Adipokines and Adipocyte Targets in the Future Management of Obesity and the Metabolic Syndrome

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Abstract: The role of adipocytes has been recently better understood. Several adipocytokines have been identified, including leptin, a main regulator of appetite and energy expenditure, adiponectin and others, as novel insulin-sensitizers/insulin-mimetics, and some others inducing insulin resistance. Adipocytokines thus represent interesting novel drug targets in the future management of obesity.

Key Words: Adipokine, diabetes mellitus, insulin resistance, obesity.

1. INTRODUCTION

Obesity is a rapidly growing nutritional disorder characterized by excessive accumulation of adipose tissue [1]. Both hyperplasia and hypertrophy of fat cells are found when weight is gained [1]. In diabetes mellitus, on the other hand, two main defects are found: insulin resistance of peripheral tissues such as liver, muscle and fat, as well as secretory failure of pancreatic β-cells [2]. Increased body weight is tightly associated with insulin resistance and type 2 diabetes mellitus [3]. From an epidemiological viewpoint, the global incidence of type 2 diabetes is projected to nearly double by the year 2025 with then about 300 million people suffering from the disease in industrialized countries due to on average increased body weight [3]. The role of various adipokines as connectors between obesity and diabetes mellitus has been better elucidated in recent years. Thus, fat-secreted proteins including adiponectin, interleukin (IL)-6, leptin, monocyte chemoattractant protein (MCP)-1, omentin, plasminogen activator inhibitor (PAI)-1, resistin, serum retinol binding protein (RBP)-4, SPARC/osteonectin, tumor necrosis factor (TNF) α, vaspin, and visfatin, influence insulin sensitivity, energy metabolism, and appetite. Extensive studies in rodents suggest their potential application in the treatment of obesity and diabetes mellitus. Furthermore, several drugs currently used to lower serum glucose levels including thiazolidinediones (TZDs) mediate part of their effects by altering expression of adipokines in fat tissue. However, data on adipokine-based therapies in humans are sparse. In this review, the current knowledge on function, regulation, and therapeutic potential of various adipokines is summarized and critically discussed.

2. INSULIN-SENSITIZING AND INSULIN-MIMETIC ADIPOKINES

2.1. Adiponectin

Adiponectin has been first described in 1995 as a novel serum protein similar to C1q, produced almost exclusively in adipocytes [4]. As early as 1996 it has been shown that adiponectin expression in fat is decreased in obesity [5]. However, not until 2001 a direct role of this adipokine in insulin sensitivity has been demonstrated (Fig. (**1**)) [6]. Thus, a globular C-terminal fragment reduces glucose levels by

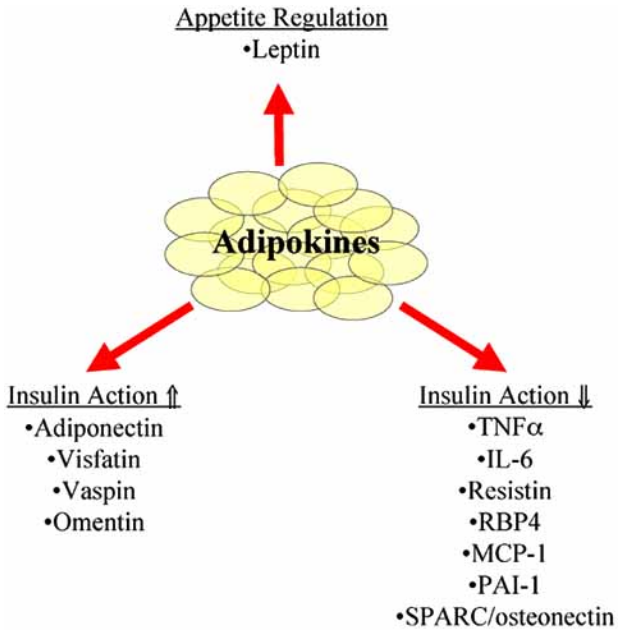

Fig. (1). Adipokines implicated in insulin sensitivity and appetite control.

increasing fatty acid combustion in myocytes [6]. Globular adiponectin also improves insulin sensitivity by paracrine action in fat cells [7]. In liver cells, however, only full-length adiponectin sensitizes to suppression of gluconeogenesis by insulin *in vivo* and *in vitro* [8,9]. Adiponectin forms several complexes and a high-molecular-weight complex appears as the active form [10]. Further support for a role of adiponectin as an endogenous insulin sensitizer comes from knockout (KO) experiments in mice. Two independent studies demonstrate impaired insulin sensitivity in adiponectin KO mice as compared to wild type (WT) controls [11,12]. In contrast, one study finds increased β-oxidation but no insulin

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resistance in adiponectin KO animals [13]. Besides its peripheral effects, adiponectin acts in the brain to increase energy expenditure and cause weight loss [14].

Regulation of adiponectin in obesity, insulin resistance, and by insulin-modulating hormones and drugs is well-elucidated by now. Thus, adiponectin levels are significantly decreased in insulin resistance and obesity [5,15,16]. Furthermore, adiponectin expression and secretion increase when insulin sensitivity and obesity improve [17-19]. Insulin-sensitizing TZDs probably mediate part of their effect *via* adiponectin since they increase plasma concentrations of this adipokine in both, subjects with normal insulin sensitivity and type 2 diabetics *in vivo* [20,21]. In contrast, various hormones associated with insulin resistance and obesity including catecholamines, insulin, glucocorticoids, TNFα and IL-6 downregulate adiponectin expression and secretion in fat cells *in vitro* [22-24].

The signaling molecules by which adiponectin leads to insulin sensitization have been better characterized in recent studies. On the cell surface, two adiponectin receptors (AdipoR) which are structurally and functionally distinct from G-protein-coupled receptors are necessary for adiponectin action [25]. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly found in liver [25]. T-cadherin is an additional receptor for hexameric and high-molecular-weight adiponectin [26]. Downstream of these receptors, activation of adenosine monophosphate kinase (AMPK) which inhibits acetyl coenzyme A carboxylase (ACC) is necessary for adiponectin action [7,27]. In hepatocytes, adiponectin-induced AMPK activity decreases expression of gluconeogenic enzymes whereas in muscle proteins involved in fatty acid transport and oxidation are induced [9,28].

Taking these studies into consideration, not only adiponectin and adiponectin receptor agonists, but also AMPK activators are interesting targets for the treatment of core components of the metabolic syndrome including insulin resistance and obesity in humans. In accordance with this view, various well-known drugs used in the treatment of type 2 diabetes and exercise stimulate AMPK activity. Thus, glucose-lowering metformin activates AMPK and inhibits ACC in hepatocytes leading to decreased levels of gluconeogenic enzymes similar to adiponectin [29]. Furthermore, insulin-sensitizing pioglitazone increases AMPK activity in liver and fat cells *in vivo* [30]. Moreover, exercise which profoundly increases insulin sensitivity independent of weight loss leads to activation of AMPK and inhibition of ACC in liver, muscle, and adipose tissue [31]. Direct activation of AMPK by a single dose of 5′-aminoimidazole 4-carboxamide riboside (AICAR) decreases hepatic triglyceride content in high-fat-fed rats leading to enhanced whole-body insulin action [32]. However, adverse effects of AICAR such as liver enlargement by 40 % in rodents have to be taken into account [33].

2.2. Visfatin

Visfatin/pre-B cell colony-enhancing factor is a novel adipokine improving glucose tolerance and playing a role in the development of obesity-associated insulin resistance and diabetes mellitus (Fig. (**1**)) [34]. In the first characterization of this adipokine it is suggested that visfatin is preferentially expressed in visceral as compared to subcutaneous adipose tissue and plasma visfatin concentrations correlate strongly with the amount of visceral fat in human subjects [34]. Most interestingly, visfatin shows insulin-mimetic effects *in vitro* and *in vivo* [34]. Thus, glucose levels are decreased in several rodent models of insulin resistance and obesity by visfatin [34]. Furthermore, visfatin increases basal glucose uptake in 3T3-L1 adipocytes, as well as L6 myocytes, and suppresses gluconeogenesis in H4IIEC3 hepatocytes *in vitro* [34]. The authors present evidence that these effects are mediated *via* direct stimulation of the insulin receptor, however, the binding site is different from insulin [34]. A recent study shows that increasing concentrations of visfatin are independently and significantly associated with type 2 diabetes mellitus independent of several known biomarkers [35]. In contrast, our group is unable to show a significant correlation between visfatin plasma concentrations and parameters of insulin sensitivity, including fasting insulin, fasting plasma glucose concentrations, and the glucose infusion rate during the steady state of an euglycemic-hyperinsulinemic clamp independent of percent body fat [36]. Furthermore, in contrast to the original report, in our hands visfatin gene expression is not different between visceral and subcutaneous adipose tissue [36]. We find a significant correlation between visceral visfatin gene expression and body mass index (BMI), as well as percent body fat whereas no significant association between BMI or percent body fat and subcutaneous visfatin mRNA expression exists [36]. Furthermore, we have elucidated hormonal regulation of visfatin expression in fat *in vitro* [37,38]. Thus, the glucocorticoid dexamethasone induces visfatin mRNA in fat possibly contributing to increased levels of this adipokine found in obesity [37]. In contrast, the β-adrenergic agonist isoproterenol, growth hormone (GH), TNFα, and IL-6 are negative regulators of visfatin expression [37,38].

2.3. Vaspin

Recently, vaspin (visceral adipose tissue-derived serpin) has been suggested as a novel adipokine with insulin-sensitizing effects toward white adipose tissue in states of obesity (Fig. (**1**)) [39]. Vaspin has been isolated from visceral white adipose tissue of OLETF rats, an animal model of abdominal obesity with type 2 diabetes [39]. Vaspin is a member of the serine protease inhibitor family and exhibits approximately 40% homology with alpha1-antitrypsin [39]. Tissue expression and serum levels of this adipokine decrease with worsening of diabetes in OLETF rats [39]. Interestingly, both, expression and serum levels, are normalized by treatment with insulin or pioglitazone in rodents *in vivo* [39]. Moreover, administration of vaspin to obese mice fed with high-fat high-sucrose chow improves glucose tolerance and insulin sensitivity [39]. Furthermore, altered expression of various insulin resistance-associated genes is reversed by vaspin treatment [39].

Most recently, our group has determined vaspin mRNA expression in paired samples of visceral and subcutaneous adipose tissue from 196 subjects with a wide range of obesity, body fat distribution, insulin sensitivity, and glucose tolerance [40]. Vaspin mRNA expression is only detectable in 23% of the visceral and in 15% of the subcutaneous adi-

pose tissue samples [40]. Visceral vaspin expression significantly correlates with BMI, % body fat, and 2 h glucose during oral glucose tolerance testing. Subcutaneous vaspin mRNA expression is significantly correlated with waist-to-hip-ratio, fasting plasma insulin concentration, and glucose infusion rate during steady state of an euglycemic-hyperinsulinemic clamp [40]. Vaspin is not detectable in 3T3-L1 adipocytes *in vitro* [Kralisch and Fasshauer, unpublished].

2.4. Omentin

Omentin is the fourth adipokine known so far which might improve insulin action (Fig. (**1**)) [41]. Similar to visfatin and vaspin, a differential expression of this protein between visceral and subcutaneous adipose tissue has been proposed with expression being restricted to the visceral fat depot [41]. Preliminary studies suggest that omentin enhances insulin-mediated glucose uptake in 3T3-L1 adipocytes, an effect that might be mediated *via* Akt/protein kinase B [41].

3. INSULIN RESISTANCE-INDUCING ADIPOKINES

3.1. TNFα

TNFα is a proinflammatory cytokine, expression of which in fat has been shown as early as 1993 by Hotamisligil and co-workers [42]. TNFα induces insulin resistance in liver, muscle, and fat and the signaling pathways mediating this effect are much clearer by now (Fig. (**1**)) [42]. Thus, TNFα induces serine phosphorylation of insulin receptor substrate (IRS)-1 *in vitro*, and serine-phophorylated IRS-1 acts as an inhibitor of insulin signaling molecules including insulin receptor (IR), phosphatidylinositol (PI) 3-kinase, and insulin-responsive glucose transporter (Glut)-4 [43,44].

Increased TNFα expression in fat and secretion can be found in obese animals including ob/ob mice, db/db mice, fa/fa rats, as well as in overweight humans [45-47]. However, TNFα serum concentrations in humans are dependent on body weight and are indistinguishable between insulin-resistant and insulin-sensitive probands when BMI is matched for [48]. The TZD troglitazone but not rosiglitazone downregulates TNFα in adipocytes [49,50].

After elucidating its profound insulin resistance-inducing effects [42], TNFα inhibition has long been regarded as a potential novel therapeutic way to improve insulin sensitivity. Initial studies in animals have supported this exciting concept since neutralization of TNFα in fa/fa rats improves glycemic control [42]. However, these findings cannot be transferred to humans. In a well-controlled trial, the recombinant human TNFα-neutralizing antibody CDP571 does not improve glucose homeostasis in obese NIDDM patients despite adequate plasma antibody levels [51]. Furthermore, TNFα antagonists such as infliximab which are used in Crohn´s disease and rheumatoid arthritis are not reported to improve insulin sensitivity despite their profound anti-inflammatory effect [52].

3.2. IL-6

IL-6, similar to TNFα, is a proinflammatory adipokine which is not exclusively expressed in adipose tissue [53]. However, it is estimated that about 25 % of systemic IL-6 is secreted by subcutaneous fat cells *in vivo* [53]. Furthermore, visceral as compared to subcutaneous fat secretes even 2- to 3-fold more IL-6 *in vitro* [54]. Epidemiological studies suggest that IL-6 plasma concentrations correlate with the development of type 2 diabetes mellitus [55]. Administration of recombinant IL-6 in rodent models and in humans results in hyperglycemia and compensatory hyperinsulinemia *in vivo* and the signaling pathways mediating these effects have been better elucidated recently (Fig. (**1**)) [56,57]. Thus, IL-6 impairs intracellular insulin signaling in fat cells and hepatocytes potentially *via* upregulation of suppressor of cytokine signaling (SOCS) proteins [58-60]. Furthermore, expression of various adipokines is affected with IL-6 downregulating adiponectin [24], and visfatin [38], as well as upregulating MCP-1 [61] *in vitro*. However, IL-6 has differential effects depending on the tissue secreting the cytokine and the target organ [62]. Thus, IL-6 secretion from muscle is upregulated after insulin-sensitizing exercise and central application of this adipokine increases energy expenditure [62]. In accordance with its overall insulin resistance-inducing effects, IL-6 levels are significantly increased when insulin sensitivity is impaired and weight is gained in both, rodents and humans [63]. Insulin resistance-inducing hormones including insulin, catecholamines, growth hormone, TNFα, and IL-6 stimulate expression of this adipokine *in vitro* [64] whereas rosiglitazone does not influence IL-6 synthesis *in vivo* [65].

3.3. Resistin

In 2001, resistin has been introduced as a novel adipokine impairing insulin sensitivity and linking insulin resistance with obesity (Fig. (**1**)) [66]. In rodents, resistin inhibits insulin signaling in skeletal muscle, liver, and adipose tissue, resulting in glucose intolerance [67,68]. Whereas resistin´s role in rodent insulin resistance is well established, its significance in humans remains obscure. Thus, human fat cells do not secrete substantial amounts of resistin and synthesis of this cytokine does not correlate to insulin resistance and obesity [69,70]. Furthermore, conflicting results exist concerning regulation of resistin in genetic and diet-induced obesity, by TZDs, and by insulin resistance-inducing hormones [55,66,71-73]

Resistin receptors have not been cloned so far and intracellular signaling molecules used are largely unknown. One study in resistin KO mice suggests downregulation of AMPK phosphorylation as a potential mode of action for this adipokine [74]. In accordance with this assumption, AMPK activity in insulin-sensitive tissues including skeletal muscle, liver, and fat is significantly downregulated by adenovirus-mediated overexpression of resistin [67].

3.4. RBP-4

Most recently, RBP4 has been introduced as a novel adipokine expression of which is upregulated in fat of insulin resistant adipose-Glut4 KO mice [75]. Several pieces of evidence suggest that RBP4 potently impairs insulin sensitivity (Fig. (**1**)) [75]. Thus, transgenic overexpression of RBP4 or injection of recombinant RBP4 in normal mice induces insulin resistance [75]. Furthermore, genetic ablation of RBP4 improves insulin sensitivity [75]. Moreover, fenretinide which increases urinary excretion of RBP4 not only normalizes serum RBP4 levels but also improves insulin resistance and glucose intolerance in obese mice [75]. The signaling path-

ways used are not well-understood so far, however, RBP4-induced expression of gluconeogenic enzymes in liver and impaired insulin signaling in muscle might contribute [75].

RBP4 levels are elevated not only in obese and diabetic mice but also in overweight humans [75]. Interestingly, RBP4 concentrations are normalized by insulin-sensitizing rosiglitazone [75].

3.5. MCP-1

MCP-1 is a member of the CC chemokine family secreted by a variety of tissues including fat [76]. MCP-1 is traditionally regarded as an important mediator recruiting monocytes and T lymphocytes into different tissues [76]. Beyond this well-studied function, MCP-1 strongly decreases insulin-stimulated glucose uptake and downregulates adipogenic genes including adipsin, Glut4, β3-adrenergic receptors, and peroxisome proliferator-activated receptor (PPAR) γ in 3T3-L1 adipocytes *in vitro* (Fig. (**1**)) [77]. The signaling pathways mediating these effects are not thoroughly understood. However, it is well established that the main receptor of MCP-1 is CCR2 which is also expressed in adipocytes [78].

Expression of MCP-1 is upregulated in genetically obese mice as compared to lean controls [77,79]. Furthermore, MCP-1 is stimulated by insulin resistance-inducing hormones including insulin, TNFα, growth hormone, and IL-6 [61,77].

3.6. PAI-1

PAI-1 is a well-studied inhibitor of fibrinolysis which contributes to increased risk of atherosclerosis found in obesity and insulin resistance. PAI-1 is upregulated in visceral adipose tissue in humans in obesity and insulin resistance [80,81]. Novel data in mice suggest that beyond its effect on vascular function, PAI-1 also influences weight gain and insulin sensitivity (Fig. (**1**)) [82]. Thus, animals with genetic ablation of this adipokine show increased resting metabolic rates, total energy expenditure, and insulin sensitivity as compared to WT controls [82]. Interestingly, adiponectin expression in fat of KO mice is preserved despite high-fat-feeding as compared to WT controls [82].

3.7. SPARC/osteonectin

Two independent studies suggest that SPARC/osteonectin is another adipokine upregulated in obesity potentially influencing glucose metabolism and vascular function (Fig. (**1**)) [83,84]. Thus, goldthioglucose-induced obesity in mice is associated with 3- to 6-fold increased SPARC mRNA levels in epididymal fat as compared to non-treated controls [83]. Furthermore, SPARC mRNA is upregulated in obese db/db mice and serum levels are increased in overweight humans [84]. Moreover, SPARC is significantly elevated in patients with coronary artery disease even if patients are matched for age and BMI [84]. SPARC increases PAI-1 expression in a paracrine manner in rat fat cells and might, therefore, mediate part of its effect *via* this adipokine [83].

4. APPETITE CONTROLLING ADIPOKINES

4.1. Leptin

Leptin which has been cloned in 1994 inhibits appetite and weight gain by decreasing orexigenic and increasing anorexigenic peptide synthesis in the hypothalamus [85]. An inactivating mutation of the leptin gene or its receptor leads to massive obesity in ob/ob and db/db mice, respectively [85]. Interestingly, leptin, like adiponectin, metformin, TZDs, and exercise, activates AMPK, however, its role in peripheral tissues including liver, muscle, and fat is only partly understood (Fig. (**1**)) [85].

Leptin expression and secretion are significantly upregulated in obesity [85]. Furthermore, body fat stores and leptin plasma levels are strongly correlated [85]. TZDs similar to feeding downregulate leptin in rodent fat *in vivo* and *in vitro* whereas leptin synthesis is stimulated upon starvation [86-90].

In normal obesity, leptin administration can further increase endogenous leptin levels, however, appetite and body weight are not significantly altered [91]. Central leptin resistance due to saturation of leptin transport across the blood-brain barrier is a potential mechanism for these disappointing clinical results [92]. However, supplementation of this adipokine is a valuable treatment option when obesity and/or insulin resistance are associated with low endogenous leptin levels. Thus, in nine female patients with lipodystrophy and serum leptin levels below 4 ng/ml leptin treatment decreases glycosylated hemoglobin by on average 1. 9 % due to improved insulin-stimulated hepatic and peripheral glucose metabolism, as well as a marked reduction in hepatic and muscle triglyceride content [93,94]. Daily caloric intake also decreases supporting leptin´s role as an appetite-suppressive adipokine [94]. Another study demonstrates convincingly that in three rare cases of genetically-based leptin deficiency in adults, administration of recombinant human leptin in physiological doses for 18 months results in a dramatic decrease in BMI from a mean of 51. 2 to 26. 9 kg/m^2 [95]. Secondary parameters studied including physical activity, hypogonadism, and type 2 diabetes mellitus also improve significantly [95]. Similar results after leptin supplementation can also be found in morbidly obese leptin-deficient children [96]. In a more recent study, leptin supplementation in women with hypothalamic amenorrhea and low leptin levels improves reproductive, thyroid, and growth hormone axes, as well as markers of bone formation [97].

CONCLUSIONS

Adipokines are an exciting new link between obesity and insulin resistance but also obesity and cardiovascular disease, hypertension, as well as hyperlipidemia. Since the influence of adipokines on obesity and insulin sensitivity has been studied most thoroughly, the current review has focused on this issue. In general, adipokines can increase or decrease insulin sensitivity (Fig. (**1**)). Furthermore, they can influence appetite and food intake (Fig. (**1**)).

Concerning insulin-sensitizing/insulin-mimetic fat-derived proteins, recent results on novel adipokines have broadened the spectrum. Thus, visfatin, vaspin, and omentin have been suggested as novel adipokines improving insulin action besides adiponectin. Various studies have dramatically increased our knowledge on adiponectin biology. We know that high plasma levels correlate with a lower incidence of type 2 diabetes mellitus and decreased expression can be found in insulin resistance. Unfortunately, no experiments in

humans have been presented so far, proving adiponectin´s role as an endogenous insulin-sensitizer directly. However, taking animal studies into consideration, adiponectin, adiponectin receptor agonists, and AMPK activators are good candidates for new treatment options of type 2 diabetes and obesity. Furthermore, drugs increasing expression of anti-inflammatory adiponectin in fat are of potential interest. Thus, TZDs inducing PPARγ-activity have already been shown to stimulate adiponectin. Other compounds including combined PPARα/PPARγ-agonist might also mediate some of their effects on insulin sensitivity *via* similar mechanisms. The role of visfatin, vaspin, and omentin in human insulin resistance and obesity has to be elucidated much more thoroughly before safe conclusions about their therapeutic potential can be drawn.

Concerning insulin resistance-inducing adipokines, the role of IL-6 in human insulin sensitivity and obesity is best understood. Thus, IL-6 impairs glucose tolerance *in vivo* and high levels at baseline are associated with increased risk to develop impaired glucose tolerance. However, therapies based on direct inhibition of IL-6 will probably not be introduced into clinical practice, because of various side-effects to be expected. TNFα and resistin appear as major links between insulin resistance and obesity in rodents, however, their role in humans is uncertain. Furthermore, TNFα inhibition has no effect on insulin sensitivity in human studies. For PAI-1, SPARC/osteonectin, MCP-1, and RBP4, the data concerning human physiology are too sparse to exactly define their role in insulin resistance and obesity. Thus, their potential cannot be evaluated until more data are available.

Leptin is the major appetite-suppressive hormone and this adipokine works well in human leptin deficiency. Here, it is important to more clearly understand the cause of leptin resistance in obesity and to find ways to reconstitute central and peripheral leptin signaling.

Taken the studies summarized in this review into consideration, various adipokines are potential targets in the treatment of insulin resistance and obesity in upcoming years. However, much more data especially on human (patho)physiology and on the newer adipokines are necessary to more clearly focus on the most promising candidates.

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