Review

CellPress

Insulin resistance and impaired adipogenesis

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The adipose tissue is crucial in regulating insulin sensitivity and risk for diabetes through its lipid storage capacity and thermogenic and endocrine functions. Subcutaneous adipose tissue (SAT) stores excess lipids through expansion of adipocytes (hypertrophic obesity) and/or recruitment of new precursor cells (hyperplastic obesity). Hypertrophic obesity in humans, a characteristic of genetic predisposition for diabetes, is associated with abdominal obesity, ectopic fat accumulation, and the metabolic syndrome (MS), while the ability to recruit new adipocytes prevents this. We review the regulation of adipogenesis, its relation to SAT expandability and the risks of ectopic fat accumulation, and insulin resistance. The actions of GLUT4 in SAT, including a novel family of lipids enhancing insulin sensitivity/secretion, and the function of bone morphogenetic proteins (BMPs) in white and beige/brown adipogenesis in humans are highlighted.

Insulin resistance: a major driver of the global type 2 diabetes epidemic

Diabetes, and particularly type 2 diabetes (T2D), is increasing at an epidemic scale worldwide. In China alone it was recently estimated that 11.6% of the adult population, around 136 million people, has diabetes [1]. Globally, it is expected to afflict around 500 million people by 2030. The epidemic of T2D is attributed to our changed life-style, with less physical activity and fast-food consumption ultimately leading to obesity. T2D develops when the insulin secretory capacity is unable to compensate for the obesity-related increase in insulin resistance.

Obesity as defined by body mass index (BMI) is a heterogeneous condition and around 30% of obese individuals do not show the associated metabolic complications and are considered metabolically healthy obese [2], although also this may not be an entirely benign condition [3]. However, a similar number of non-obese individuals exhibit markers of a dysmetabolic state and reduced insulin sensitivity [4]. Thus, BMI *per se* is not a sufficiently sensitive marker of individual risk for obesity-related metabolic complications. In addition, adipose tissue distribution is important and an abdominal distribution, defined as a large waist circumference, markedly enhances both cardiovascular and diabetes risk for a given BMI [5,6].

The molecular abnormalities associated with obesity-induced insulin resistance in insulin-responsive tissues and organs (i.e., skeletal muscle, adipose tissue, and the liver) have been investigated extensively and are well established. Increased lipids plays an important role and can promote insulin resistance through the activation of various signaling pathways including protein kinase C (PKC), ceramide, and other lipid molecules and the accumulation of lipids in target tissues can induce insulin resistance (lipotoxicity) [7]. One important factor that precipitates this is the inability to store excessive lipids in SAT, which leads to 'lipid overflow' into ectopic sites that are able to accumulate lipids but at the expense of inducing lipotoxicity and negative metabolic consequences associated with insulin resistance.

Here we review current knowledge about the ability of SAT to store excess lipids and prevent accumulation in ectopic sites, as well as the possibility that white adipose tissue (WAT) under appropriate signaling conditions can assume an oxidative beige/brown phenotype and thereby also promote weight loss and increase insulin sensitivity.

SAT expandability and consequences for ectopic fat accumulation and insulin resistance

SAT is the largest adipose tissue depot in humans and also the preferred site to store excess fat. However, it has limited ability to expand and, when its storage capacity is exceeded, fat is stored in other metabolically more harmful ectopic lipid depots, including intra-abdominal/visceral sites, liver, myocardium, epi/pericardial and perivascular sites, and skeletal muscles. The importance of SAT expansion in accommodating excess lipids safely has been clearly demonstrated in several different genetically engineered animal models. For instance, overexpressing adiponectin in adipose tissue in mice leads to profound subcutaneous obesity, but with hyperplastic 'healthy' adipose tissue, and the mice are at least as insulin sensitive as their lean littermates [8]. Similarly, inhibiting adipose tissue development in lipoatrophic or lipodystrophic animal models leads to marked insulin resistance, ectopic liver fat accumulation, and reduced glucose tolerance similar to what is seen in human lipoatrophic/lipodystrophic diabetes [9]. This concept was supported by a recent study using isotope-based tracing of murine adipogenesis *in vivo*. It was shown that age-dependent inhibition of SAT hyperplastic potential in obesity was associated with the

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Keywords: obesity; type 2 diabetes; insulin resistance; BMP4; Gremlin1; adipogenesis.

1043-2760/
 http://dx.doi.org/10.1016/j.tem.2015.01.006

development of insulin resistance and thus that hypertrophic SAT is an important link to obesity-induced metabolic dysfunction [10].

The individual 'set point' and ability to expand SAT is probably both genetically determined and modified by prepubertal lifestyle factors [11]. At present, it can be determined only indirectly from, for instance, SAT cell size in relation to the amount of body fat/BMI and/or the accumulation of ectopic fat. In contrast to males, females seem to maintain an ability to recruit new cells in the thigh/gluteal region in adulthood [12], which is consistent with the reduced accumulation of ectopic visceral fat in obese women compared with obese men [7]. By contrast, a reduced ability to expand SAT is seen in Asian populations and this is accompanied by early accumulation (i.e., at low BMI) of ectopic visceral fat [13].

The association between increased visceral fat, the various metabolic complications of obesity, and the risk of developing diabetes and cardiovascular disease is well established and waist circumference is used as an easily measured clinical indicator of this [5,6]. There is also a close correlation between the amounts of fat accumulated in the various ectopic depots, indicating that they are all used for storage when SAT is unable to accommodate more excess fat [14]. This multicompartmentalization of ectopic fat also amplifies the dysmetabolic consequences associated with insulin resistance as seen in the MS, with, for instance, increased hepatic very-low-density lipoprotein (VLDL) cholesterol and triglyceride (TG) release and lower high-density lipoprotein (HDL) cholesterol levels [15]. Consistent with this concept, the large Dallas Heart Study showed that the amount of ectopic fat rather than the amount of SAT correlated with the metabolic complications, including degree of insulin resistance and the prospective risk of developing T2D in obese individuals [16].

SAT adipose cell size and phenotype are related to insulin resistance and T2D

The capacity of SAT to accommodate excess fat is regulated by the ability of the existing adipose cells to expand (hypertrophy) and/or recruit precursor cells into adipogenic differentiation (hyperplasia). Large clinical studies have shown that SAT adipose cell size expansion is limited to an upper maximal size and that hypertrophic, rather than hyperplastic, obesity is associated with insulin resistance and dyslipidemia also for a given BMI [17,18]. Inability to recruit new adipose precursor cells (both mesenchymal stem cells and committed preadipocytes) during caloric excess leads to inappropriate expansion of the available adipose cells and induction of the associated negative metabolic consequences.

Many studies, both in humans and in animal models, have shown that hypertrophic expansion of SAT adipose cells leads to a dysfunctional adipose tissue associated with increased tissue fibrosis, infiltration and activation of immune/inflammatory cells, increased lipolysis, local and systemic insulin resistance, and altered adipokine secretion [19]. As expected, hypertrophic obesity and adipose cell size are also related to the various aspects of the MS [17–20] (Figure 1). Waist circumference, a well-established marker of ectopic visceral fat accumulation and future risk of developing T2D [6,16], is also positively correlated with SAT adipose cell size.

In a detailed study of obese individuals with and without insulin resistance, Kloting *et al.* [2] demonstrated that insulin-sensitive obesity is characterized by smaller SAT adipocytes, higher secretion of the adipocyte differentiation marker adiponectin, and reduced adipose tissue inflammation and number of infiltrating macrophages. The strongest predictor of insulin sensitivity was the combination of circulating adiponectin and infiltrating macrophages in the adipose tissue [2].

An important finding relating SAT adipogenesis to insulin sensitivity and risk of T2D came from looking at individuals with a family history of T2D. Healthy first-degree relatives (FDRs) of individuals with T2D have a larger waist circumference and inappropriately enlarged SAT adipose cells for a given BMI compared with matched subjects lacking known heredity for T2D or having heredity for overweight/obesity [21]. These findings are consistent with reduced SAT adipogenesis and ability to recruit new adipose cells during caloric excess and would thus

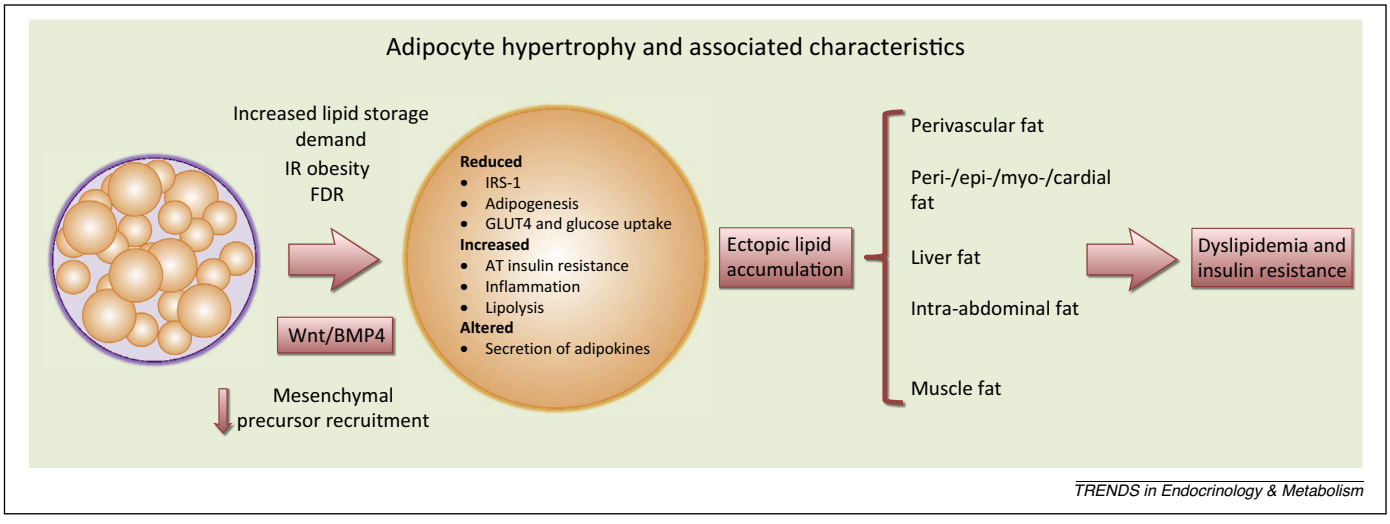

Figure 1. Characteristics of adipocyte cell hypertrophy. Adipocyte expansion with dysregulated subcutaneous adipose tissue (SAT) promotes ectopic fat accumulation and the metabolic syndrome. Adipocyte hypertrophy characterizes the SAT of insulin-resistant obesity and nondiabetic first-degree relatives of type 2 diabetes (T2D) patients.

favor ectopic fat accumulation. FDRs are insulin resistant also when non-obese and are highly susceptible to the negative metabolic consequences of increased body fat. Indeed, FDRs show an obese metabolic phenotype associated with markers of increased ectopic fat accumulation even when non-obese and have a markedly increased risk of developing T2D in follow-up studies [22,23]. Furthermore, FDRs are at increased risk of becoming overweight/obese and thus have double risks for developing T2D [22,23].

Interestingly, young and non-obese individuals with identified diabetes risk genotypes are also characterized by reduced insulin sensitivity, smaller SAT, and markers of ectopic fat accumulation [4]. Taken together, these findings support a close association between hypertrophic obesity, ectopic fat accumulation, and genetic predisposition for T2D.

Consistent with this, it was shown that individuals with inappropriately enlarged SAT adipocytes for a given BMI are characterized by reduced recruitment of precursor cells into the adipogenic pathway [24]. A similar result was found *in vivo* by examining adipose cell turnover [18]. Thus, understanding the basic mechanisms of the regulation of adipogenesis in SAT and why it is impaired in FDRs/hypertrophic obesity can open new avenues to preventing ectopic fat accumulation and T2D in obesity.

Another recent study [25] provided additional support for the importance of functional adipogenesis to prevent the development of insulin resistance and T2D. The authors evaluated various genetic peroxisome proliferator-activated receptor gamma (PPARγ) polymorphisms as nonfunctional or as loss of function based on both bioinformatics and their ability to induce adipogenesis when expressed in preadipocytes. It was shown that the loss-of-function genotypes, in contrast to the non-loss-of-function genotypes, had both a low ability to increase adipogenesis and a seven- to eightfold increased risk for T2D, while there was no increased risk associated with the functional variants [25].

Impaired glucose uptake and lipogenesis in adipose tissue relate to insulin resistance

It should be emphasized that the ability of the adipose tissue to regulate whole-body insulin sensitivity is not a consequence of its capacity to take up glucose on insulin stimulation, as it only accounts for around 10% of the glucose load [26]. However, glucose uptake and metabolism are crucial for normal adipose tissue function and genetic deletion of the insulin-regulated glucose transporter GLUT4 from adipose tissue produces a similar degree of whole-body insulin resistance in mice as does deleting GLUT4 from skeletal muscle, the tissue responsible for most insulin-stimulated glucose uptake [27]. This effect is unrelated to any changes in lipolysis or circulating free fatty acid (FFA) levels [27] but leads to alterations in adipose tissue lipid biosynthesis via pathways related to the transcription factor carbohydrate-responsive element-binding protein (CHREBP)-related pathways [28].

Reduced GLUT4 protein and impaired glucose uptake and metabolism in adipose cells lead to functional changes in the adipocytes such as the esterification of fatty acids and lipid biosynthesis and marked alterations of their endocrine functions. One example is retinol-binding protein 4 (RBP4), which is increased in individuals with low GLUT4 in adipose tissue [29] and which exerts negative effects on systemic insulin sensitivity, at least in part by promoting inflammation [30]. Secretion of several other molecules may also be altered, including a family of novel lipids (branched fatty acid esters of hydroxy fatty acids) recently shown to exert positive effects on cellular insulin sensitivity and glucose metabolism. Circulating levels of these lipids are also closely associated with systemic insulin sensitivity in humans [31].

Interestingly, GLUT4 protein is markedly reduced in SAT adipose cells from individuals with T2D and in around 30% of individuals with a genetic predisposition for T2D (FDRs) long before T2D develops [32,33]. Thus, healthy and well-functioning adipose tissue is crucially dependent on functional glucose uptake and metabolism. The reduced GLUT4 protein levels seen in adipose cells in T2D and in FDRs could thus be an early defect contributing to the dysfunctional SAT and associated alterations in adipokine secretion. Alternatively, it may be yet another marker of the impaired (pre)adipocyte differentiation and activation of the master regulator of adipogenesis PPARγ seen in hypertrophic obesity. Current studies are aimed at clarifying this important issue.

White adipocyte differentiation

Differentiation of adipocytes is a complex event with many factors and signaling pathways involved. White and brown cells are regulated differently and so are the two types of thermogenic cells, brown and beige adipocytes. The exact mechanisms that induce adipogenesis *in vivo* remain not fully elucidated. One of the earliest known events in the commitment of early stem/precursor cells into the white adipogenic lineage is repression of zinc-finger protein 521 (ZNF521) [34]. ZNF521 acts upstream of the PPARγ transcriptional activator ZNF423 and inhibits its expression by repressing early B-cell factor 1 (Ebf1) [35]. BMP4 is secreted by differentiated adipose cells [36,37] and can, in a paracrine fashion, target uncommitted precursor cells in the tissue leading to their adipogenic commitment (Figure 2). BMP4 induces the dissociation of a complex comprising wingless-type MMTV integration site (WNT) family member 2 (WISP2) and ZNF423, thereby allowing nuclear entry of ZNF423 and PPARγ induction [38,39]. Adipogenesis includes the subsequent activation of several C/EBP transcription factors – C/EBPβ, δ, and α – which are induced in a sequential manner together with numerous transcriptional cofactors. Repression of genes inhibiting adipogenesis (i.e., β-catenin and preadipocyte factor-1) is a main action of many proadipogenic factors. When expressed, PPARγ and C/EBPα act in a feedback loop to maintain their expression (Figure 2). Activation of PPARγ and C/EBPα drives the cells toward terminal differentiation and expression of adipocyte-specific genes such as FABP4, adiponectin, GLUT4, and lipoprotein lipase (LPL). Activation of PPARγ also leads to improvement of insulin sensitivity, regulates tissue partitioning of lipids, and is anti-inflammatory.

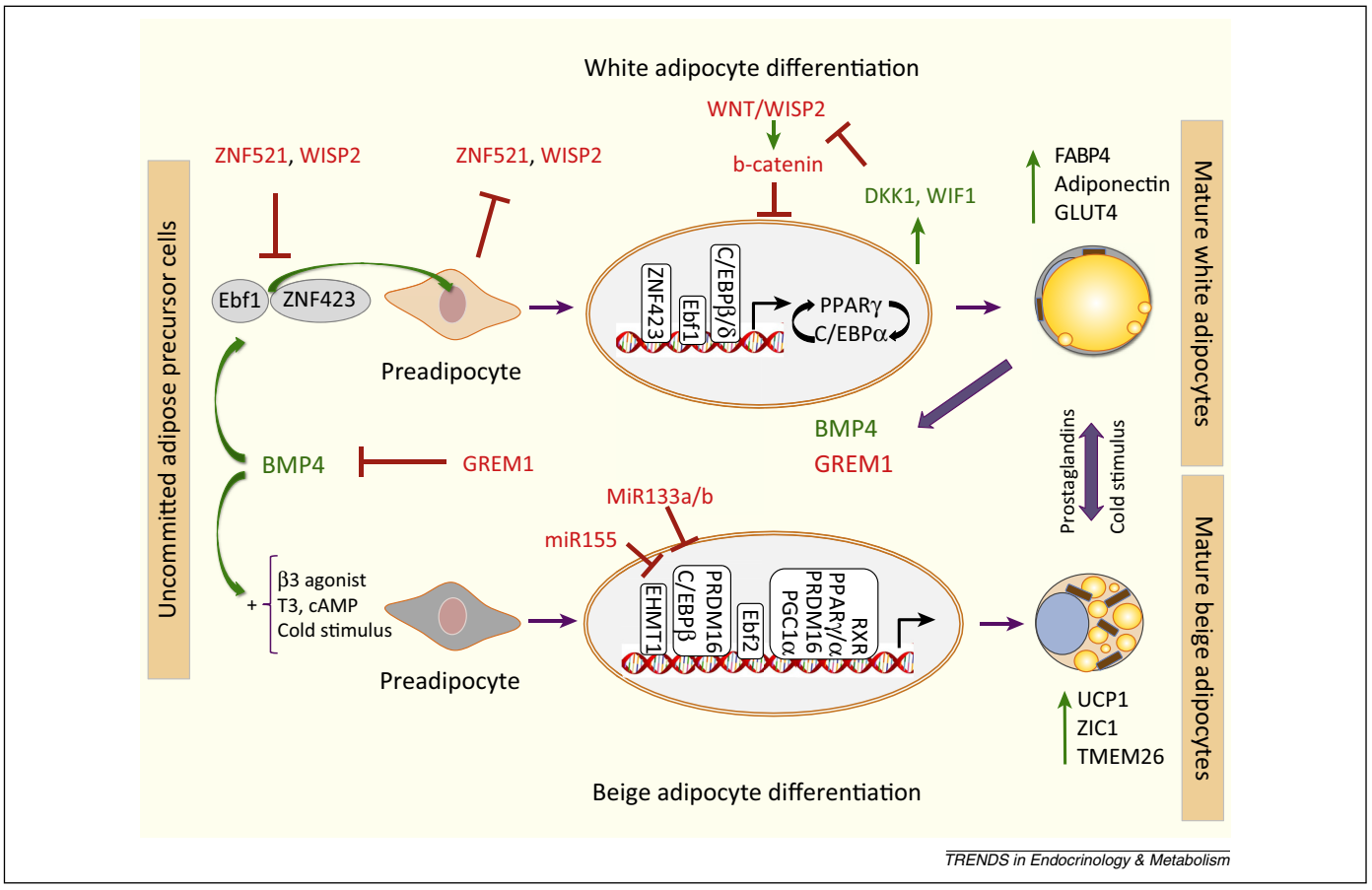

Figure 2. Regulation of commitment and differentiation of adipose mesenchymal precursor cells. Mesenchymal precursor cells are maintained uncommitted by several inhibitory complexes. Important for commitment are dissociation of the inhibitory complexes zinc-finger protein 521 (ZNF521)/early B-cell factor 1 (Ebf1) and wingless-type MMTV integration site (WNT) family member 2 (WISP2)/ZNF423. These complexes prevent ZNF423 and Ebf1/2 from entering the nucleus and activate peroxisome proliferator-activated receptor gamma (PPARγ) transcription. Differentiated white (pre)adipocytes secrete bone morphogenetic protein 4 (BMP4) and its antagonist Gremlin1 (GREM1). BMP4 receptor activation leads to dissociation of the WISP2/ZNF423 complex, while the molecular mechanisms leading to the release of Ebf1 are currently unknown. PPARγ plays a key role in white and brown adipogenesis and is the master regulator of the adipogenic differentiation of preadipocytes. Early differentiation activates Dickkopf-1 (DKK1) and WNT inhibitory factor-1 (WIF1), inhibitors of the WNT/WISP2 signaling pathway, which allows the degradation of β-catenin. This is necessary for the cells to undergo terminal differentiation. BMP4 also induces the commitment of beige adipose precursor cells which, in combination with a β3 agonist, T3, or cold stimulation, induces transcriptional activation of genes specific for thermogenesis such as uncoupling protein-1 (UCP1), Zic family member 1 (ZIC1), and PR domain-containing 16 (PRDM16). It is unclear whether white and beige adipocytes arise from the same precursor cell. The secreted BMP inhibitor GREM1 inhibits the activity of BMP4 in a feedback loop that leads to repression of commitment and adipogenesis. Abbreviations: TMEM26, transmembrane protein 26; EHMT1, euchromatic histone-lysine *N*-methyltransferase 1; PGC1α, PPARγ coactivator 1 alpha.

The reduced ability to recruit new adipose cells in SAT in FDRs and associated hypertrophic obesity is a key fundamental defect, secondary to impaired PPARγ activation and adipocyte differentiation [21,40].

The detailed mechanisms of the regulation of adipogenesis are described in several excellent recent reviews [41,42]. Figure 2 gives a comprehensive overview of the regulation of adipogenesis and the mechanisms we focus on here.

BMPs regulate white/beige/brown adipogenesis

Adipose tissue mesenchymal stem cells (MSCs) serve as a reservoir and allow the continued renewal of precursor cells that can differentiate into adipocytes [43]. BMPs are of particular interest since some members have been shown to recruit mesenchymal precursor cells into the adipose lineage. BMP7 is a regulator of brown adipogenesis [44] and BMP4 is related to white adipogenesis [40,43]. BMP7 is poorly expressed in stromal cells isolated from human SAT and BMP2 is downregulated during adipogenesis [36]. This emphasizes the role of BMP4 in human adipogenesis. BMP4 is induced by human precursor cells undergoing adipogenesis and can, in a paracrine feedback loop, induce human precursor cell commitment and differentiation [36,40] and thereby prevent hypertrophic obesity.

BMPs are secreted molecules that act in an autocrine and/or paracrine fashion and may also enter the nucleus [45]. They transduce their signals through BMP receptors type I and II, leading to activation of receptor-associated kinase activity, autophosphorylation, and downstream SMAD1/5/8 phosphorylation. Exposing the mesenchymal stem cell-like murine cell line C3H10T1/2 to BMP4 committed the cells to the adipocyte lineage and, similarly, BMP4 increased adipogenesis in human SAT precursor cells [40,43]. Inhibiting the effect of BMP4 with the BMP inhibitor Noggin reduced adipogenic differentiation, further supporting the important role of BMP4 [40].

A recent study investigated whether hypertrophic obesity is associated with reduced induction of BMP4 and/or

increased activation of the various endogenous BMP inhibitors. BMP4 is increased in adipose tissue in hypertrophic obesity [36], suggesting that the precursor cells may be less responsive due to increased activity of endogenous BMP4 antagonists. It was found that the secreted BMP inhibitor GREMLIN1 (GREM1) is highly expressed in, and secreted by, human preadipocytes and with a considerably higher expression in hypertrophic obesity. GREM1 is a secreted and potent extra- and, potentially, intracellular inhibitor of BMP4 [46] but little is known about its functions except that it is considered to be involved in fibrosis and inflammation [47]. It inhibits both BMP4 and BMP7 and, when silenced with siRNA, BMP4 markedly increases PPARγ and enhances both white and beige/brown markers for adipogenesis in human subcutaneous preadipose cells [36]. Taken together, these findings suggest that it is not lack of BMP4 but rather the presence of high levels of the endogenous inhibitor GREM1 that prevents the expected effect of BMP4 in dissociating the WISP2/ZNF423 complex and the induction of adipogenesis. This inability then favors the development of hypertrophic obesity and associated metabolic consequences including insulin resistance and T2D (Box 1).

Physiological significance of beige and brown adipose cells

The possibility that the adipose tissue could be turned into an oxidizing tissue similar to brown adipose tissue (BAT) has attracted much recent interest. This includes efforts to activate brown fat in adult humans and/or to enhance an intermediate white/brown adipose cell phenotype (beige/brite cells) primarily in SAT, which is likely to have positive systemic effects on insulin sensitivity and body weight regulation.

BAT is specialized for energy expenditure and the maintenance of body temperature. BAT was first thought to be present only in newborns and that it regressed during childhood. Later, cells with a BAT phenotype were found interspersed in various fat depots in rodent models and in 2009 several groups demonstrated the presence of small amounts of functional brown fat in adult humans [48–51] in the cervical, supraclavicular, paravertebral, and perirenal areas. Brown adipocytes can be activated by cold exposure and express uncoupling protein-1 (UCP1), specific for thermogenesis. Furthermore, brown adipocytes are distinct from white adipocytes, with multiple lipid droplets and high mitochondrial density [52].

Until now, the physiological significance of BAT for whole-body metabolism in adult humans has been unclear, although increased glucose disposal in BAT has been reported [53,54]. Recently, Chondronikola *et al.* showed for the first time that it is possible to increase resting energy expenditure, plasma glucose oxidation, whole-body glucose disposal, and insulin sensitivity in men with a significant amount of BAT after prolonged cold exposure [55]. The differences were also verified by showing that individuals with low BAT had minimal UCP1 staining of the largely unilocular white adipose cells in the supraclavicular adipose tissue. Furthermore, cold exposure increased insulin-stimulated glucose disposal only in the high-BAT group [55]. These findings suggest that BAT activation can play an important role in peripheral glucose disposal. However, the study was small and needs to be verified in larger studies.

Box 1. Regulation of white and beige/brown adipogenesis

The molecular mechanisms leading to reduced white adipogenesis in SAT in hypertrophic obesity are partly understood. MSCs in adipose tissue serve as a reservoir that can be committed into the adipogenic lineage by BMP4 [40,43]. However, there are several BMP antagonists in the adipose tissue, such as GREM1, secreted by (pre)adipocytes. GREM1 is a potent inhibitor of BMP4 and is upregulated in hypertrophic obesity [36].

Interestingly, BMP4 not only regulates white adipogenesis but also plays an important role in regulating beige/brown SAT adipogenesis. Beige or brite (brown-in-white) adipocytes have been found within human SAT and were shown to have many of the properties of oxidative brown adipocytes, although their origin is different [56,57]. It is likely that transdifferentiation of white preadipocytes into beige cells can occur or, alternatively, that there is a subpopulation of precursor cells that can undergo differentiation into beige cells. Beige cells express brown adipose cell markers such as UCP1 and Zic family member 1 (ZIC1) and can increase lipid oxidation [36,56,57].

Increasing amounts and/or activity of brown adipose tissue or increasing oxidative beige/brown adipocytes in the large SAT may be potential strategies to counteract obesity and its metabolic consequences.

Regulation of brown and beige adipose cells

A distinct population of brown-like oxidative cells, beige adipocytes, has been demonstrated in humans and mice WAT exposed to cold or PPARγ ligands [56,57]. Studies in rodents show that these cells can be induced to express typical brown markers like UCP1 and PR domain-containing 16 (PRDM16) [56]. However, in the unstimulated state they more resemble white adipocytes, with negligible expression of UCP1 and PRDM16. Although differently regulated, beige cells also have the capacity to activate UCP1 and induce oxidative genes [58]. Characterization of brown and beige cells demonstrates that they are distinct cell types; the 'real' constitutive brown adipocytes arise from the $Myf5^+$ skeletal muscle lineage and $Myf5^+$ cells switch from myoblastic precursors to brown fat cells when PRDM16 and C/EBPβ, a critical binding partner, form a transcriptional complex to induce brown fat gene expression [58].

Beige cells are suggested to arise either from transdifferentiation of white cells or from a population of smooth muscle-like cells residing in the WAT (for a comparison of white, brown, and beige adipocytes, see Table 1) [59,60]. Jespersen *et al.* found upregulation of beige markers in the supraclavicular region of humans and this may suggest that beige and brown adipocytes colocalize [52]. Attempts to induce brown differentiation in preadipocytes isolated from human subcutaneous, mesenteric, and omental fat with BMP7 showed that it was only subcutaneous preadipocytes that had the potential to induce UCP1 [61]. Current information is primarily from animal studies and it is clear that results from animal studies are in many ways different and cannot be directly translated to humans.

Adipose tissue inflammation and insulin resistance

A well-established consequence of hypertrophic obesity is chronic inflammation involving both the innate and

Table 1. Differences between human white, brown, and beige adipocytes

	White adipocytes	Brown adipocytes	Beige adipocytes	Refs
Origin	Adipose MSCs	Myf5$^+$	Adipose MSCs Transdifferentiation of (pre)adipocytes?	[43,58]
Location	Subcutaneous, ectopic sites	Cervical, supraclavicular, paravertebral, perirenal	Supraclavicular, subcutaneous	[48–57]
Function	Energy storage as TG	Energy dissipation	Adaptive thermogenesis	[48–57]
Morphology	Unilocular lipid droplets	Multilocular lipid droplets	Multilocular lipid droplets	
Mitochondrial content	Few	Abundant	Few, increases on stimulation	[52]
Activation	Food intake, thiazolidinediones	Cold, β-adrenoceptor agonists, thyroid hormones	Cold, β-adrenoceptor agonists, thyroid hormones, catecholamines	[55]
UCP1	Undetectable or low expression	Highly expressed	Induced after stimulation	[48]
Expressed markers[a] *(bold indicates specific markers)*	**ASC1**, **LEP**, HOXC8, HOXC9	PAT2, P2RX5, **ZIC1**, **LHX8**, **HOXC4**, HOXA1, PGC1α, CIDEA, PRDM16, CITED1, FGF21	PAT2, P2RX5**, TBX1**, **TMEM26**, **CD137**, **SHOX2**, PGC1α, CIDEA, PRDM16, CITED1, HOXC8, HOXC9, FGF21	

[a]Markers for beige adipocytes are expressed at very low levels in nonactivated cells but increase after stimulation. There is an overlap in gene expression patterns between white and beige adipocytes as well as between brown and beige adipocytes. Bold indicates markers considered specific for the cell phenotype.

adaptive immune systems in the adipose tissue following infiltration and activation of immune/inflammatory cells [62,63]. Several studies have shown that adipose tissue inflammation and macrophage cell recruitment are important drivers of insulin resistance in obesity and cytokines released from proinflammatory M1 macrophages in a hypertrophic environment negatively influence adipose cell function and impair adipose cell insulin signaling pathways (Box 2) [64,65]. Resident macrophages in SAT characterized by adipose cell hypertrophy have been observed. This infiltration is reduced by weight loss following diet, exercise, or surgery [66,67]. These studies have also shown that the macrophages, and not the adipocytes *per se*, are the predominant tissue source of the proinflammatory cytokines and that these cells may be recruited to adipose tissue via chemoattractants such as monocyte chemotactic protein 1 (MCP-1) [68]. Circulating MCP-1 levels as well as macrophage-specific markers such as CD68 and CD36 are increased in T2D and are associated with poor blood glucose control [69]. Thus, macrophage-derived cytokines directly or indirectly affect SAT function and lipid storage capacity by increasing lipolysis, reducing glucose uptake, and impairing insulin receptor signaling [68–71].

However, inflammation is not only a negative factor but is also essential for tissue repair and remodeling. It was recently shown that inhibition of an acute inflammatory response in adipose tissue negatively affects adipose tissue expansion/remodeling in mouse models challenged with a high-fat diet and leads to metabolic dysfunction and increased ectopic lipid accumulation [72].

Based on their properties, adipose tissue macrophages in lean and obese subjects have different cellular localizations and inflammatory potentials [68,70]. As mentioned above, the classical proinflammatory M1 macrophages are considered the major source of inflammatory mediators in adipose tissue hypertrophy, whereas the alternative M2 macrophages promote anti-inflammatory effects and tissue remodeling/repair and insulin sensitizing properties [70,73]. Interestingly, following cold exposure or exercise of the animals, M2 macrophages are able to increase thermogenesis and beige adipocyte recruitment in WAT, findings that show novel crosstalk between these cells and adipose tissue [74].

Box 2. Inflammation in hypertrophic obesity promotes insulin resistance

In hypertrophic adipose tissue, preadipocytes and immune cells crosstalk to induce chronic inflammation and insulin resistance. This leads to increased FFA release, cellular stress, and chemokine/cytokine secretion [e.g., MCP-1, interleukin-6 (IL-6)], further promoting immune cell infiltration in the adipose tissue. Interestingly, recent studies have shown that inflammation also is important for functional tissue remodeling [72].

The classical proinflammatory M1 macrophages are accumulated in hypertrophic adipose tissue and release proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), IL1β, and IL-6, which stimulate the IκB kinase beta (IKKβ)/nuclear factor kappa B (NF-κB), and c-Jun N-terminal kinase (JNK) pathways. IKKβ and JNK induce specific insulin receptor substrate 1 (IRS-1) serine phosphorylation, which results in reduction of IRS proteins and downstream insulin receptor signaling by interruption of the insulin receptor–IRS interaction, thereby promoting insulin resistance [68,70,71,75].

In lean and healthy adipose tissue, the anti-inflammatory M2 macrophages are predominant and also play a role in maintaining insulin sensitivity [70,73]. Studies in animal models have also revealed important roles for PPARγ and PPARδ in regulating the M2 macrophage phenotype [76,77]. A recent study has shown a significant association between improved insulin sensitivity and reduction in adipose tissue macrophages in T2D treated with thiazolidinediones (PPARγ ligands) [78].

Currently, there are several ongoing studies of various anti-inflammatory molecules examining their potential positive effects on insulin resistance and the development of T2D. However, the only group of anti-inflammatory agents that has so far been shown to have positive effects are the non-acetylated salicylates (e.g., salsalate) [79,80], which can be given in high doses with fewer side effects than the acetylated forms. A high dose of a non-acetylated salicylate is able to inhibit the NF-κB/IKKβ axis without modifying COX proteins and thus carries a reduced bleeding risk. Several independent studies of salsalate-treated T2D, prediabetic, and obese nondiabetic subjects have demonstrated markers of metabolic improvements such as increased serum adiponectin levels, lower glycemia, reduced lipolysis, decreased C-peptide, and reduced adipose tissue NF-κB activity [79,80]. However, the outcomes of ongoing large clinical studies are needed to better validate the potential benefits of anti-inflammatory therapy in T2D.

Concluding remarks and future perspectives

SAT plays a key role in the obesity-associated metabolic complications of the MS through its endocrine effects (adipokines), its ability to store/release lipids as well as its involvement in thermogenic regulation via the beige adipose cells. Expansion of existing subcutaneous fat cells leads to inflamed, dysregulated, and dysfunctional adipose tissue promoting ectopic fat accumulation and insulin resistance, while the ability to recruit new adipose cells is protective. Importantly, individuals with a genetic predisposition for/family history of T2D exhibit markers of intrinsic problems in their ability to recruit new SAT adipose cells accompanied by increased waist circumference, insulin resistance, and increased ectopic fat.

BMP4 is induced and secreted by human differentiated (pre)adipocytes and plays a pivotal role in both enhancing white adipogenesis and regulating the induction of an oxidative beige/brown phenotype. However, the effect of BMP4 is antagonized by endogenous BMP inhibitors such as GREM1, which is overexpressed in hypertrophic obesity. Understanding how SAT can be turned into both an adequate lipid storing and an oxidative tissue is a future challenge that could help combat the consequences of the global obesity epidemic. Much current work is also focused on understanding the role of brown fat in adult humans and whether increasing brown fat activation and/or amount can have preventive effects on obesity and insulin resistance.

Another important future challenge is to understand the role of GLUT4/glucose metabolism in regulating adipose tissue biosynthesis and the secretion of lipids and other adipokines with profound effects on whole-body insulin sensitivity. The recent identification of a novel class of lipids secreted by SAT and with positive effects on both insulin sensitivity and insulin secretion opens new avenues in our understanding of why dysfunctional SAT is associated with insulin resistance and T2D. Low circulating levels are closely related to the degree of insulin resistance in humans, suggesting that these lipids may become novel therapeutic targets. Low-grade inflammation is a well-recognized characteristic of hypertrophic obesity and T2D and the outcome of large clinical studies with various anti-inflammatory agents in T2D are eagerly awaited. The current ongoing global epidemics of obesity and T2D must be met with better preventive and therapeutic options.

Acknowledgments

The studies referred to in the authors' laboratory were supported by the Swedish Research Council, the Swedish Diabetes Foundation, the Torsten Söderberg Foundation, the Novo Nordisk Foundation, the IngaBritt and Arne Lundberg Foundation, and the Edgar Sjölund Foundation.

References

1 Xu, Y. *et al.* (2013) Prevalence and control of diabetes in Chinese adults. *JAMA* 310, 948–959
2 Kloting, N. *et al.* (2010) Insulin-sensitive obesity. *Am. J. Physiol. Endocrinol. Metab.* 299, E506–E515
3 Twig, G. *et al.* (2014) Diabetes risk among overweight and obese metabolically healthy young adults. *Diabetes Care* 37, 2989–2995
4 Scott, R.A. *et al.* (2014) Common genetic variants highlight the role of insulin resistance and body fat distribution in type 2 diabetes, independently of obesity. *Diabetes* 63, 4378–4387
5 Fox, K.A. *et al.* (2009) Does abdominal obesity have a similar impact on cardiovascular disease and diabetes? A study of 91,246 ambulant patients in 27 European countries. *Eur. Heart J.* 30, 3055–3063
6 Despres, J.P. and Lemieux, I. (2006) Abdominal obesity and metabolic syndrome. *Nature* 444, 881–887
7 Virtue, S. and Vidal-Puig, A. (2010) Adipose tissue expandability, lipotoxicity and the metabolic syndrome – an allostatic perspective. *Biochim. Biophys. Acta* 1801, 338–349
8 Kim, J.Y. *et al.* (2007) Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J. Clin. Invest.* 117, 2621–2637
9 Reitman, M.L. *et al.* (2000) Lipoatrophy revisited. *Trends Endocrinol. Metab.* 11, 410–416
10 Kim, S.M. *et al.* (2014) Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. *Cell Metab.* 20, 1049–1058
11 Spalding, K.L. *et al.* (2008) Dynamics of fat cell turnover in humans. *Nature* 453, 783–787
12 Tchoukalova, Y.D. *et al.* (2010) Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18226–18231
13 Kadowaki, T. *et al.* (2006) Japanese men have larger areas of visceral adipose tissue than Caucasian men in the same levels of waist circumference in a population-based study. *Int. J. Obes. (Lond.)* 30, 1163–1165
14 Graner, M. *et al.* (2013) Cardiac steatosis associates with visceral obesity in nondiabetic obese men. *J. Clin. Endocrinol. Metab.* 98, 1189–1197
15 Boren, J. *et al.* (2013) Ectopic lipid storage and insulin resistance: a harmful relationship. *J. Intern. Med.* 274, 25–40
16 Neeland, I.J. *et al.* (2012) Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *JAMA* 308, 1150–1159
17 Krotkiewski, M. *et al.* (1983) Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J. Clin. Invest.* 72, 1150–1162
18 Arner, E. *et al.* (2010) Adipocyte turnover: relevance to human adipose tissue morphology. *Diabetes* 59, 105–109
19 Gustafson, B. *et al.* (2009) Inflammation and impaired adipogenesis in hypertrophic obesity in man. *Am. J. Physiol. Endocrinol. Metab.* 297, E999–E1003
20 Weyer, C. *et al.* (2000) Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 43, 1498–1506
21 Arner, P. *et al.* (2011) Genetic predisposition for type 2 diabetes, but not for overweight/obesity, is associated with a restricted adipogenesis. *PLoS ONE* 6, e18284
22 InterAct Consortium *et al.* (2012) Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: the EPIC–InterAct case-cohort study. *PLoS Med.* 9, e1001230
23 Cederberg, H. *et al.* (2014) Family history of type 2 diabetes increases the risk of both obesity and its complications: is type 2 diabetes a disease of inappropriate lipid storage? *J. Intern. Med.* Published online July 12, 2014. http://dx.doi.org/10.1111/joim.12289
24 Isakson, P. *et al.* (2009) Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-α, and inflammation. *Diabetes* 58, 1550–1557
25 Majithia, A.R. *et al.* (2014) Rare variants in PPARG with decreased activity in adipocyte differentiation are associated with increased risk of type 2 diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13127–13132
26 Virtanen, K.A. *et al.* (2002) Glucose uptake and perfusion in subcutaneous and visceral adipose tissue during insulin stimulation in nonobese and obese humans. *J. Clin. Endocrinol. Metab.* 87, 3902–3910
27 Abel, E.D. *et al.* (2001) Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409, 729–733
28 Herman, M.A. *et al.* (2012) A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. *Nature* 484, 333–338
29 Graham, T.E. *et al.* (2006) Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med.* 354, 2552–2563
30 Norseen, J. *et al.* (2012) Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and Toll-like

receptor 4-dependent and retinol-independent mechanism. *Mol. Cell. Biol.* 32, 2010–2019
31 Yore, M.M. *et al.* (2014) Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. *Cell* 159, 318–332
32 Carvalho, E. *et al.* (2001) Insulin resistance with low cellular IRS-1 expression is also associated with low GLUT4 expression and impaired insulin-stimulated glucose transport. *FASEB J.* 15, 1101–1103
33 Smith, U. (2002) Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance – is insulin resistance initiated in the adipose tissue? *Int. J. Obes. Relat. Metab. Disord.* 26, 897–904
34 Addison, W.N. *et al.* (2014) Direct transcriptional repression of Zfp423 by Zfp521 mediates a bone morphogenic protein-dependent osteoblast versus adipocyte lineage commitment switch. *Mol. Cell. Biol.* 34, 3076–3085
35 Gao, H. *et al.* (2014) Early B cell factor 1 regulates adipocyte morphology and lipolysis in white adipose tissue. *Cell Metab.* 19, 981–992
36 Gustafson, B. *et al.* (2015) BMP4 and BMP antagonists regulate human white and beige adipogenesis. *Diabetes* Published online January 20, 2015. http://dx.doi.org/10.2337/db14-1127
37 Elsen, M. *et al.* (2014) BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. *Am. J. Physiol. Cell Physiol.* 306, C431–C440
38 Hammarstedt, A. *et al.* (2013) WISP2 regulates preadipocyte commitment and PPARγ activation by BMP4. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2563–2568
39 Gupta, R.K. *et al.* (2010) Transcriptional control of preadipocyte determination by Zfp423. *Nature* 464, 619–623
40 Gustafson, B. and Smith, U. (2012) The WNT inhibitor Dickkopf 1 and bone morphogenetic protein 4 rescue adipogenesis in hypertrophic obesity in humans. *Diabetes* 61, 1217–1224
41 Rosen, E.D. and MacDougald, O.A. (2006) Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* 7, 885–896
42 Rosen, E.D. and Spiegelman, B.M. (2014) What we talk about when we talk about fat. *Cell* 156, 20–44
43 Bowers, R.R. *et al.* (2006) Stable stem cell commitment to the adipocyte lineage by inhibition of DNA methylation: role of the BMP-4 gene. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13022–13027
44 Tseng, Y.H. *et al.* (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004
45 Felin, J.E. *et al.* (2010) Nuclear variants of bone morphogenetic proteins. *BMC Cell Biol.* 11, 20
46 Topol, L.Z. *et al.* (2000) Biosynthesis, post-translation modification, and functional characterization of Drm/Gremlin. *J. Biol. Chem.* 275, 8785–8793
47 Costello, C.M. *et al.* (2010) Role of Gremlin in the lung: development and disease. *Am. J. Respir. Cell Mol. Biol.* 42, 517–523
48 Virtanen, K.A. *et al.* (2009) Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525
49 Cypess, A.M. *et al.* (2009) Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517
50 van Marken Lichtenbelt, W.D. *et al.* (2009) Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508
51 Zingaretti, M.C. *et al.* (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120
52 Jespersen, N.Z. *et al.* (2013) A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. *Cell Metab.* 17, 798–805
53 Orava, J. *et al.* (2011) Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab.* 14, 272–279
54 Ouellet, V. *et al.* (2012) Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J. Clin. Invest.* 122, 545–552
55 Chondronikola, M. *et al.* (2014) Brown adipose tissue improves whole body glucose homeostasis and insulin sensitivity in humans. *Diabetes* 63, 4089–4099
56 Sharp, L.Z. *et al.* (2012) Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS ONE* 7, e49452
57 Wu, J. *et al.* (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150, 366–376
58 Kajimura, S. *et al.* (2009) Initiation of myoblast to brown fat switch by a PRDM16–C/EBP-β transcriptional complex. *Nature* 460, 1154–1158
59 Long, J.Z. *et al.* (2014) A smooth muscle-like origin for beige adipocytes. *Cell Metab.* 19, 810–820
60 Rosenwald, M. and Wolfrum, C. (2014) The origin and definition of brite versus white and classical brown adipocytes. *Adipocyte* 3, 4–9
61 Schulz, T.J. *et al.* (2011) Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 143–148
62 Andersson, C.X. *et al.* (2008) Inflamed adipose tissue, insulin resistance and vascular injury. *Diabetes Metab. Res. Rev.* 24, 595–603
63 Richardson, V.R. *et al.* (2013) Adipose tissue inflammation: feeding the development of type 2 diabetes mellitus. *Immunobiology* 218, 1497–1504
64 Weisberg, S.P. *et al.* (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808
65 Xu, H. *et al.* (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* 112, 1821–1830
66 Bruun, J.M. *et al.* (2006) Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *Am. J. Physiol. Endocrinol. Metab.* 290, E961–E967
67 Cancello, R. *et al.* (2005) Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 54, 2277–2286
68 Kanda, H. *et al.* (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* 116, 1494–1505
69 Mine, S. *et al.* (2006) Increased expression levels of monocyte CCR2 and monocyte chemoattractant protein-1 in patients with diabetes mellitus. *Biochem. Biophys. Res. Commun.* 344, 780–785
70 Lumeng, C.N. *et al.* (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* 117, 175–184
71 Hotamisligil, G.S. *et al.* (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. *Science* 271, 665–668
72 Wernstedt Asterholm, I. *et al.* (2014) Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab.* 20, 103–118
73 Gordon, S. (2007) Macrophage heterogeneity and tissue lipids. *J. Clin. Invest.* 117, 89–93
74 Qiu, Y. *et al.* (2014) Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* 157, 1292–1308
75 Rui, L. *et al.* (2001) Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J. Clin. Invest.* 107, 181–189
76 Bouhlel, M.A. *et al.* (2007) PPARγ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab.* 6, 137–143
77 Odegaard, J.I. *et al.* (2008) Alternative M2 activation of Kupffer cells by PPARδ ameliorates obesity-induced insulin resistance. *Cell Metab.* 7, 496–507
78 Koppaka, S. *et al.* (2013) Reduced adipose tissue macrophage content is associated with improved insulin sensitivity in thiazolidinedione-treated diabetic humans. *Diabetes* 62, 1843–1854
79 Yuan, M. *et al.* (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ. *Science* 293, 1673–1677
80 Goldfine, A.B. *et al.* (2008) Use of salsalate to target inflammation in the treatment of insulin resistance and type 2 diabetes. *Clin. Transl. Sci.* 1, 36–43