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REVIEW

Adipose tissue and its role in organ crosstalk

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Abstract

The discovery of adipokines has revealed adipose tissue as a central node in the interorgan crosstalk network, which mediates the regulation of multiple organs and tissues. Adipose tissue is a true endocrine organ that produces and secretes a wide range of mediators regulating adipose tissue function in an auto-/paracrine manner and important distant targets, such as the liver, skeletal muscle, the pancreas and the cardiovascular system. In metabolic disorders such as obesity, enlargement of adipocytes leads to adipose tissue dysfunction and a shift in the secretory profile with an increased release of pro-inflammatory adipokines. Adipose tissue dysfunction has a central role in the development of insulin resistance, type 2 diabetes, and cardiovascular diseases. Besides the well-acknowledged role of adipokines in metabolic diseases, and the increasing number of adipokines being discovered in the last years, the mechanisms underlying the release of many adipokines from adipose tissue remain largely unknown. To combat metabolic diseases, it is crucial to better understand how adipokines can modulate adipose tissue growth and function. Therefore, we will focus on adipokines with a prominent role in auto-/paracrine crosstalk within the adipose tissue such as RBP4, HO-1, WISP2, SFRPs and chemerin. To depict the endocrine crosstalk between adipose tissue with skeletal muscle, the cardiovascular system and the pancreas, we will report the main findings regarding the direct effects of adiponectin, leptin, DPP4 and visfatin on skeletal muscle insulin resistance, cardiovascular function and β-cell growth and function.

Keywords adipokines, adipose tissue, interorgan crosstalk.

Obesity is acknowledged as a global health problem as it is frequently associated with a number of chronic disorders, such as insulin resistance, type 2 diabetes and cardiovascular (CV) disease. The intensive research to find new therapeutic strategies to combat these global epidemics has led to a deep insight into adipose tissue (AT) biology and the discovery of its central role in the interplay with other organs or tissues, the so-called interorgan crosstalk. This concept was initially demonstrated in studies using coculture models of human adipocytes and myocytes (Dietze *et al.* 2002). It is now evident that AT plays a central role in a complex and multidirectional network of auto-/paracrine and endocrine crosstalk between organs and tissues such as liver, skeletal muscle, pancreas or heart. The central role of white AT, and more specifically visceral AT (VAT), in the pathogenesis of metabolic diseases is widely acknowledged (Wronska & Kmiec 2012). However, there are new adipose depots arising in interorgan crosstalk. Other fat depots such as the epicardial and perivascular AT may additionally contribute to the complexity of interorgan crosstalk (Ouwens *et al.* 2010).

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Previously regarded as a passive depot for lipid storage and release of energy-rich substrates, AT is nowadays considered as major active endocrine organ that secretes heterogeneous bioactive factors, the so-called adipokines (Trayhurn & Wood 2004, Scherer 2006). Adipokines comprise, among others, classical cytokines and chemokines, vasoactive and coagulation factors, regulators of lipoprotein metabolism and proteins more specifically secreted by the adipocytes, such as leptin or adiponectin (Mohamed-Ali *et al.* 1998). Since the discovery of leptin, the number of adipokines has notably increased in the last years with new molecules such as visfatin, apelin, omentin or dipeptidyl peptidase 4 (DPP4) among others (Fain *et al.* 2010, Lamers *et al.* 2011). Recently, our group demonstrated the complexity of the human adipokinome consisting of hundreds of different factors (Lehr *et al.* 2012). Despite the extensive research on novel adipokines characterization, the precise mechanisms leading to the secretion of many adipokines are still not fully understood (some of the different adipokine secretory routes are summarized in Fig. 1).

Adipokines can act locally within the AT, but they can also reach distant organs through the systemic circulation, where they can exert a wide range of biological actions, including the regulation of food intake and body weight, insulin sensitivity, inflammation, coagulation or vascular function (Trayhurn & Wood 2004, Guzik *et al.* 2006). Thus, adipokines represent key signalling and mediator molecules for AT to establish a complex network of feedback loops with other distant target organs or tissues. Importantly, an imbalanced adipokine production with prominent secretion of pro-inflammatory adipokines, as observed in clinical metabolic conditions such as obesity and type 2 diabetes mellitus, has been proposed to play a role in the pathogenesis of insulin resistance (Kahn & Flier 2000). AT inflammation results from the combination of adipocyte hypertrophy, cellular stress, hypoxia and increasing infiltration of pro-inflammatory immune cells (Sell *et al.* 2012). Adipocyte enlargement is in turn associated with a shift towards a more pro-inflammatory secretome, with increased expression and secretion of pro-inflammatory adipokines such as TNF-α, IL-6, IL-8 or MCP-1, while the anti-inflammatory adipokine adiponectin is decreased (Skurk *et al.* 2007; as depicted in Fig. 2).

The association between obesity and the metabolic syndrome seems to be critically dependent on body fat distribution. Clinical evidence supports that abdominal obesity is more strongly correlated with the development of the metabolic syndrome than peripheral body fat distribution. Thus, visceral fat exerts a prominent role in the development of insulin resistance, diabetes and CV diseases (Ferrannini *et al.* 1997). Firstly, due to its vascularization, it can modulate peripheral organs through the release of FFA and adipokines into the portal vein, and secondly, because this depot is more prone to macrophage infiltration and inflammation. The visceral and the subcutaneous compartments are the two most abundant fat depots, and it has been shown that they produce a unique profile of adipokines. In this review, we will focus on the role of AT in interorgan crosstalk analysing the mechanisms of adipokine secretion, providing an overview on the main findings on the auto-/paracrine role of AT and its endocrine actions on the skeletal muscle, the CV system and the pancreas.

Secretory function of adipose tissue

In the last years, proteomic profiling of the AT secretome has extensively increased the number of newly identified adipokines (Lehr *et al.* 2012). Nowadays AT is accepted to be a major endocrine organ, which releases soluble factors, the so-called adipokines. Proteins that contain a N-terminal signal peptide are exported via a classical or ER/Golgi-dependent

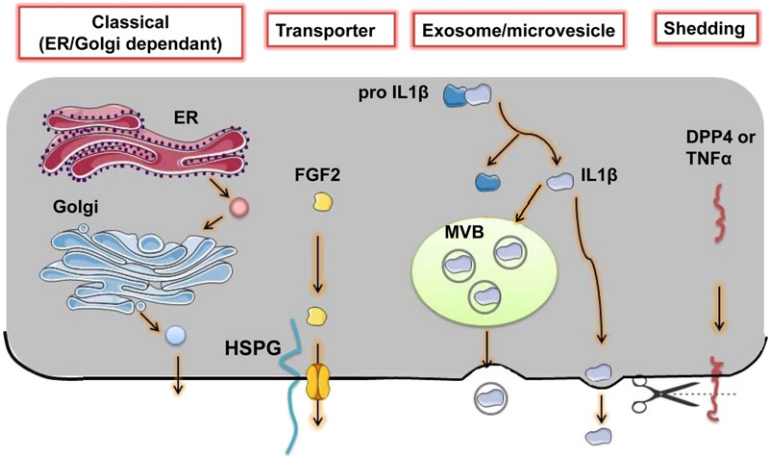

Figure 1 Different routes of protein release out of the cell. Despite the classical or Golgi/ER-dependent pathway, there are non-classical secretory pathways such as transporter-mediated export (e.g. FGF2), vesicular export mechanisms (e.g. IL-1β) and the release by ectodomain shedding (e.g. DPP4 or TNF-α). ER, endoplasmatic reticulum; HSPG, heparan sulphate proteoglycan; MVB, multi-vesicular body.

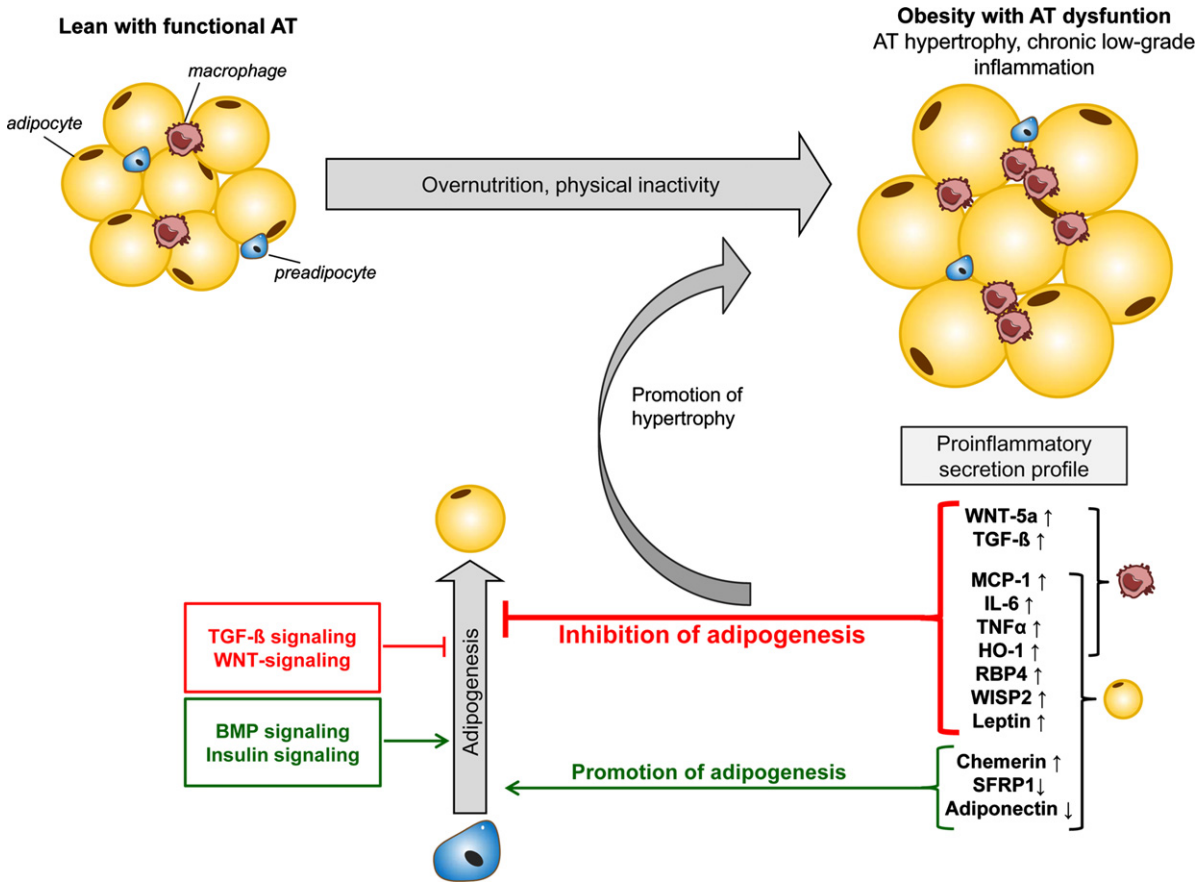

Figure 2 Auto-/paracrine role of adipokines and cytokines in the development of AT dysfunction and hypertrophy. AT consists of different cell types, such as pre-adipocytes, macrophages and mature adipocytes. Under lean conditions, AT displays a normal metabolic function, AT-resident macrophages have an anti-inflammatory M2 phenotype and adipocytes have a small size, resulting in a rather anti-inflammatory secretion profile. Under overnutrition and physical inactivity, obesity starts to develop and adipocytes become enlarged with increased macrophage infiltration and a switch towards the pro-inflammatory M1 phenotype resulting in a low-grade chronic inflammation. In the obese state, the secretion profile shifts towards a rather pro-inflammatory secretion profile, with decreased secretion levels of the insulin-sensitizing adipokine adiponectin and increased release of pro-inflammatory cytokines and adipokines such as TNF-α. Most of those adipokines which are upregulated in obesity have been shown to inhibit adipogenesis and thereby further promote AT hypertrophy. On the other hand, factors promoting adipogenesis, such as adiponectin and SFRP1, are decreased in the obese state.

secretory pathway. This can be blocked by the use of the inhibitors brefeldin A or monensin. Despite this classical export route, there is an increasing number of adipokines that are secreted through alternative routes. These proteins are characterized by a lack of the signal peptide sequence and by insensitivity to brefeldin A or monensin treatment (Nickel 2003). Moreover, several studies support that adipokines can be released by constitutive secretion, regulated or both. The different routes of protein export from the AT are the topic of the following sections (as summarized in Fig. 1). As examples of adipokines secreted by the classical mechanism we have chosen adiponectin and leptin. On the other hand, adipokines released through non-classical secretory mechanisms are depicted with specific examples of transporter-mediated export such as FGF2, shedding like TNF-α and DPP4, multiple secretion routes such as IL-1β or yet unknown mechanisms such as visfatin.

Classical or ER/Golgi-dependent pathway

The classical ER/Golgi secretion mechanism comprises proteins which contain a signal peptide to lead their translocation to the ER and further secretion (Palade 1975). In summary, mRNA of the respective protein is translated at the ribosome of the rough ER and translocated into this compartment. Inside the ER, the correct folding and assembly of the proteins is assured (Vitale & Denecke 1999). Via COPII-coated vesicles proteins exit the ER and enter the Golgi complex, where they are further processed and sorted for trans-

port to their destination. Subsequently vesicles fuse with the plasma membrane and release the proteins into the extracellular space. Two classical adipokines such as leptin and adiponectin are classically secreted. However, the precise mechanisms of adiponectin and leptin secretion are poorly understood.

Adiponectin

There are three secreted oligomeric isoforms of adiponectin: trimeric, hexameric or high-molecular weight (HMW). Adiponectin is an insulin-sensitizing adipokine that reduces glucose production by the liver and increases fatty acid oxidation in skeletal muscle (Wang *et al.* 2008, Dadson *et al.* 2011). Adiponectin serum levels, and especially adiponectin HMW levels, are reduced in obese and type 2 diabetes mellitus patients (Wang *et al.* 2008, Dadson *et al.* 2011). Adiponectin multimerisation and secretion is initially controlled via thiol-mediated retention in the transit through the ER. Via direct interactions with chaperones such as ERp44, adiponectin is retained, while the oxidoreductase Ero1-Lα interacts with ERp44 promoting adiponectin release. The expression of both proteins is tightly regulated by the metabolic state of the cell. Thus, PPARγ activation enhances Ero1-Lα gene expression leading to increased HMW-adiponectin secretion in 3T3-L1 adipocytes (Wang *et al.* 2008). Once adiponectin is moving through the Golgi and trans-Golgi network, a pool of the adipokines is packaged into GGA1 (Golgi localizing γ-adaptin ear homology domain ADP ribosylating factor – ARF – binding)-coated vesicles and delivered to endosomes (Xie *et al.* 2006).

Insulin-stimulated adiponectin release in rat adipocytes may be mediated by activation of the endosomal membrane recycling with the participation of Rab5 and Rab11 which are markers for the early sorting and recycling compartments respectively (Xie *et al.* 2008). A recent study demonstrated that the novel protein FIP1 (family of interacting proteins 1), an effector of Rab11, negatively regulated adiponectin trafficking and secretion. Interestingly, FIP1 expression is downregulated during adipogenesis and by thiazolidinediones stimulation, while TNF-α upregulated FIP1 expression in the 3T3L1 adipocytes. In contrast, FIP1 positively correlated with BMI in humans (Carson *et al.* 2013).

Leptin

Leptin, the product of the ob gene, was the first adipokine to be identified (Zhang *et al.* 1994). Leptin is a multi-functional adipokine with a key role in regulating food intake, energy expenditure and neuroendocrine function among others (Ahima & Flier 2000). Localization analysis by velocity and density gradient ultracentrifugation revealed that leptin is found in the high-density microsomes, which are positive for ER markers (Cammisotto *et al.* 2005). As a classically transported adipokine, leptin secretion is abolished by brefeldin A treatment. The serine/threonine protein kinase D1 (PKD1) has been proposed to mediate the fission at the trans-Golgi network of leptin transport vesicles to the plasma membrane (Xie *et al.* 2008). Leptin secretion seems to take place through both constitutive and regulated secretory pathways.

Regulated secretion of leptin in the adipocytes was highly induced by a cocktail of glucose, insulin and pyruvate (GIP). The concentrations used mimicked hyperglycemia and hyperinsulinaemia, and leptin secretion was stimulated through vesicular exocytosis. Both the constitutive secretion and the GIP-stimulated secretion of leptin were Ca^{2+} dependent. Moreover, forskolin, an activator of adenylate cyclase, abolished the facilitating effects of GIP, suggesting that cAMP/PKA may act downstream of GIP. In this line, insulin-stimulated leptin secretion is inhibited by β-agonists via adenylate cyclase activation (Cammisotto & Bukowiecki 2002). Beside other pathways, insulin mainly acts through the PI3K/mTOR signalling pathway on the translation and translocation of leptin and thereby enhancing leptin secretion (Tsai *et al.* 2012).

Non-classical protein secretion

Proteins secreted by non-classical pathways lack a N-terminal signal peptide leading to ER/Golgi transport and are therefore called 'leaderless' (Nickel 2003). Several well-known adipokines, such as TNF-α, visfatin, Pref1 or DPP4, are released through non-classical mechanisms from AT. To date, there are at least three distinct non-classical release mechanisms known, namely transporter-mediated export, microvesicle/exosome release and the selective post-translational hydrolysis from the cell surface, which is also termed 'shedding' (Hooper *et al.* 1997). The following section deals with some representatives for each kind of non-classical export pathway.

Transporter-mediated export of FGF2

The adipokine FGF2 (fibroblast growth factor 2, also known as basal FGF) is mainly released by pre-adipocytes and plays a role in adipogenesis (Kakudo *et al.* 2007, Xiao *et al.* 2010, Hutley *et al.* 2011). FGF2 circulating levels negatively correlate with BMI and are restored by 6-month exercise intervention (Seida *et al.* 2003). FGF2 lacks a transient signal sequence and is insensitive to monensin or brefeldin A treatment. Methylamine, which inhibits exocytosis, showed a

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decrease in FGF2 release, which indicates externalization via a mechanism of exocytosis independent of ER/Golgi complex (Mignatti *et al.* 1992). Drug treatment with ouabain, which is able to inhibit the plasma membrane Na^+/K^+ATPase, points to an export mechanism through this transporter (Florkiewicz *et al.* 1995, Dahl *et al.* 2000). It was also shown that FGF2 is recruited to the inner leaflet of the plasma membrane before it is translocated to the extracellular space (Schafer *et al.* 2004). Important factors in the translocation process seem to be the ECM components heparin sulphate proteoglycans, which was shown by Zehe *et al.* (2006). So for FGF2 different export routes still seem to be possible.

Shedding of TNF-α and DPP4

It is now widely accepted that tumour necrosis factor (TNF)-α is one important mediator, which triggers obesity-related insulin resistance. During obesity, the expression of TNF-α is upregulated in AT, muscle and macrophages. In obese mice, it was shown that complete removal or blockade of TNF-α is able to improve the metabolic profile. TNF-α might act mainly in an auto-/paracrine fashion at its target sites in obesity (Xu *et al.* 2002). By proteolytic cleavage at Ala76-Val77 in the extracellular part of the transmembrane protein, the soluble TNF-α is released. This process is sensitive to hydroxamic acid-based inhibitors, which are broad spectrum inhibitors for matrix metalloproteases (MMP). Therefore, TNF-α shedding seems to involve one or more MMPs. Black and colleagues identified TACE (TNF-α converting enzyme; also known as Adam17) as the major shedding enzyme of TNFα, by cloning and purification. Downregulation of the gene in mouse cells leads to a marked decrease in soluble TNF-α (Black *et al.* 1997).

Dipeptidyl peptidase 4 (DPP4) is an ubiquitously expressed type II transmembrane protease, which cleaves and inactivates N-terminal dipeptides from a variety of substrates, including growth factors and hormones (like the incretin hormones), neuropeptides and chemokines (Yazbeck *et al.* 2009). DPP4 is not only present on the cell surface, but is also found in the circulation (Cordero *et al.* 2009). Our group identified DPP4 as a novel adipokine by proteomic profiling of the adipocyte secretome. Elevated serum levels of DPP4 were found in obese patients and correlate with the size of adipocytes and risk factors for the metabolic syndrome (Lamers *et al.* 2011). Thus, DPP4 arises as a marker for visceral obesity, insulin resistance and the metabolic syndrome (Sell *et al.* 2013). Like most type II transmembrane proteins, DPP4 contains a predicted signal sequence, which is located at the transmembrane domain (revealed by bioinformatic analysis with the SignalP webtool). We were able to show that DPP4 is insensitive to brefeldin A treatment in different cell types (Raschke *et al.* 2013, Röhrborn *et al.* 2013). It is most likely that DPP4 is released by ectodomain shedding, which means that the extracellular part of this type II transmembrane protein is selectively hydrolysed from the cell surface (Hooper *et al.* 1997). Our group showed for the first time that DPP4 release from human adipocytes is blocked by the MMP inhibitor batimastat, suggesting the involvement of MMPs. Our results point towards MMP9 as a potential player in DPP4 shedding in adipocytes (Röhrborn *et al.* 2013). Further research will determine what are the mechanisms behind the enhanced DPP4 release from AT in patients with the metabolic syndrome.

Multiple export routes of IL-1β

Since 1987 it is known that IL-1 lacks a classical signal peptide (Dinarello 1987). There are two isoforms of IL-1, IL-1α and IL-1β. Both are secreted in an unconventional way, but much more is known about IL-1β release. The mechanism of production and maturation of IL-1β was in the focus of many studies during the last years and is therefore understood to a great extent. However, the mechanism by which IL-1β is released into the extracellular space is still very controversially discussed. At least four possible active export routes are proposed in the literature (as reviewed by Eder 2009). The group of Rubartelli could propose three of these routes in several studies on IL-1β secretion in human macrophages namely via different kinds of vesicles, which might be lysosomes, shed plasma membrane vesicles or exosomes from multi-vesicular bodies (MVB) (Rubartelli *et al.* 1990, Andrei *et al.* 1999, Carta *et al.* 2013). Additionally, it was shown that the ABC transporter inhibitor glyburide selectively inhibits IL-1β release in murine macrophages, which proposes that at least partly IL-1β is exported via an active, transporter-mediated export (Hamon *et al.* 1997, Brough & Rothwell 2007). The multiple export routes of IL-1β need to be further investigated to elucidate if several mechanisms are possible or if the used route is cell or species specific.

Non-classical secretion of visfatin

Visfatin was originally described as an adipokine with insulin-mimetic properties, although this later affirmation was retracted (Fukuhara *et al.* 2005, 2007). Visfatin is identical to the cytokine pre-B-cell colony-enhancing factor (PBEF), and the enzyme nicotinamide phosphoribosyltransferase (Nampt), catalysing

the rate-limiting step leading to the synthesis of nicotinamide adenine dinucleotide (NAD; as reviewed by Romacho *et al.* 2013a). In the context of metabolic diseases, although there are conflicting results, several studies have reported elevated circulating levels of visfatin in obesity, type 2 diabetes mellitus and the metabolic syndrome (Romacho *et al.* 2013a). Due to the lack of the signal peptide, visfatin was initially proposed not to be actively secreted but released by passive cellular death (Kitani *et al.* 2003, Hug & Lodish 2005, Stephens & Vidal-Puig 2006), although no evidence for this affirmation was ever provided. On the contrary, other studies demonstrated that it is not classically secreted (Revollo *et al.* 2007, Tanaka *et al.* 2007). Tanaka *et al.* excluded other non-classical mechanisms for visfatin secretion such as transport system or microvesicles. Furthermore, other studies have reported that visfatin is actively secreted from several cell types such as 3T3-L1, amniotic epithelial and endothelial cells (Ognjanovic *et al.* 2005, Tanaka *et al.* 2007, Romacho *et al.* 2013b).

Auto- and paracrine crosstalk within adipose tissue

AT is composed of different cell types. In addition to mature adipocytes, other cell types such as pre-adipocytes, vascular cells and macrophages are present in AT (as depicted in Fig. 2). Adipokines and cytokines secreted from these different AT-resident cell types influence each other in an intra-organ crosstalk scenario. It is well described that cytokines secreted from macrophages, like TNF-α, influence adipocyte metabolic and secretory function. This crosstalk is bidirectional, as adipocyte-derived factors, such as leptin, also modulate function of AT-resident immune cells (Ouchi *et al.* 2011). The risk to develop type 2 diabetes and other metabolic complications is increased in obesity and related to AT dysfunction. Furthermore, the degree of macrophage infiltration positively correlates with adipocyte size (Xu *et al.* 2003), and adipocyte size itself is linked with metabolic dysfunction (Gustafson *et al.* 2013). Enlarged adipocytes may contribute to the development of metabolic dysfunction by a decreased lipid buffering capacity, leading to ectopic fat accumulation (Goossens 2008). Additionally, there is a shift towards a more pro-inflammatory secretion profile with increased adipocyte size (Skurk *et al.* 2007). There is a constant turnover of the number of adipocytes during life with ~10% of all adipocytes being renewed per year (Spalding *et al.* 2008). Thus, *de novo* adipogenesis is crucial to maintain adipocyte number and to provide new adipocytes in case of excess energy supply. Interestingly, both the ability to recruit and differentiate new adipocytes are impaired in individuals with hypertrophic AT (Gustafson *et al.* 2013). Adipocyte size seems to be a pivotal factor, and understanding the mechanisms which regulate adipocyte differentiation is therefore essential to prevent AT hypertrophy and dysfunction.

Differentiation into adipocytes requires a temporally regulated transcriptional cascade. Key transcription factors in adipogenesis are the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and the CCAAT-enhancer-binding proteins (C/EBPs) (Rosen & Macdougald 2006). The transcriptional cascade can be influenced by several pro- and antiadipogenic extracellular factors. Bone morphogenetic proteins (BMP) promote adipogenesis (Bowers & Lane 2007, Schulz & Tseng 2009), while TGF-β inhibits adipogenesis (Zamani & Brown 2011). Another inhibitory pathway in the regulation of adipocyte differentiation is the Wnt-signalling pathway, which is activated by the Wnt family of secreted glycoproteins (Ross *et al.* 2000).

The auto-/paracrine modulation of AT inflammation and metabolic function of AT by adipokines has been thoroughly reviewed (Karastergiou & Mohamed-Ali 2010), and we will not focus on that in this part. The important role of impaired adipogenesis in the development of hypertrophic obesity and metabolic dysfunction has been recently summarized (Gustafson *et al.* 2013). However, the auto-/paracrine regulation of adipogenesis has not been so extensively explored. Secretion of a more pro-inflammatory adipokine profile occurring in obesity does not only impair other organs, but also adipogenesis within AT. Cytokines released from macrophages and hypertrophic adipocytes, such as TNF-α and IL-6, have been shown to inhibit adipocyte differentiation. The classical adipokine leptin inhibits adipogenesis (Rhee *et al.* 2008), while adiponectin has been described to promote adipogenesis (Fu *et al.* 2005, do Carmo *et al.* 2008). Most of the adipokines which are upregulated in the obese state inhibit de novo adipogenesis, while promoters of adipogenesis such as adiponectin are downregulated (Fig. 2). This section will focus on novel AT-derived factors regulating adipogenesis in an auto-/paracrine manner. More specifically, we will address the auto-/paracrine effects of several adipokines which are upregulated in obesity and exert a negative effect on adipogenesis such as RBP4, WISP2 and the recently identified adipokine HO-1. On the other hand, we summarize here the main effects of novel adipokines that may positively regulate adipogenesis such as SFRPs and chemerin. For all these factors, both the role in adipogenesis and their regulation in AT by intra-organ crosstalk will be described in the following section (Fig. 2).

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Retinol-binding protein 4 (RBP4)

RBP4 belongs to the lipocalin family of transport proteins and is the only retinol transporter in the circulation (Quadro *et al.* 1999). The primary site of RBP4 synthesis is the liver, followed by AT (Tsutsumi *et al.* 1992). Within AT, mature adipocytes represent the main source of RBP4 (Cheng *et al.* 2013). RBP4 was identified as an adipokine in 2005, and serum RBP4 levels are elevated in several obese and diabetic mouse models (Yang *et al.* 2005). However, the relation between RBP4 serum levels and insulin resistance in human clinical studies remains controversial (Kotnik *et al.* 2011). Circulating RBP4 is about 90% bound to retinol (holo-RBP4), and 10% is present in the unbound form (apo-RBP4). As sole retinol transport protein, RPB4 coordinates cellular retinol uptake (Quadro *et al.* 1999). Retinoids are retinol derivates and activate the nuclear receptor retinoid acid receptor (RAR) and retinoid X receptor (RXR), which are also involved in the regulation of adipogenesis (Ziouzenkova & Plutzky 2008). RBP4 overexpression in primary porcine pre-adipocytes has been shown to decrease adipogenesis, mediated by impaired AKT and mTOR phosphorylation (Cheng *et al.* 2013). In addition to insulin signalling, RBP4 might also affect adipogenesis by activating RAR. Differentiation of 3T3-L1 pre-adipocytes treated with retinol-bound holo-RBP4 is blocked, accompanied by increased RARα activation. This effect was dependent on retinol, as treatment of 3T3-L1 pre-adipocytes with the retinol-free apo-RBP4 increased retinol efflux and enhanced differentiation (Muenzner *et al.* 2013). Expression of RBP4 in AT is regulated by several adipocyte- and macrophage-derived factors in an auto-/paracrine manner. Thus, RBP4 protein expression is downregulated in primary human adipocytes by TNF-α (Sell & Eckel 2007, Kotnik *et al.* 2013) and IL-1β (Kotnik *et al.* 2013). Leptin, which is mainly secreted from mature adipocytes, increases RPB4 protein level in human AT explants (Kotnik *et al.* 2011). The insulin-sensitizing PPARγ agonists troglitazone and pioglitazone have been shown to increase RBP4 expression in human adipocytes (Sell & Eckel 2007, Yao-Borengasser *et al.* 2007). It is likely that RBP4 is dysregulated in states of AT inflammation, and indeed, a positive association between expression of RBP4 and the macrophage marker CD68 was found in subcutaneous human AT (Yao-Borengasser *et al.* 2007). In addition to its effects on adipogenesis, RBP4 impairs macrophage secretory function. Macrophages challenged with apo-RBP4 or holo-RBP4 express increased levels of MCP1, IL-6 and TNF-α, which was independent of retinol and the RBP4 receptor STRA6 (Norseen *et al.* 2012). In conclusion, the adipokine RBP4 may contribute to the development of metabolic dysfunction by impairing adipocyte differentiation and increasing secretion of pro-inflammatory cytokines by macrophages. The RBP4-mediated induction of TNF-α may further inhibit adipocyte differentiation. However, it is surprising that RBP4 expression in adipocytes is decreased by TNF-α and increased by PPARγ agonists. Moreover, some of the described effects of RBP4 are dependent on retinol and some are not, complicating the role of RBP4 in AT function.

Heme oxygenase-1

Heme oxygenases (HO) cleave heme to biliverdin, iron and carbon monoxide (CO) and are present in two isozymes. While HO-2 is constitutively expressed in several tissues, HO-1 is a stress responsive isoform inducible by oxidative stress, cytokines, UV-Light and other factors. Increased HO-1 activity protects against oxidative stress, as present in diabetes and atherosclerosis (Abraham & Kappas 2008). HO-1 is secreted from primary human adipocytes, and HO-1 protein levels increase during adipocyte differentiation. As HO-1 protein abundance is substantially higher in adipocytes compared with macrophages, this adipokine is rather an adipocyte-derived factor in AT (Lehr *et al.* 2012). The protein level of HO-1 is increased in SAT and VAT from obese patients compared with matched lean controls (Lehr *et al.* 2012). Moreover, serum HO-1 levels are upregulated in human obesity (Lehr *et al.* 2012) and type 2 diabetic subjects (Bao *et al.* 2010). Recently, positive effects of HO-1 on AT have been reported. Induction of HO-1 by cobalt protoporphyrin IX (CoPP) injection reverts AT inflammation in Zucker obese rats (Kim *et al.* 2008) and lowers weight gain and body fat content (Kim *et al.* 2008, Nicolai *et al.* 2009), indicating a role for HO-1 in the regulation of adipogenesis. In line, adipogenic differentiation is increased in mesenchymal stem cells (MSCs) from HO-2-deficient mice, which display lower HO-1 protein expression. Treatment of HO-2-deficient MSCs with CoPP decreases adipogenesis, reduces lipid droplet size and improves adipokine secretion profile (Burgess *et al.* 2012). Similar effects on adipogenesis and adipokine secretion were observed in human MSCs. Human MSCs treated with the HO-1 inducer CoPP show an increased number of small lipid droplets, while large lipid droplets are reduced. Moreover, the secretion of adiponectin was increased and TNF-α release strongly decreased by CoPP (Vanella *et al.* 2013). The inhibitory effect of HO-1 on adipogenesis has been proposed to be mediated by increased expression of the WNT-signalling components Wnt10b and β-catenine (Vanella *et al.*

2013). Although inhibiting adipogenesis, HO-1 seems to improve the adipokine secretion profile and prevent HFD-induced obesity in animal models (Kim *et al.* 2008, Nicolai *et al.* 2009). However, a recent study showed that AT-specific overexpression of HO-1 could not prevent HFD-induced obesity and insulin resistance and did not affect expression of pro-inflammatory cytokines or adipocyte size (Huang *et al.* 2013). Thus, HO-1 is likely to be important in the early induction of adipogenesis, as HO-1 induction in pre-adipocytes decreases adipogenesis (Tanaka *et al.* 2009, Burgess *et al.* 2012, Vanella *et al.* 2013). On the other hand, aP2 gene promotor driven expression of HO-1, which does not increase HO-1 expression in pre-adipocytes, does not impair adipogenesis (Huang *et al.* 2013).

WNT1 inducible signalling pathway protein 2 (WISP2)

WISP2, also known as CCN5, belongs to the CCN (connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed) family (Brigstock 2003) and is induced by Wnt/β-catenine signalling (Jackson *et al.* 2005). Canonical WNT/β-catenine signalling is activated by several extracellular WNT ligands, leading to β-catenine stabilization and interaction with the nuclear transcription factors TCF/LEF. The inhibition of WNT/β-catenine signalling is necessary to induce adipogenic differentiation. Thus, harmine-mediated promotion of 3T3-L1 pre-adipocyte differentiation is accompanied by suppression of Wisp2 and other components of the Wnt-signalling pathway, suggesting a role for Wisp2 in the regulation of adipogenesis (Waki *et al.* 2007). Recent evaluation of the fat cell secretome, regarding the expression of adipokines during adipogenesis and their regulation in obesity, revealed Wisp2 as a top candidate which is upregulated in obesity (Dahlman *et al.* 2012). Wisp2 has recently been validated and characterized as novel adipokine (Hammarstedt *et al.* 2013). Expression of Wisp2 mRNA in subcutaneous AT from human subjects correlates positively with adipocyte size and waist circumference. Knockdown of Wisp2 in 3T3-L1 cells induces spontaneous differentiation, while treatment with recombinant Wisp2 suppresses adipogenesis (Hammarstedt *et al.* 2013). Thus, Wisp2 is a potent new regulator of adipocyte differentiation. Interestingly, the positive regulator of white adipocyte differentiation BMP4 (Schulz & Tseng 2009) is able to revert the inhibitory effect of Wisp2 on adipogenesis (Hammarstedt *et al.* 2013). Other Wnt ligands such as Wnt5a are secreted from macrophages and inhibit adipocyte differentiation in a paracrine manner (Bilkovski *et al.* 2011). Future studies will determine whether Wisp2 is secreted by other AT-resident cells and how secretion is regulated in states of AT inflammation.

Secreted frizzled-related proteins

WNT-signalling inhibits adipogenesis and can be regulated on several levels. Members of the family of secreted frizzled-related proteins (SFRPs) are extracellular regulators of Wnt-signalling, by binding and modifying the activity of Wnt ligands (Park *et al.* 2008). The SFRP family comprises five members in mammals (SFRP1-5). SFRP1 is induced during adipogenesis and primarily expressed in mature adipocytes, indicating a role in the regulation of adipogenesis (Lagathu *et al.* 2010). Overexpression of SFRP1 in 3T3-L1 pre-adipocytes inhibits the canonical WNT-signalling pathway and promotes adipogenesis. Interestingly, the stimulatory effect of SRFP1 on adipogenesis was only observed when differentiation was induced with a mild differentiation cocktail, but not with a maximal differentiation cocktail (Lagathu *et al.* 2010). SFRP1 seems to play an important role in the regulation of adipocyte number during progression of AT hypertrophy and dysfunction. Thus, SFRP1 expression in AT initially rises in mice under HFD to compensate the increased demand for fat storage, but decreases again at later time points when animals start to develop AT dysfunction metabolic complications (Lagathu *et al.* 2010). In line, SFRP1 expression in subcutaneous AT is increased in mild obese humans and falls to levels equal to lean subjects in morbidly obese humans (Lagathu *et al.* 2010). However, a recent study showed a negative correlation between SFRP1 expression in subcutaneous AT and BMI and no effect of SFRP1 on differentiation of primary human adipose-derived stem cells (hADSCs) (Ehrlund *et al.* 2013). In addition to SFRP1, SFRP2 has been shown to promote adipogenic differentiation of murine bone marrow stromal cells (Cianferotti & Demay 2007) and SFRP4 increases differentiation of hADSCs (Park *et al.* 2008). Again, the recent study of Ehrlund *et al.* (2013) could not observe any effect of SFRP2 and SFRP4 and adipogenesis in hADSCs. The role of SFRP5 as an adipokine is very controversial. SFRP5 expression has been reported to be decreased in several obese mouse models and in VAT of obese individuals (Ouchi *et al.* 2010). $Sfrp5^{-/-}$ mice under high-fat diet develop insulin resistance and AT inflammation and have enlarged adipocytes, suggesting that SFRP5 promotes adipogenesis and has anti-inflammatory properties (Ouchi *et al.* 2010). However, another study showed increased SFRP5 expression in AT of obese mouse models and $Sfrp5^{Q27stop}$ mutant mice with a non-functional Sfrp5 allele have less fat under high-fat diet and a lower percentage of large adipocytes

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(Mori *et al.* 2012). Moreover, SFRP5 has been shown to be expressed in human AT but is not secreted time-dependently from human AT explants and therefore not a real adipokine (Ehrlund *et al.* 2013). In summary, most studies observed a positive effect of SFRPs on adipogenesis, suggesting that they are negative regulators of WNT signalling. The different results on the regulation of SFRPs in obesity may be explained by a potential gradual regulation during progression of obesity, as described for SFRP1 (Lagathu *et al.* 2010). SFRPs seem to be promising targets, and their regulation and function in AT has to be further investigated.

Chemerin

The chemoattractant protein chemerin (RARRES2 or TIG) binds to chemokine-like receptor 1 (CMKLR1) and plays a role in innate and adaptive immunity (Bondue *et al.* 2011). White AT, liver and placenta express high levels of chemerin mRNA, and chemerin has recently been identified as a novel adipokine secreted by 3T3-L1 adipocytes (Goralski *et al.* 2007). Chemerin is mainly derived from mature adipocytes, as expression of chemerin mRNA is strongly upregulated during differentiation of 3T3-L1 adipocytes (Bozaoglu *et al.* 2007, Roh *et al.* 2007) and human pre-adipocytes (Sell *et al.* 2009). As many other adipokines, chemerin is dysregulated in obesity and has been proposed as a potential link between obesity and T2DM (Roman *et al.* 2012). Thus, expression of chemerin and its receptor CMKLR1 are increased in AT from obese rats compared with lean counterparts and positively correlate with BMI (Bozaoglu *et al.* 2007). Moreover, chemerin serum levels are increased in obese human subjects (Bozaoglu *et al.* 2007), and secretion of chemerin is higher from human AT explants of obese subjects (Sell *et al.* 2009). Chemerin is another factor regulating adipogenesis in an auto-/paracrine manner. Knockdown of chemerin in 3T3-L1 pre-adipocytes (Goralski *et al.* 2007) and human multi-potent bone marrow-derived stromal cells (BMSCs) (Muruganandan *et al.* 2010) abrogates adipogenesis, suggesting that chemerin is necessary for adipocyte differentiation. Chemerin mainly impacts the mitotic clonal expansion phase in the first days of differentiation (Muruganandan *et al.* 2011) and has no effect on adipogenesis when knocked down in later phases of differentiation (Goralski *et al.* 2007, Muruganandan *et al.* 2011). Interestingly, chemerin is differentially regulated in pre-adipocytes and mature adipocytes. PPARγ agonists enhance chemerin expression in pre-adipocytes (Muruganandan *et al.* 2011), while treatment of mature adipocytes with PPARγ agonists decreases chemerin mRNA expression (Muruganandan *et al.* 2011) and release (Sell *et al.* 2009). Furthermore, chemerin release from human adipocytes is increased by the pro-inflammatory cytokine TNF-α (Sell *et al.* 2009). Lipid loading of 3T3-L1 adipocytes by treatment with oleic acid and palmitic acid during differentiation increases chemerin protein levels (Bauer *et al.* 2011). In conclusion, chemerin is a positive regulator of adipogenesis and is upregulated in obesity. Enhanced expression of chemerin in adipocytes with enlarged lipid droplets may be a physiological stimuli in AT to promote adipogenesis and thereby provide new adipocytes.

Endocrine crosstalk by adipose tissue

Through the release of adipokines, AT establishes a crosstalk with other key target tissues and organs such as the central nervous system, the liver, skeletal muscle, the CV system and the pancreas. On the other side, factors released from these targets of AT can interact with AT itself. The identification of novel mediators released from the heart such as cardiokines, or the skeletal muscle such as myokines, further complicates the picture of this bidirectional interorgan crosstalk. In this section, we will specifically focus on the crosstalk between AT and skeletal muscle, the vascular wall and the pancreas from an adipocentric point of view (as summarized in Fig. 3). As outlined in previous sections, we will focus on the effects of two classical adipokines such as adiponectin and leptin on these crosstalk scenarios. On the other hand, we will summarize the current main findings of two novel adipokines with enzymatic activity such as DPP4 and visfatin that may play beneficial or detrimental roles depending on the crosstalk scenario.

The adipo-myocyte axis

Skeletal muscle plays a key role in insulin sensitivity as a main target for insulin-stimulated glucose disposal. Increased AT mass and insulin resistance are closely related. In metabolic diseases, skeletal muscle insulin resistance results from an increased release of pro-inflammatory adipokines, cytokines and chemokines and free fatty acids (FFA) as a result of AT dysfunction (as reviewed in Sell *et al.* 2006b). In a pioneer coculture model with human adipocytes and myocytes, our group demonstrated that adipocytes directly induced insulin resistance in human skeletal muscle cells (Dietze *et al.* 2002). The coculture reduced insulin-mediated Akt phosphorylation, GLUT4 translocation and glucose uptake (Dietze *et al.* 2002) in a similar way to what reported in skeletal muscle of diabetic patients (Krook *et al.* 2000). Conditioned medium (CM) from adipocytes increased

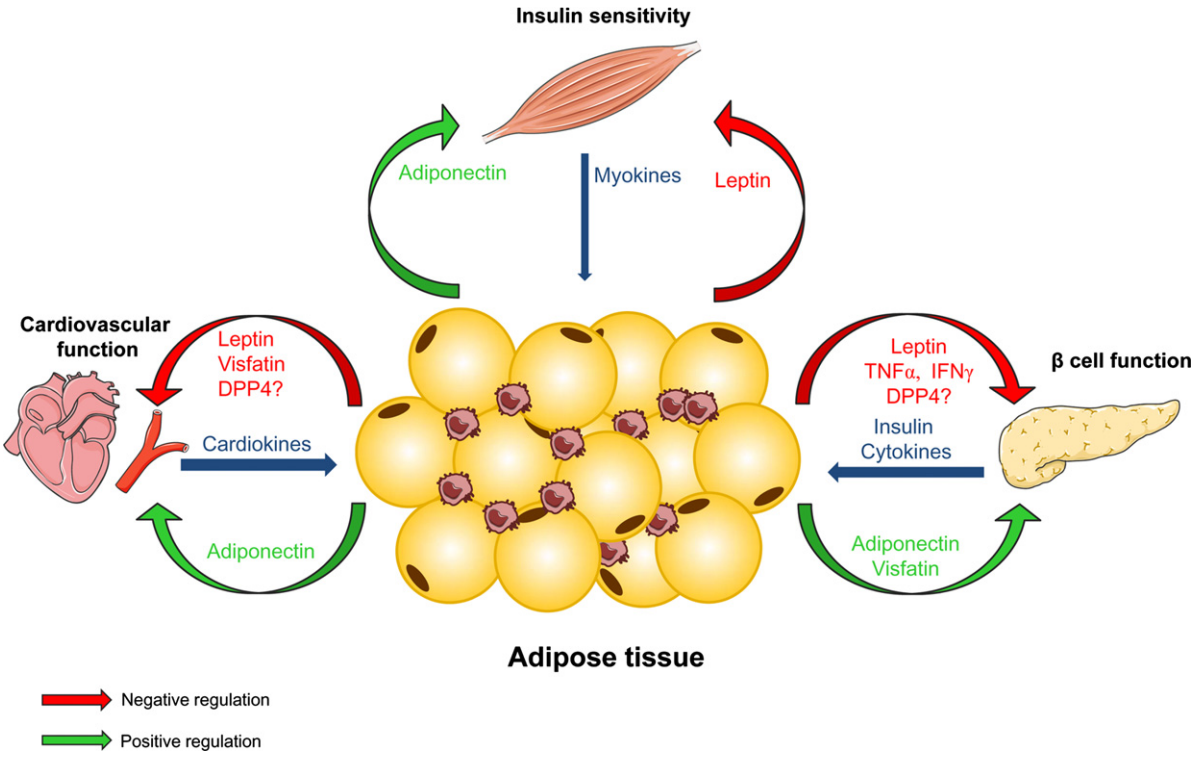

Figure 3 Endocrine role of adipose tissue in interorgan crosstalk. Through the release of adipokines, adipose tissue can exert a positive or negative regulation on the function of other distant targets such as the cardiovascular system, skeletal muscle and the pancreas. On the other hand, these targets can also affect adipose tissue function through the release of their specific '-kines' such as cardiokines, myokines, hormones like insulin or cytokines. Adipokines that are upregulated in obesity such as leptin, visfatin and potentially DPP4 exert a deleterious effect on cardiovascular function, while adiponectin whose levels are downregulated in obesity exerts an anti-inflammatory and antiatherogenic effect in the vascular wall. Adiponectin promotes insulin sensitivity in skeletal muscle while pathological concentrations of leptin impair insulin sensitivity in this tissue. While adiponectin and visfatin have been proposed to exert beneficial effects on β-cell function, other adipokines and cytokines upregulated in obesity such as leptin, TNF-α, IFNγ and potentially DPP4 negatively affect β-cell mass and function.

oxidative stress, ceramide content, reduced mitochondrial capacity and potentiated the lipotoxic potential of palmitate in skeletal muscle cells (Sell *et al.* 2006a). These findings underpin the crucial role of adipokines in skeletal muscle dysfunction and in the pathogenesis of type 2 diabetes. Skeletal muscle has been identified as a real endocrine organ, and novel skeletal muscle-derived mediators termed myokines are being identified (Hartwig *et al.* 2013, Raschke *et al.* 2013). Interestingly, some of these factors overlap with the adipocyte secretome and may also contribute to the circulating levels of some adipokines (as reviewed in Raschke & Eckel 2013).

Adiponectin and leptin

The circulating levels of adiponectin are downregulated in obesity and are inversely correlated with insulin resistance (Kadowaki & Yamauchi 2005). Thus, adiponectin improves whole body insulin sensitivity (Turer & Scherer 2012). This adipokine has also been reported as a myokine released from L6 muscle cells (Liu *et al.* 2009). Adiponectin directly prevented insulin resistance in myocytes cocultured with adipocytes (Dietze-Schroeder *et al.* 2005). One proposed mechanism is that the globular C-terminal fragment of adiponectin can reduce glucose levels by increasing fatty acid oxidation in myocytes (as reviewed in Turer & Scherer 2012). Interestingly, Iwabu *et al.* demonstrated that decreased levels of adiponectin and the adiponectin receptor AdipoR1 in obesity may have causal roles in mitochondrial dysfunction and insulin resistance observed in diabetes. Adiponectin increased PGC-1α expression and mitochondrial content in myocytes via the AdipoR1 receptor. Analogously, a transgenic mouse with skeletal muscle-specific disruption of AdipoR1 displayed a decreased expression of PGC-1α, mitochondrial content and detoxifying enzymes in skeletal muscle, which were associated with insulin resistance and decreased exercise endurance in these mice (Iwabu *et al.* 2010). Most of the reported effects of adiponectin in skeletal muscle have been mediated

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by globular adiponectin, which may have a reduced contribution to overall circulating adiponectin (Bluher 2012). Therefore, the real physiological impact of adiponectin on skeletal muscle needs to be further explored.

Leptin circulating levels positively correlate with fat mass (Schwartz *et al.* 1996). Leptin has been proposed to be an adipomyokine, as it is secreted both from adipocytes and myocytes. However, data from our group showed that leptin is rather a true adipokine (Raschke & Eckel 2013). Leptin receptors are abundant in human skeletal muscle (Guerra *et al.* 2007), and their expression is upregulated by both exercise and atrophy (Chen *et al.* 2007). Leptin promotes insulin sensitivity by enhancing fatty acid oxidation and decreasing triglyceride storage in muscle (Ahima & Flier 2000). Several studies with murine models support an anabolic role for leptin in the regulation of muscle mass. A novel mouse model lacking all functional leptin receptor isoforms, the POUND mouse ($Lepr^{db/lb}$), showed increased body weight and decreased muscle mass. In POUND mice, myogenic differentiation was probably impaired due to an increase in muscle-wasting myostatin and decreased IGF-1 and Akt expression in skeletal muscle (Arounleut *et al.* 2013). On the contrary, leptin treatment increased skeletal muscle mass in aged mice (Hamrick *et al.* 2010). Only future research will determine whether leptin could be a therapeutic target to improve declined skeletal muscle function in degenerative diseases and ageing.

DPP4 and visfatin impact on skeletal muscle

Our group has characterized DPP4 as an adipokine potentially linking obesity to the metabolic syndrome (Lamers *et al.* 2011) and has recently validated it as a myokine (Raschke *et al.* 2013). In mice lacking DPP4, both insulin secretion and glucose tolerance are improved (Marguet *et al.* 2000). In a recent study, DPP4 inhibition with sitagliptin upregulated GLUT4 expression in the skeletal muscle of spontaneous hypertensive rats (SHR) (Giannocco *et al.* 2013). Nevertheless, the impact of adipose-derived DPP4 on skeletal muscle remains fully unknown.

Little is known on the potential impact of this adipokine on skeletal muscle. Visfatin may have a deleterious effect on skeletal muscle since it induced oxidative stress in C2C12 myotubes through nuclear factor (NF)-κB activation independently of MAPK and Akt phosphorylation (Oita *et al.* 2010). Costford *et al.* have demonstrated that exercise increases intracellular Nampt (iNampt) expression in human skeletal muscle and that iNampt induction correlates with an enhancement of mitochondrial content and PGC-1α expression (Costford *et al.* 2010). Visfatin has been also identified as a myokine in chicken and rat (Krzysik-Walker *et al.* 2008, Wang *et al.* 2010), although in humans the potential role of visfatin as an adipomyokine remains to be further explored (Scheler *et al.* 2013).

The adipo-vascular axis

Vascular complications are the main cause for morbimortality in obese and type 2 diabetic patients. Indeed, obesity is an independent risk factor for atherosclerosis (Williams *et al.* 2002). Endothelial dysfunction is a key initial step in the development of atherosclerosis, which is characterized by reduced bioavailability of the anti-atherogenic molecule nitric oxide (NO), impaired vascular homeostasis leading to increased vasoconstriction, leucocyte adherence, platelet activation, smooth muscle proliferation, permeability, prooxidation, coagulation and inflammation (Verma *et al.* 2003). Atherogenesis is a complex process beginning with endothelium activation. The increased expression of adhesion molecules promotes leucocyte adhesion and transmigration. After lipid accumulation, activated monocytes become foam cells. Later they are joined by smooth muscle cells that migrate and proliferate towards the intima layer and increase extracellular matrix secretion and the release of pro-inflammatory cytokines leading to fibrous cap formation. When the fibrous cap weakens by the action of MMPs, the plaque becomes unstable and breaks leading to thrombus formation and ischaemic accidents (Ross 1999). NF-κB plays a pivotal role in atherothrombotic diseases, as it regulates the expression of other key molecules in this process such as adhesion molecules, pro-inflammatory cytokines and the inducible form of NO synthase (iNOS), a key enzyme related to impaired vascular reactivity in diabetes (Gunnett *et al.* 2003). A pro-inflammatory secretion profile of AT has been associated with chronic systemic inflammation and endothelial dysfunction (Karastergiou & Mohamed-Ali 2010). Adipocytokines such as TNF-α, several interleukins and many other adipokines lead to the activation of pro-inflammatory signalling pathways in vascular cells. AT can additionally affect vascular homeostasis through the release of classical vasoactive factors such as NO or angiotensin II, agents controlling fibrinolysis such as PAI-1 and vasoactive adipokines (Lau *et al.* 2005, Guzik *et al.* 2006). Several adipokines are now considered as markers or predictors of CV disease (Taube *et al.* 2012), but more importantly, adipokines are starting to be acknowledged as active promoters of atherothrombotic diseases. The main direct CV effects of adiponectin, leptin, DPP4 and visfatin will be addressed here.

Adiponectin and leptin: two opposite players in the vascular wall?

Considered the cardioprotective adipokine *par excellence*, adiponectin circulating levels are downregulated in patients suffering cardiometabolic diseases (Kumada *et al.* 2003). This adipokine inhibits the initial steps of the atherogenic process by downregulating TNF-α induction of adhesion molecules, preventing macrophage-to-foam cell transformation and smooth muscle cell proliferation (Ouchi *et al.* 1999, Arita *et al.* 2002). The adipokine prevents endothelial activation by inhibiting NF-κB signalling and directly ameliorating endothelial dysfunction by increasing nitric oxide (NO) production (as reviewed in Taube *et al.* 2012). The anti-atherogenic effect of adiponectin has been further demonstrated in adiponectin knockout models (Ouedraogo *et al.* 2007).

Leptin receptors are expressed in both endothelial cells and smooth muscle cells and thus leptin can exert direct actions on the vascular wall. The correlation between leptin levels and CV diseases remains controversial; therefore, it has been proposed that the positive or negative correlations reported may depend on the absence or presence of the specific CV pathophysiology (Koh *et al.* 2008, Sweeney 2010). Leptin also increases the expression of PAI-1, CRP and caveolin-1 in human coronary artery endothelial cells (Singh *et al.* 2007, 2010, 2011). Within physiological concentrations, leptin promotes the release of vasodilator factors such as NO and endothelium-derived hyperpolarizing factor (EDHF) (Vecchione *et al.* 2002, Beltowski 2012) while at concentrations in the pathophysiological range (hyperleptinaemia), leptin impairs NO-mediated vasodilation by acetylcholine both *in vitro* and *in vivo* (Knudson *et al.* 2005). However, the *in vivo* effects of leptin on atherogenesis remain elusive. Leptin-deficient hyperlipidaemic mice develop less atherosclerosis than the proatherogenic apoE$^{-/-}$ mice on an atherogenic diet. On the contrary, low-density lipoprotein–receptor knockout mice (LDLR$^{-/-}$) lacking leptin develop more atherosclerotic lesions than LDLR$^{-/-}$ control mice (Hasty *et al.* 2001). The conflicting results from *in vivo* clinical and animal studies demonstrate that the contribution of leptin to obesity-related CV complications is complex and may depend on the pathological scenario, the degree of hyperleptinaemia and the progression of the CV disease.

DPP4 and visfatin: promising therapeutic targets in cardiovascular diseases

DPP4 is an exoprotease that cleaves and inactivates several substrates such as the members of the incretin family glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) (Drucker & Nauck 2006). On the other hand, DPP4 is associated with immunomodulatory functions (Augustyns *et al.* 1999, Iwata *et al.* 1999). The soluble form of DPP4 displays enzymatic activity and is found in plasma (Iwaki-Egawa *et al.* 1998), but its direct effects on the vascular wall remain unknown. Our group demonstrated that DPP4 directly induces proliferation of human vascular smooth muscle cells (Lamers *et al.* 2011). DPP4 increases superoxide generation and the receptor of advanced glycation end products (AGEs) gene expression in HUVECs in a concentration-dependent manner (Ishibashi *et al.* 2013). AGEs are products of non-enzymatic glycation and oxidation of proteins and lipids that accumulate in hyperglycaemia. Thus, both AGEs and its receptor RAGE play a key role in diabetic-related vascular complications. Both DPP4 pharmacological inhibition and genetic deletion have been proposed to upregulate cell survival pathways in cardiomyocytes and endothelial cells through a GLP-1 dependent mechanism (Bose *et al.* 2005, Sauve *et al.* 2010). However, increasing evidence suggests that DPP4 inhibitors exert vasoprotective effects independent of GLP-1, through endothelial repair, anti-inflammatory effects and inhibition of ischaemic injury (Fadini & Avogaro 2013). Thus, DPP4 inhibition delays the onset of atherosclerosis in a murine model of atherosclerosis and insulin resistance (Shah *et al.* 2011). On the contrary, it has been described that loss of DPP4 enzymatic activity in HUVEC can induce a prothrombotic status (Krijnen *et al.* 2012). Although DPP4 inhibition represents a promising therapeutic approach, further research on the direct action of DPP4 on the vascular wall is required.

Visfatin circulating levels are increased in obesity, type 2 diabetes mellitus and athero-thrombotic diseases. This adipokine promotes angiogenesis, smooth muscle cell proliferation and triggers endothelial cell proliferation and migration. Furthermore, in the latter cell type, visfatin upregulates adhesion molecules, cytokine secretion and NADPH oxidase activation via NF-κB, while in vascular smooth muscle cells it activates the ERK1/2-NF-κB–iNOS axis. Visfatin promotes atheroma plaque destabilization through MMP-2 and 9 in both endothelial cells and monocytes. Regarding effects on the vascular tone, visfatin induces relaxation in rat aortic rings, while in bovine coronary arteries, human and murine microvessels visfatin impairs endothelium-dependent relaxation to ACh (as reviewed in Romacho *et al.* 2013a). On the contrary, visfatin has an anti-apoptotic effect on cardiomyocytes, and its proangiogenic properties are beneficial in peripheral limb ischaemia (Romacho *et al.* 2013a). Both clinical and basic research evidence support a role for visfatin as a promising therapeutic target in metabolic-related CV diseases.

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The adipo-insular axis

Type 2 diabetes results from pancreatic β-cell failure to secrete enough insulin to compensate for increasing insulin resistance (Leahy *et al.* 2010). In early stages of the disease, insulin resistance is compensated by increasing β-cell function and mass. Over disease progression, there is an increased deterioration of both β-cell function and mass partially due to accelerated apoptosis. The main factors contributing to this loss of β-cell function and mass are glucotoxicity, lipotoxicity, and islet cell amyloid (Wajchenberg 2007). Thus, the maintenance of β-cell function and mass are crucial targets to prevent the onset of type 2 diabetes.

The interaction between AT and pancreas was previously thought to be restricted to insulin-mediated glucose uptake leading to increased triacylglycerol storage in the adipocytes (Weir *et al.* 2001). Now it is accepted that the pro-inflammatory adipokine profile in obese AT contributes to pancreatic β-cell injury. While the induction of β-cell apoptosis by IL-1, TNF-α and IFN-γ is well established (Pukel *et al.* 1988). Other rather adipose-specific adipokines such as leptin, resistin and adiponectin have been proposed as important players in the development of pancreatic β-cell dysfunction and type 2 diabetes. In this part, the main direct effects of the classical adipokines adiponectin and leptin and the novel adipokines visfatin and DPP4 will be discussed.

Adiponectin and leptin: antagonist effects on β cell

The adiponectin receptors AdipoR1 and AdipoR2 are expressed in primary (Kharroubi *et al.* 2003) and clonal β-cells (Brown *et al.* 2010a, Wijesekara *et al.* 2010). The C-terminal globular domain of adiponectin inhibited the apoptotic effect of cytokines and palmitate and prevented the impairment of insulin secretion induced by FFA and cytokines in INS-1 rat clonal cell line (Rakatzi *et al.* 2004). Similarly, adiponectin reversed apoptosis triggered by both intermittent and sustained high glucose in INS-1 cells (Lin *et al.* 2009). However, adiponectin did not inhibit FFA-induced apoptosis in isolated human islets (Staiger *et al.* 2005). In isolated islets from mice under high-fat diet, adiponectin stimulates glucose-stimulated insulin secretion (GSIS) (Gu *et al.* 2006). Furthermore, several studies have provided evidence that adiponectin stimulates insulin secretion through direct exocytosis of insulin granules and upregulation of insulin gene expression (as reviewed in Lee *et al.* 2013). It has been recently proposed that adiponectin beneficial effects on β-cell survival rely on ceramidase activity leading to formation of the anti-apoptotic metabolite sphingosine-1-phosphate (S1P) in *in vitro* and *in vivo* (Holland *et al.* 2011). All this bulk of evidence supports the notion that adiponectin positively affects β-cell function and growth.

Leptin was the first adipokine suggested to directly exert pancreatic effects. In addition to its central action on food intake and energy expenditure, leptin has been proposed to regulate glucose homeostasis by modulating the synthesis and release of insulin and glucagon, through direct actions on β- and α-cells of pancreatic islets respectively (Tuduri *et al.* 2009, Dunmore & Brown 2013). The leptin receptor ObR is highly expressed in the endocrine pancreas (Kieffer *et al.* 1996), and clinical data suggested a negative correlation between leptin circulating concentrations and β-cell function (Ahren & Larsson 1997). In most studies with perfused rat pancreas and rodent islets, physiological concentrations of leptin induced GSIS from pancreatic β-cells (as reviewed in Seufert 2004). Furthermore, leptin suppressed insulin gene expression (Pallett *et al.* 1997) and GSIS (Brown *et al.* 2002). On the other hand, leptin has been shown to inhibit ectopic fat accumulation, to prevent β-cell dysfunction and to protect the β-cell from cytokine- and fatty-acid-induced apoptosis (Brown & Dunmore 2007). The impaired leptin signalling in the leptin-deficient mouse (*ob/ob)* and the leptin-receptor-deficient mouse (*db/db)* leads to hyperinsulinaemia even before the development of the obese and diabetic phenotype (Coleman 1978, 1982, Chen & Romsos 1995). Although initial studies provided conflicting results on the effects of leptin on insulin secretion, probably due to the U-shaped effect depending on the leptin concentrations, it is currently accepted that leptin inhibits insulin secretion in β-cells both *in vitro* and *in vivo* (as reviewed in Dunmore & Brown 2013).

DPP4 and visfatin: potential mediators of β-cell dysfunction?

The direct effects of DPP4 on β-cell function remain unclear, but due to its enzymatic cleavage of GLP1, this novel adipokine may have a crucial effect on β-cells. GLP1 has a prominent role in β-cell function, as it is responsible for approx. 70% of post-prandial insulin secretion (Kazafeos 2011). GLP-1 also contributes to glucose homeostasis through its effects on insulin biosynthesis and its inhibition of glucagon release. Furthermore, GLP1 promotes pancreatic β-cell proliferation (Buteau 2008). The beneficial effects of GLP-1 on pancreatic β-cells have been also reported in studies with diabetic animals (as reviewed in Garber 2011). Similarly, DPP4 pharmacological inhibition has been related to improved β-cell survival and neogenesis in streptozotocin-treated diabetic rats (Pospisilik *et al.* 2002). The DPP4 inhibitors sitagliptin, saxagliptin and

vildagliptin improved β-cell function as proinsulin-to-insulin ratios decreased and HOMA-B increased in type 2 diabetic patients (as reviewed in Cernea & Raz 2011). In the light of these evidences, this novel adipokine may have negative effect on the pancreatic β-cell counteracting the positive effects of GLP1 on pancreatic β-cell mass and glucose homeostasis.

Visfatin has intrinsic enzymatic activity as Nampt (Revollo *et al.* 2007). Visfatin can directly increase insulin secretion in β- and βTC6 cells (Revollo *et al.* 2007, Brown *et al.* 2010b). Visfatin effects on β-cells may be mediated by its intrinsic enzymatic activity, as visfatin effects on insulin secretion can be blocked by FK866, the enzymatic inhibitor of Nampt and the enzymatic product of visfatin, NMN, has been proposed to regulate insulin transcription through PDX1 expression (Sheng *et al.* 2011). In MIN6 cells, visfatin stimulated β-cell proliferation and inhibited palmitate-induced β-cell apoptosis through ERK1/2- and PI3K/Akt activation (Cheng *et al.* 2011). On the contrary, Nampt and NMN did not have an effect on β-cell survival but rather both potentiated GSIS in human islets in a recent study (Spinnler *et al.* 2013). Nampt heterozygous female mice (Nampt+/−) showed impaired glucose tolerance due to reduced GSIS (Revollo *et al.* 2007). NMN administration reverted impairment of β-cell function (Revollo *et al.* 2007) and prevented the inflammation-induced islet dysfunction in mice under a fructose-rich diet (Caton *et al.* 2011). These potential protective effects are in conflict with one clinical study that correlated visfatin circulating levels with progressive β-cell deterioration (Lopez-Bermejo *et al.* 2006). It has been speculated that visfatin beneficial effects on β-cell function occur at lower physiological levels, whereas higher concentrations within the pathophysiological range may have harmful effects (Brown *et al.* 2010b). All these studies underpin the relevant role of visfatin through its Nampt enzymatic activity regulating β-cell function.

Conclusion

The intensive research in last years to characterize the AT secretome has led to the identification of a growing number of adipokines, which represent key mediators in interorgan crosstalk. Through interorgan crosstalk, AT regulates its own function and communicates with other organs and tissues. The importance of these factors as auto-/paracrine mediators of AT dysfunction in pathological conditions such as obesity is now well accepted (Trayhurn & Wood 2004). Nevertheless, their causative role in obesity and type 2 diabetes and related complications is still under investigation.

Some key questions on AT crosstalk have not developed in parallel to the identification of novel adipokines. It is still poorly understood how these adipokines are released from AT, and thus, the secretory pathways for many of these adipokines are not fully characterized (e.g. leptin and adiponectin) or even totally unknown (e.g. visfatin). Moreover, our knowledge on how many of these novel adipokines can modulate adipogenesis, as well as AT tissue growth and function need to be characterized in more detail.

Adipokines are now acknowledged as links between metabolic diseases and many of their complications. However, the direct effects of adipokines and of their combination leading to key metabolic-related complications such as skeletal muscle insulin resistance and both impairment of CV function and β-cell function require to be further explored. Importantly, it is not understood how some of these factors can exert adverse or beneficial effects depending on the target tissue/organ or the circulating concentration (physiological vs. pathological).

In the already complex network of interorgan crosstalk, other organs besides AT arise as novel secretory players that can in turn affect AT and other targets. Thus, pancreatic β-cells also secrete cytokines, skeletal muscle secretes myokines while the heart secretes proteins termed cardiokines (Cao *et al.* 2003, Brandt *et al.* 2012, Shimano *et al.* 2012).

Future investigations addressing these questions will shed light on the mechanisms underlying the physio- and pathological role of adipokines in AT dysfunction-related complications and may provide novel therapeutic approaches to prevent and or treat them.

Conflict of interest

The authors declare they have no conflict of interest exist.

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