molecular & Microbiology Department, VA, USA
Tel.: +1 703 993 85 12
Fax: +1 703 993 43 93
mrandha1@gmu.edu
- Mohammed Jarrar, MS
George Mason University, Center for the Study of Genomics in Liver Diseases, Molecular & Microbiology Department, VA, USA
Tel.: +1 703 993 85 12
Fax: +1 703 993 43 93
mjarrar@gmu.edu
- Zobair M Younossi, MD, MPH
Inova Fairfax Hospital, Center for Liver Diseases, Department of Medicine, 3300 Gallows Road, VA22042, USA
Tel.: +1 703 698 3182;
+1 703 208 6650
Fax: +1 703 698 3482;
+1 703 208 6655
zobair.younossi@inova.com

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.