Physiological, pathological and potential therapeutic roles of adipokines

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Formerly regarded purely as passive energy storage, adipose tissue is now recognized as a vital endocrine organ. Adipocytes secrete diverse peptide hormones named adipokines, which act in a autocrine, paracrine or endocrine way to influence several biological functions. Adipokines comprise diverse bioactive substances, including cytokines, growth, and complement factors, which perform essential regulatory functions related to energy balance, satiety and immunity. Presently adipokines have been widely implicated in obesity, diabetes, hypertension and cardiovascular diseases.
In this article we aim to present a brief description of the roles and potential therapeutic modulation of adipokines, such as leptin, resistin, adiponectin, apelin, visfatin, FABP-4, tumor necrosis factor-α (TNF-α), interleukin-6 and plasminogen activator inhibitor-1 (PAI-1).

Introduction

Since the identification of the adipose tissue as a major site for the metabolism of steroids and the characterization of leptin, adipose tissue can no longer be viewed as a simple storage site of energy, but rather as a fundamental endocrine organ [1,2]. Research on the biology of adipocytes during the past decade has revealed their capacity to secrete a variety of bioactive substances, such as leptin, adiponectin and resistin. Some of these so-called 'adipokines', such as adiponectin and leptin, despite being expressed by other tissues, are predominantly produced by adipocytes. Contrarily other adipokines, namely tumor necrosis factor-α (TNF-α) and plasminogen activator inhibitor-1 (PAI-1), are mostly expressed by other tissues and organs, rather than in adipose tissue, regardless of their high levels in the latter. Adipose tissue is the largest organ in the body and an extensive vascular network supplies each adipocyte. Consequently, even a slight cellular secretion is physiologically capable of regulating development, metabolism, eating behavior, fat storage, insulin sensitivity, hemostasis, blood pressure, immunity and inflammation. Indeed, adipose tissue macrophage number increases in obesity and participates in inflammatory pathways that are activated in adipose tissues of obese individuals [3]. Moreover, besides promoting fibrosis and adipocyte hypertrophy [4], obesity alters adipose tissue metabolic and endocrine function and leads to an increased release of fatty acids, hormones and proinflammatory molecules that contribute to obesity-associated complications, such as the metabolic syndrome, type 2 diabetes, vascular sclerotic processes and hypertension. Regarding fibrosis, it has recently been suggested differential clinical consequences of fibrosis in human white adipose tissue (WAT). In omental WAT, fibrosis could contribute to limit adipocyte hypertrophy and is associated with a better lipid profile, whereas subcutaneous WAT fibrosis might hamper fat mass loss induced by surgery [4].

Indeed, the redundant adipose tissue maintains a proinflammatory state in obesity both by lipotoxicity and by the direct secretion of inflammatory mediators. Nevertheless, it also releases anti-inflammatory and beneficial adipokines, possibly as part of an adaptive and counter-regulatory response. Additionally, adipose tissue anatomical distribution has a major role in cardiovascular risk, to which visceral adipose tissue mostly contributes [5]. This anatomical remark has a cellular counterpart, since visceral and subcutaneous adipocytes markedly differ in their biology [5]. In this regard, excessive accumulation of visceral adipose tissue is associated to an increase incidence in metabolic disturbances, elevated risk of cardiovascular diseases and premature death. The mechanisms underlying the role of visceral adipose tissue

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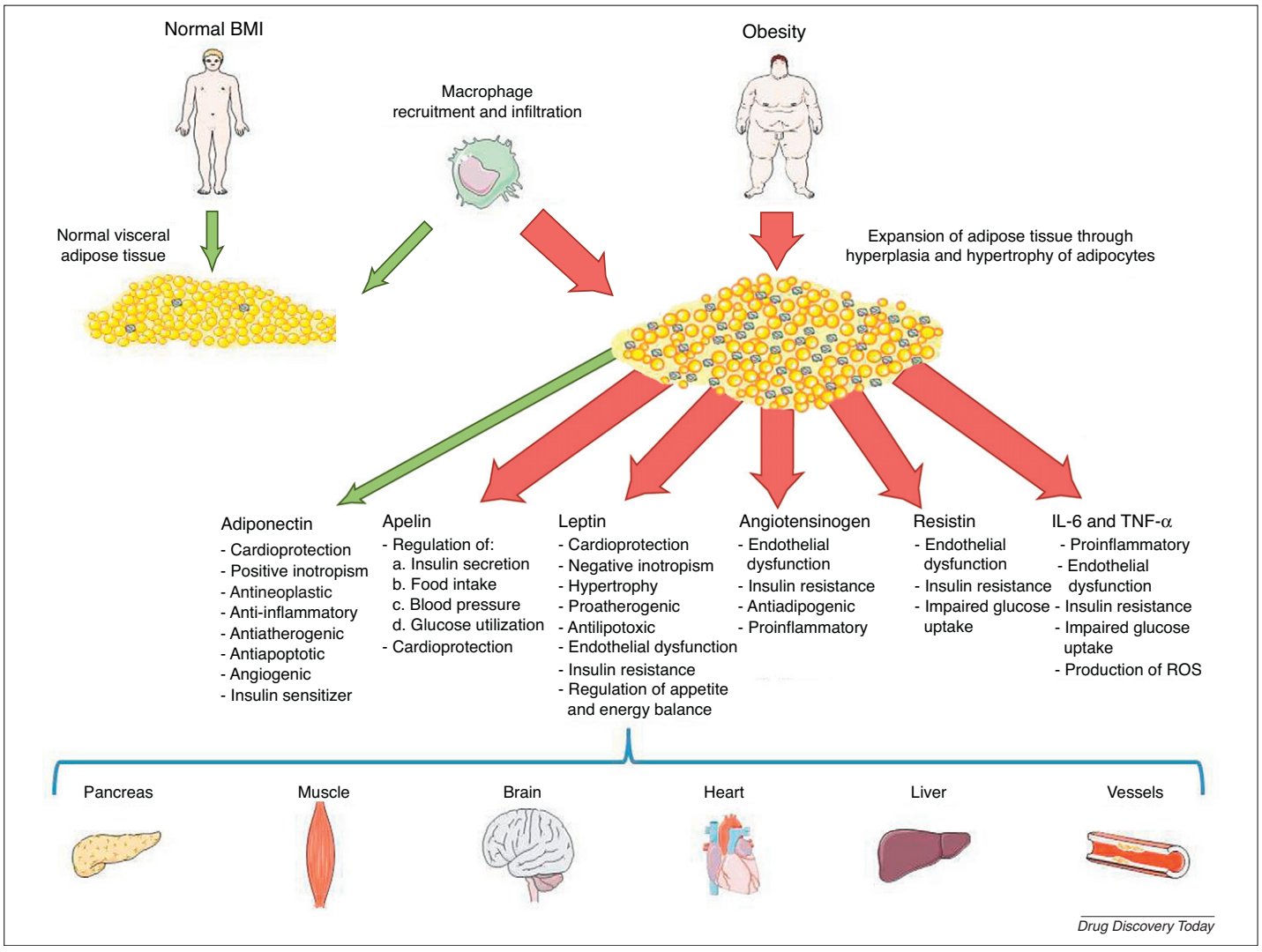

FIGURE 1

Contribution of adipokines to obesity and metabolic syndrome abnormalities. The schematic overview illustrates some adipokines' actions in peripheral and central metabolic processes. Macrophage-infiltration of the expanding adipose tissue is crucial in the inflammatory response modulation, but their interaction with endothelial cells and adipocytes is also fundamental. The change of resident macrophages from the M2 status, that preserves adipocyte function, to the M1 status, that express several inflammatory mediators, seems to have a fundamental role in the development of insulin resistance. *Abbreviations*: BMI: body mass index; IL-6: interleukin-6; RAS: renin–angiotensin system; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α. This figure was produced using Servier Medical Art.

and obesity-associated diseases include the increased portal release of free fatty acids (FFA), in addition to an altered secretion of adipokines (Fig. 1) [6].

In this article we aim to address the updated pathophysiological roles of adipokines, such as leptin, adiponectin, apelin, visfatin, FABP-4, TNF-α, IL-6 and PAI-1 and to provide an overview of their potential use as therapeutic targets.

Adiponectin

Adiponectin, the most abundant protein secreted by WAT [7], is composed of two structurally distinct domains, the C-terminal collagen-like fibrous domain and the C1q-like globular domain [8]. Adiponectin is able to form several stable complexes of different-molecular weights, such as the intracellular higher-molecular weight (HMW) multimer or the circulating trimer, hexamer [lower molecular weight (LMW)] forms [7,9].

Three different receptors have been identified: the structurally highly related G-protein-coupled seven-transmembrane-domain receptors, AdipoR1 and AdipoR2 [10], and also T-cadherin, a glycosylphosphatidylinositol-anchored extracellular protein responsive to hexameric and HMW-adiponectin [7,11]. AdipoR1 is ubiquitously expressed in muscle, whereas AdipoR2 is liver specific. T-cadherin is widespread in muscle, cardiovascular and nervous systems [11].

Accumulating evidence suggests that adiponectin presents cardioprotective, antineoplastic, anti-inflammatory, antiatherogenic and antiapoptotic properties [12] and is extensively involved in obesity-related disorders [13]. In normal human subjects, adiponectin's major source is the adipose tissue [11], although it can be produced by other organs such as bone marrow, bone-forming cells, fetal tissue, myocytes and salivary glands [13]. Its plasma levels typically range from 5 to 10 μg/ml (~0.01% of all plasma

protein) [7] and, unlike most of the other adipokines, its plasma concentration is inversely correlated with body mass index (BMI) and with visceral fat accumulation [14]. Circulating adiponectin levels are reduced in obesity and type 2 diabetes [14], positively correlates with high-density lipoprotein (HDL-C) and negatively correlates with triglycerides and apolipoprotein B-100 [15]. Mice lacking adiponectin develop insulin resistance, glucose intolerance, hyperglycemia and hypertension [13]; however, the underlying mechanisms remain unclear. Moreover, adiponectin seems to have potential to become a therapeutic target against obesity and metabolic syndrome. In rat adipose tissue and cultured mouse adipocytes, rimonabant, an antiobesity drug, significantly increases the levels of this protein and these results are mediated through a cannabinoid type 1 receptor pathway [16].

The beneficial actions of adiponectin on insulin resistance appear to be mediated by phosphorylation and activation of AMP-activated-protein kinase (AMPK) in skeletal muscle and liver [17]. Adenovirus-mediated transcription of adiponectin reduces TNF-α levels and decreases insulin resistance in *adiponectin*-knockout mice [18]. Therefore, the insulin-sensitizing effects of adiponectin may result from its ability to suppress inflammatory cytokine production [19]. Indeed, in *in vitro* experiments adiponectin inhibits nuclear factor-κB (NF-κB) activation, effectively reducing TNF-α and interleukin-8 (IL-8) in endothelial cells [20]. These anti-inflammatory actions are further confirmed by the fact that hypoadiponectinemia is associated with augmented C-reactive protein (CPR) in human aortic endothelial cells and rat hepatocytes [21].

Adiponectin's cardioprotective effects were confirmed by the reduced apoptosis and inflammation levels observed in cultured cardiomyocytes and *in* vivo animals exposed to hypoxia-reoxygenation cycle after its administration. This effect was modulated by AMPK and cyclooxygenase-2 activation, in addition to prevent excessive peroxynitrite-induced oxidation and nitrative stress [18,22]. In endothelial cells adiponectin has antiapoptotic actions also by activating AMPK signaling [20] and by preventing angiotensin-II-actions, reversing endothelial nitric oxide synthase (eNOS) and/or heat-shock protein-90 (HSP90) coupling [23]. Moreover, adiponectin decreased the size of cardiac lesion through modulation of pro-survival reactions, cardiac energy metabolism and inhibition of hypertrophic remodeling [20]. Interestingly, higher levels of adiponectin are correlated with severity and mortality of systolic heart failure (HF) [12], possibly due to the fact that higher BMI favors survival in end-stage HF [20,24] meaning that, paradoxically, obesity is a predictor of better prognosis in patients with systolic HF [24]. Hypoadiponectinemia contributes to the worsening of diastolic dysfunction in hypertension-induced diastolic HF patients. Moreover, recent studies suggested that aldosterone negatively regulates adiponectin, thereby contributing to the reduction of adiponectin sensitivity in the heart possibly through interferon-γ modulation [25] and that spontaneously hypertensive rats exhibited an impaired adiponectin-induced activation of AMPK signaling in insulin-sensitive tissues, such as liver, skeletal muscle and blood vessels [26,27]. Therefore hyperadiponectinemia together with overexpression of adiponectin receptors in skeletal muscle may reflect a defective compensatory mechanism to overcome adiponectin resistance in hypertensive rats [26].

Adiponectin was found to be reduced in patients with coronary artery disease (CAD) and its serum levels are indicative for symptomatic CAD, but are not further reduced with the progression of this disease [28]. Moreover, adiponectin has been suggested to have antiatherosclerotic properties [29] and, despite its serum levels being decreased in incipient atherosclerosis [30], it has recently been recognized as a potential biomarker of atherosclerotic lesions [31]. Adiponectin suppresses foam cell formation by macrophages [32], decreases the expression of endothelial adhesion molecules [33], and inhibits vascular smooth muscle migration [20]. Furthermore, adiponectin modulates inflammatory processes by inhibition of endothelial nuclear transcription factor NF-κB signaling that mediates the effects of several cytokines [34]. Animal studies showed that *adiponectin*-knockout mice developed atherosclerotic lesions [35] and that adiponectin adenovirus-mediated delivery reduced the size of atherosclerotic lesions in *apolipoprotein-E*-knockout mice [12].

Also, adiponectin subfractions seem to have different roles in the pathogenesis of atherosclerosis: while the HMW subfraction correlates to intima-media thickness in pre-atherosclerosis of obese juveniles and adolescents [36], the LMW is positively associated with nuchal subcutaneous adipose tissue thickness [37].

Despite these pleiotropic effects on numerous physiological processes, the use of adiponectin or its receptors as therapeutic targets is complex due to the presence of different adiponectin isoforms and production sites, multiple receptors with different affinities for adiponectin isoforms, and by cell-type-specific effects in different tissues [13]. However, adiponectin injection has been recently shown to reverse pulmonary emphysema-induced dysfunction with few adverse side effects [38]. Also lately a new kit for assessing the risk of a type 1 diabetes patient to suffer from a cardiovascular event and/or terminal renal failure and/or death has been developed based on the determination of adiponectin levels [39].

Apelin

Apelin is derived from a 77 amino-acid prepropeptide that produces four active isoforms (apelin-12, 13, 17 and 36), each showing different receptor-binding affinities, from which (Pyr^1)-apelin-13 has the highest abundance and activity [40,41]. Apelin exerts its paracrine function by binding and activating the apelin receptor (Aplnr) (formerly named APJ) [42], initiating the downstream phosphoinositide 3-kinase (PI3K), which subsequently stimulates protein kinase B (PKB/AKT) and extracellular signal-regulated kinases (ERKs) pathways [43]. Increasing evidence suggests that apelin regulates multiple physiological functions, including fluid homeostasis, food intake, cell proliferation, blood pressure regulation, angiogenesis and glucose utilization [44–47] and therefore is capable of interfering with diabetes, obesity, hypertension or cardiovascular diseases [47,48].

Apelin system is widely expressed in various tissues, including adipose tissue, brain, heart, lungs and kidneys [48]. Apelin production in adipose tissue is regulated by factors, such as fasting and refeeding, insulin [44], hypoxia [49], growth hormone [50] and TNF-α [50]. Whereas insulin stimulates adipose tissue apelin expression [44], apelin inhibits insulin secretion [50], presenting an interesting interaction between the two systems. Interestingly, apelin-13 has been found to have beneficial effects on high-fat-diet-induced obese

mice, improving glucose tolerance and increasing glucose utilization in normal and insulin-resistant mice through AMPK and AKT signaling pathways [51]. Furthermore, intraperitoneal administration of apelin in normal and obese mice for 14 days reduced body adiposity without altering food intake or insulin, leptin and triglyceride levels whereas it increased adiponectin levels [52]. In a well-established mice model of type 1 diabetes, chronic apelin-13 treatment significantly ameliorated pancreatic islet mass and insulin production [43].

Considering these physiological actions in the control of glucose homeostasis, it is tempting to propose a link between apelin and obesity-associated insulin resistance. Accordingly, apelin overproduction in the obese might represent a protective mechanism before the emergence of type 2 diabetes or cardiovascular diseases. Thus, apelin becomes a potential therapeutic target in diabetes and obesity.

Increasing evidences suggest that apelin signaling mediates important effects in cardiovascular homeostasis. Apelin acutely promotes a potent positive inotropic effect in healthy and HF rats [53,54] accompanied by a decrease of left ventricular (LV) preload and afterload without signs of cellular hypertrophy. Despite normal cardiac development to adulthood, *Aplnr* and *apelin*-knockout mice develop a progressive impairment of contractility both with aging and in response to pressure overload. While *apelin*-knockout mice reverts the decreased contractility due to aging after a continuous 2-week infusion of apelin-13 [55], *Aplnr*-knockout mice present marked decreased of exercise capacity [56] and diminished insulin sensitivity [57]. In humans, plasma *apelin* levels have been reported as a marker of pulmonary disease [58] and in HF, which was recently patented [59]. In monocrotaline-induced pulmonary hypertension (PH) rats, chronic (Pyr^1)-apelin-13 treatment attenuated neurohumoral activation and right ventricular hypertrophy [60]. A unique characteristic of apelin in PH, when compared to other inotropic agents, is the fact that it enhances right ventricular function, while decreasing myocardial hypertrophy [53,60] and fibrosis [60].

An emerging feature is the interaction between the apelinergic system and the renin–angiotensin system (RAS) regarding receptors homology, apelin-angiotensin-II receptor type 1 (AT_1) receptor heterodimers formation, physiological counteractions and angiotensin-converting enzyme 2 (ACE2)-mediated apelin inactivation [48]. Such interaction assumes biological relevance since *Aplnr*-knockout mice display an increased angiotensin-II vasopressor effect [45]. Modulation of apelin's breakdown enzyme, ACE2, may in the future, be a therapeutic target in major cardiovascular diseases.

Apelin is a vasodilator both *in vivo* [61] and *ex vivo* models employing human arteries [40], veins [40] and resistance vessels [46]. Accordingly, intravenous administration in rodents reduces mean arterial pressure [45], systemic venous tone [62] and cardiac preload and afterload [53]. Such vasodilation is endothelium-dependent [40] and predominantly mediated through nitric oxide (NO)-dependent pathways ranging from 5% for apelin-36 to 25% for apelin-12 [63]. Moreover, apelin stimulates transcription [64] and phosphorylation [45] of eNOS-synthase *in vitro* and increases plasma nitrate and nitrite concentrations *in vivo* [63].

At present, apelin represents an angiogenic factor similar to vascular endothelial growth factor (VEGF) and has been investigated as a new target to limit tumor angiogenesis. Indeed, apelin is overexpressed in one third of human tumors and promotes lymphangiogenesis and tumor growth *in vivo* [65]. Thus, its inhibition might represent an interesting mechanism to restrain tumor growth. Conversely, apelin's angiogenic properties, which affect both existing and newly developing blood vessels, together with its capacity to enhance superoxide dismutase activity and to protect against ischemic heart disease after hypoxia-reperfusion [66] might be helpful for functional recovery after ischemia [67,68]. Several patents concerning apelin-induced angiogenesis role, mostly related to wound healing, acquired immune deficiency syndrome (AIDS) or tumor-induced excessive angiogenesis have been proposed. Recently, the first synthetic molecules capable of interfering with the Aplnr were reported: the first nonpeptidic agonist, E339-3D6 and the antagonists CXCR4 and ALX40-4C [42].

Leptin

Although it was also identified in many other tissues, leptin, the product of the obese (ob) gene, is mostly expressed in the adipose tissue, and is present in human serum at levels ranging from 1 to 15 ng/mL in non-obese individuals, but reaching levels higher than 30 ng/mL in subjects with a BMI $\geq$30 kg/m^2 [69]. Moreover, there is a gender dimorphism in leptin levels with women showing higher leptin concentrations than men [70]. It has a cytokine-like structure [71] and acts in at least six isoforms of ubiquitously expressed single membrane-spanning receptors (OB-Rs) that belong to the cytokine receptor family [72]. Indeed, leptin induces macrophage TNF-α production and modulates the immune response [73]. Moreover, leptin correlates with IL-6 plasma levels in juvenile patients with metabolic syndrome [74]. Leptin-deficient animals are more susceptible to infections [75] and display impaired cell-mediated immunity [76]. Nevertheless, the fundamental role of leptin seems to be the regulation of body weight and appetite [77]. Leptin reduces food intake and increases energy expenditure, by enhancing thermogenesis and metabolic rate, mainly by central nervous system effects [78]. Leptin increases physical activity and reduces insulinemia [79]. Basal levels of leptin in the fed state signal energy sufficiency, whereas food deprivation rapidly increases leptin inducing starvation behavior [71]. Its circulating and adipose tissue mRNA levels are strongly associated to fat mass [80] and it is mainly expressed in subcutaneous adipose tissue [81]. Circulating leptin levels mainly reflect the amount of energy stored in adipose tissue and direct the central nervous system in regulating energy homeostasis, neuroendocrine function and metabolism. Leptin causes leanness in wild-type and reverses obesity in leptin-deficient animals. Nevertheless, despite high leptin levels in human obesity, weight gain and adiposity increases. This acquired leptin resistance that accompanies most cases of obesity and facilitates energy storage in periods of abundance [80] might be partly mediated by limited blood–brain barrier permeation and acquired altered hypothalamic signaling [82], but most likely also to changes in subcellular pathways, namely a negative feedback regulation by suppressor of cytokine signaling-3 acting at both Janus-activated kinases and leptin receptors [83]. Recombinant leptin was administered safely to obese patients, including children [84,85] and yielded benefits in female patients with lipodystrophy and leptin deficiency, as reflected by improvements in glycaemic control and serum triglycerides levels [86]. Still, most trials with leptin have been disappointing and efforts are now

directed at the discovery of drugs that may circumvent the mechanism of leptin resistance [87]. Several combination therapies including leptin have been tested in obesity and particularly the combination with pramlintide, an amylin analog, has resulted in sustained weight loss prompting a currently undergoing phase III trial [88]. One of the current major constraints is the lack of effective enteral formulations.

Beyond appetite suppression, several other important roles have been attributed to leptin. Acting in peripheral receptors it modulates the sympathetic nervous system, cardiac metabolism and overall cardiovascular physiology. Leptin exerts vasodilator effects in aorta and coronary arteries in addition to in mesenteric arteries through NO or endothelium-derived hyperpolarizing factor, respectively [89,90]. However, these vascular responses are impaired in spontaneously hypertensive rats [91]. Therefore, leptin may be an essential link between obesity and hypertension both by sympathetic system activation [92], impaired vasodilation [91] and by promoting vascular smooth muscle cell proliferation [93]. Although increased plasma levels correlate with worse prognosis in HF and myocardial ischemia and stimulates pathological hypertrophy pathways [94], leptin activates molecular mechanisms that provide protection after coronary artery ligation [95] and it minimizes lipotoxicity in peripheral tissues as well as in the heart by changing intracellular metabolism [96].

Adipose tissue renin–angiotensin system

Copper *et al.* showed that hypertensive obese individuals had higher serum angiotensin-converting enzyme (ACE) and angiotensinogen levels [97] after multivariate analyses controlling for several risk factors, providing a potential mechanistic link between obesity and hypertension [97]. The first reference to adipose tissue RAS dates back to 1987, when angiotensinogen mRNA was first identified in rat periaortic brown adipose tissue [98].

Angiotensinogen release has since been reported in adipose tissue deposits from blood vessel walls, atria and mesenterium in animal models, but also in human adipocytes [99]. Interestingly, angiotensinogen release is a feature characteristic of preadipocyte differentiation and therefore a late marker of differentiation [100]. The production of other RAS elements, namely, ACE, angiotensin-II, ACE2, the expression of AT_1, AT_2 and rennin and/or prorenin receptors have been detected in adipocytes [101,102]. Furthermore, human adipose tissue also expresses non-RAS enzymes, like cathepsin D and cathepsin G [99]. To define a functional local RAS it is not only important to show the local synthesis of its several elements, but also to demonstrate its activity and regulation by different factors [99]. In accordance adipose tissue RAS activity may be modulated by fasting or excessive alimentary intake, which reduces and increases angiotensinogen production, respectively [103]. In preadipocyte cell lines angiotensinogen expression is also modulated by fatty acids, glucocorticoids and TNF-α [104,105], while in animal models sympathetic stimulation, nephrectomy and enalapril increase angiotensinogen release [106].

Several pathophysiological roles have been attributed to adipose tissue RAS. Angiotensin-II inhibits insulin-induced adipogenesis in human preadipocytes [107], through the AT1 receptor and activation of NF-κB and mitogen-activated protein kinase (MAPK) ERK pathways, in addition to peroxisome proliferator-activated receptor-γ (PPAR-γ) phosphorylation [108]. Moreover, angiotensin-II inhibits the formation of insulin-sensitive adipocytes and inhibits insulin-mediated effects on PI3K, thus contributing to the development of insulin resistance. RAS blockade might help to prevent the loss of adipogenic potential [101,107]. Adipose tissue RAS also promotes lipolysis or lipogenesis in WAT through AT_1 or AT_2 receptor activation, respectively [109]. In humans, however, lipolytic effects are weak [110]. Angiotensin-II promotes the release of prostacyclin and NO and adipokines like leptin and PAI-1 [101]. Moreover adipocyte overexpression of angiotensinogen increases the release of inflammatory cytokines [111]. Interestingly, the phenotype of animals with angiotensinogen adipose tissue overexpression is characterized by increased fat mass and higher blood pressure [112] and the loss of AT_2 expression is sufficient to rescue obesity induced by adipose tissue angiotensinogen overexpression. However, despite a reduction of adipose mass, AT_2 receptor deficiency increases renin production, further worsening the hypertension caused by angiotensinogen overexpression [111]. The role of new RAS pathways, namely ACE2/mas receptor/angiotensin 1–7, at this level is still largely unresolved. In a recent report, a *mas receptor*-knockout model revealed 50% increase in abdominal fat mass and dramatic changes in glucose and lipid metabolism inducing a metabolic syndrome-like state [113]. All these experimental studies thus point to the existence of a functional tissue RAS with complex interactions at several levels. However, the role of adipose tissue RAS in the development and treatment of human obesity-associated hypertension still warrants further studies.

In clinical studies, it is known that the renin, ACE and AT_1 receptor genes are significantly upregulated in obese hypertensives [114] and that in post-menopausal women a 5% reduction in body weight can lead to a meaningfully reduced RAS in plasma and adipose tissue [115]. However, in spite of the high prevalence of obesity-related hypertension, randomized clinical studies evaluating specific effects of different pharmacological agents, namely modulating RAS activity, are scarce, with small samples and short duration and thus, at the moment, there is not enough evidence to recommend a particular type of drug in the treatment of these patients.

Resistin

Resistin is a 12.5-kD cysteine-rich protein expressed during adipocyte differentiation, but downregulated in the mature adipocytes exposed to glitazone. Mouse resistin contains 114 amino acids and circulates as a homodimer of two peptides [116]. In mice the major source of resistin are the adipocytes, followed by the pancreatic islets, the pituitary gland and the hypothalamus [117]. Its secretion can be regulated by factors such as epinephrine, somatropin, insulin and glucose [117,118].

Previous studies suggested that resistin might constitute the link between obesity [116] and insulin resistance [117]. Later, resistin was shown to have an important role in adipogenesis, inflammation and cardiovascular disease [119].

Experimental studies suggest that resistin can be a factor causing insulin resistance in obese rodents: resistin plasma concentrations are higher in genetic and diet-induced obese and insulin-resistant mice and its administration to normal mice induces impaired glucose tolerance and insulin resistance. Furthermore, administration of the neutralizing antibody against resistin increases

insulin sensitivity and reduces blood glucose in obese diabetic mice [116,120,121]. This insulin resistance induced by resistin is likely due to an upregulation of suppressors of cytokine signaling, which interferes with the activation of insulin-receptor substrate [117]. However, subsequent studies have not supported these findings. Way *et al.* reported decreased resistin levels in obese rodent models and showed that its expression was suppressed by FFA, and increased by PPAR-γ agonists stimulation [122]. Therefore the role of resistin as a mediator of insulin resistance in rodents remains highly controversial.

In human adipose tissue resistin levels are high not due to adipocytes [121], but mostly to monocytes and macrophages contribution [123]. Moreover, it seems to be involved in the recruitment of other immune cells and in the secretion of proinflammatory factors [117,123]. Like in rodents, several conflicting reports have attempted to establish a relation between resistin and obesity-related disorders. Nagaev *et al.* reported that resistin levels were undetectable in human fat cells from subjects with varying degrees of insulin resistance and obesity [124]. Also Yamauchi *et al.* did not find any relationship between high-fat diet and serum resistin concentration and reported that high resistin levels did not contribute to insulin resistance in humans [121]. Contrarily, other studies demonstrated increased resistin levels in adipose tissue from obese humans [121], particularly in cultured preadipocytes when compared to mature adipocytes [125]. Moreover, while some studies clearly show that resistin serum levels correlate with its expression in abdominal adipose tissue [121], others report similar levels in subcutaneous and omental adipose tissue, challenging the idea that resistin is the link between visceral adiposity and insulin resistance [120,126]. Moreover, serum resistin does not seem to affect adipocyte differentiation [120].

Thiazolidinediones inhibit resistin expression in human macrophages [127] and lower serum resistin levels in rodents [116,123] as well as in humans [128], suggesting that the insulin-sensitizing effect of glitazone can be attributed partly to its inhibition of resistin expression. Moreover, rosiglitazone-induced decrease in resistin levels highlights a direct role of PPAR-γ in the regulation of resistin expression [127]. We can conclude that most recent human studies do not support a role for resistin as an important mediator of insulin sensitivity and diabetes.

Resistin also seems to be involved in the development of atherosclerosis in humans by promoting the formation of foam cells, the proliferation and migration of vascular endothelial and smooth muscle cells [117], endothelial dysfunction possibly by decreasing eNOS and/or NO expression, oxidative stress in human coronary arteries [119] and increasing the expression of adhesion molecules in human saphenous vein [123]. This resistin proinflammatory role appears to be regulated by activation of the NFκB transcription factor [117]. Furthermore correlations between increased serum resistin levels and atherosclerosis were observed in Japanese and American patient studies [129,130]. Recently clinical studies demonstrated an increase in circulating resistin levels in humans with premature CAD [123] and indicated that high plasma levels of resistin are associated with the severity of coronary disease and hypertension [119]. The divergent roles of resistin in humans and rodents are evident at the moment. The discovery of its receptor will likely contribute to a better understanding of this adipokine.

TNF-α, interleukin-6 and cytokines

TNF-α and IL-6 are the most widely studied cytokines produced by the adipose tissue. TNF-α production site was identified well before the production of leptin [131]. Overproduction of TNF-α from visceral adipose tissue induced by infiltrating macrophages [132] is at the heart of insulin resistance [133], impaired insulin-mediated glucose transport in adipose tissue [134] and lipoprotein lipase excessive activity [135]. Perivascular adipose tissue importantly contributes to TNF-α production [136], whose actions include vessel tone modulation [137]. Moreover, TNF-α activates proinflammatory subcellular pathways and induces the production of reactive oxygen species (ROS) [138], thus promoting not only endothelial dysfunction and obesity-related disorders [139,140], but also perpetuating the proinflammatory activity in adipose tissue [141]. However, in healthy and obese humans TNF-α production by adipocytes may not be quantitatively relevant and increased circulating levels in the obese might depend on the systemic activation by other adipokines.

As part of the proinflammatory activity, IL-6 expression and secretion are also increased in adipose tissue [142] and, like TNF-α, the main source are infiltrating macrophages [3]. Nevertheless, contrarily to TNF-α, adipocytes production, especially visceral adipose tissue, is quantitatively important in obese humans [143]. IL-6 decreases insulin-dependent glucose uptake by down-regulating glucose transporter type 4 (GLUT4) and the insulin-receptor substrate-1 [144] and it may reinforce hyperlipidemia by direct hepatic effects through the portal circulation [145]. Sustained increased levels of IL-6 are associated both with the release of acute phase response proteins and with systemic arterial hypertension, myocardial infarction and cardiovascular mortality [146]. Despite some controversy, most studies suggest an active and important role in vascular dysfunction [147] and vascular smooth muscle proliferation [148] mediated by Janus Kinases, signal transducers and activators of transcription [149]. Many other cytokines are released by the adipose tissue, namely proinflammatory cytokines such as IL-1 and IL-8 and anti-inflammatory cytokines such as IL-10, all of which can participate in the modulation of the low grade inflammatory response that accompanies obesity [143].

Other adipokines

Fatty acid-binding proteins (FABPs)

Fatty acid-binding proteins (FABPs) are predominantly expressed in adipose tissue and macrophages and accounts for 1% of total cytosolic protein in human adipose tissue [150]. In macrophages, *FABP-4* expression is induced by low-density lipoprotein (LDL) and Toll-like receptor activators [151]. FABP-4 regulates inflammatory activity and cholesterol trafficking [152]. Recently it has been demonstrated that FABP-4 elicits a direct and acute Ca^{2+}-dependent suppressing effect on cardiomyocyte contraction [150]. Studies in *FABP-4*-null mice have demonstrated its role in glucose and lipid metabolism maintenance and protection from development of insulin resistance in diet-induced obesity, type 2 diabetes, and atherosclerosis in models of hypercholesterolemia [151]. Contrarily, a small-molecule inhibitor of FABP-4 was found to be an effective therapeutic agent against severe atherosclerosis and type 2 diabetes [153] and at improving insulin sensitivity in *Leptin*-deficient mice [154]. Humans with a functional genetic variant of the *FABP-4* gene present reduced adipose tissue expression of

FABP-4, have lower serum levels of triglycerides and present a significant reduced risk for developing type 2 diabetes mellitus and CAD [150]. Higher serum FABP-4 has been reported to be useful for the prediction and diagnosis of obesity-related metabolic syndrome and type 2 diabetes mellitus [151].

Plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is the most important inhibitor of tissue plasminogen activator and is a main determinant of fibrinolytic activity. Recent studies established that PAI-1 is expressed in adipose tissue and that its plasma and adipocytes levels correlate with the amount of visceral fat and triglycerides [155] and are increased in type 2 diabetes. The reason why adipocytes secrete a large amount of PAI-1 has not been clarified. However, several authors indicate that plasmin may destroy cells basement membrane facilitating adipocytes enlargement. PAI-1 may inhibit the activity of plasminogen activators to prevent the overproduction of plasmin.

Visfatin or pre-B-cell colony-enhancing (PBEF)

Visfatin or pre-B-cell colony-enhancing (PBEF) was identified in 2005 as a new 'antidiabetic' adipokine in adipose tissue [156]. This 52 kDa protein circulates in plasma at levels approximately 15 ng/ml and is increased in type 2 diabetics, although it is uncertain whether this increase is related to diabetes or, merely, the level of adiposity in these patients [157]. Initially, visfatin was discovered as a cytokine for the differentiation of B cells. Later it showed to inhibit apoptosis of neutrophils in sepsis and was discussed as a novel biomarker for acute lung injury. Visfatin was suggested to present a role in vascular smooth muscle cell maturation and therefore to be involved in vascular pathology in addition to in the physiologic pathways leading to labor. Furthermore, it was found to be upregulated in colorectal cancer and was linked to cell cycle regulation [158]. More recently, visfatin was reported to regulate insulin secretion in the pancreas suggesting that the actions of visfatin may be insulin-receptor mediated [159]. However, its physiological relevance remains controversial, because several other authors were not able to reproduce visfatin insulin-mimetic effects neither *in vitro* nor *in vivo* [160]. In relation to atherogenesis, visfatin expression was shown to be increased in plaques from patients with unstable carotid and coronary atherosclerosis [161]. In the heart, visfatin exhibited direct cardioprotective effects in a murine *in vivo* ischemia and/or reperfusion model by reducing apoptosis and the infarct size as much as 50%, following treatment with a single intravenous bolus dose of the peptide [162].

Concluding remarks

Adipocytes secrete a variety of adipokines that perform vital regulatory functions with respect to energy balance, satiety, fluid balance and immune function. Production and secretion of these substances are considered to be dynamically regulated, mainly

TABLE 1

Overview of the most important physiological functions of several adipokines and target diseases that may benefit in the future from their modulation as therapeutic targets. AngII, angiotensin-II; AT1, angiotensin-II receptor 1; AT2, angiotensin-II receptor 2; DM, diabetes mellitus; IL-6, interleukin-6; FABP-4, fatty acid-binding protein-4.

Adipokine	Target	Potential clinical application	Refs
Adiponectin		Anti-inflammatory effects	[19,20]
		Predictor of the risk of cardiovascular event and/or terminal renal failure and/or death in a type 1 diabetic patients	[39]
	Muscle	Insulin sensitizer in metabolic syndrome	[13]
	Vessels	Atherosclerotic lesion size reduction	[12]
	Heart	Cardioprotection during ischemia-reperfusion	[18]
	Lungs	Regression of pulmonary emphysema dysfunction	[38]
Apelin		Glucose homeostasis and insulin sensitivity regulation	[18]
	Heart	Positive inotropic effect	[54,56]
		Right ventricle function improvement in pulmonary hypertension	[60]
	Vessels	Arterial vasodilatation	[61]
		Plasma marker of pulmonary disease	[58]
		Antiangiogenic properties in tumor growth (by inhibiting apelin receptor)	[65]
		Angiogenic properties in ischemic hearts (by stimulating apelin receptor)	[67,68]
Leptin		Increases physical activity and reduces insulinemia and appetite	[79]
		Treatment of leptin resistance in obesity	[79]
	Heart	Regulation of cardiac metabolism and cardioprotection during ischemia	[95]
RAS		Inhibition of AngII-induced antiadipogenic actions and insulin resistance	[101,107]
		Inhibition of AngII-induced inflammation	[111]
	Adipocytes	Lipolysis *via* AT_1 receptor	[109]
		Lipogenesis *via* AT_2 receptor	[109]
Resistin		Increase insulin sensitivity by administration of antibodies against resistin	[116]
TNF-α and IL-6		Amelioration of arterial hypertension, myocardial infarction and cardiovascular mortality by inhibition of IL-6 release	[146]
FABP-4	Vessels	Anti-atherosclerotic effects by FABP-4 inhibition	[153]
	Muscle	Improved insulin sensitivity by FABP-4 inhibition	[154]
		Predictor of obesity-related metabolic syndrome and type 2 diabetes mellitus	[151]
Visfatin	Pancreas	Insulin secretion regulation	[159]
	Heart	Cardioprotection and antiapoptotic effects during ischemia and/or reperfusion	[162]

according to nutritional condition. Oversecretion of 'deleterious' adipokines such as PAI-1, resistin, TNF-α or IL-6, and hyposecretion of defense adipokines such as adiponectin and apelin, might constitute major mechanisms of life-style related diseases, including diabetes mellitus, hyperlipidemia, hypertension and atherosclerosis.

It has been proposed that the elucidation of the molecular and pathophysiological mechanisms underlying the actions of the adipokines could lead to the development of strategies for the treatment of obesity-related disorders. In this context this article described the most important potential therapeutic applications of the above-mentioned adipokines in its respective section. Table 1 summarizes those major potential therapeutic applications.

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