Adipose tissue in control of metabolism

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Abstract

Adipose tissue plays a central role in regulating whole-body energy and glucose homeostasis through its subtle functions at both organ and systemic levels. On one hand, adipose tissue stores energy in the form of lipid and controls the lipid mobilization and distribution in the body. On the other hand, adipose tissue acts as an endocrine organ and produces numerous bioactive factors such as adipokines that communicate with other organs and modulate a range of metabolic pathways. Moreover, brown and beige adipose tissue burn lipid by dissipating energy in the form of heat to maintain euthermia, and have been considered as a new way to counteract obesity. Therefore, adipose tissue dysfunction plays a prominent role in the development of obesity and its related disorders such as insulin resistance, cardiovascular disease, diabetes, depression and cancer. In this review, we will summarize the recent findings of adipose tissue in the control of metabolism, focusing on its endocrine and thermogenic function.

Introduction

Adipose tissue, which is primarily composed of adipocytes as well as pre-adipocytes, macrophages, endothelial cells, fibroblasts, and leucocytes, has been increasingly recognized as a major player of systemically metabolic regulation. As the fuel reservoir, adipose tissue conserves the heat of the body and controls the lipid mobilization (Sethi & Vidal-Puig 2007). The surplus energy is efficiently deposited in the form of neutral triglycerides (TGs) in adipose tissue through the lipogenic pathway. However, the storage of neutral TGs in adipocytes increases the lipid droplet size, which results in adipose expansion and subsequent obesity (Tan & Vidal-Puig 2008). By contrast, TGs reserved in adipocytes are broken down into glycerol and fatty acids through lipolytic pathway when food is scarce, energy expenditure requirements are stimulated or the storage of neutral TGs exceeds the capacity of adipocytes (Lafontan & Langin 2009). The released glycerol and fatty acids from adipose tissue can then be transported in the blood and subsequently infiltrate into muscle, liver and other organs, which drives lipid distribution and modulates whole-body energy balance (Frayn 2002).

Adipose tissue is not only a passive fuel reservoir, but also an endocrine organ. Extensive effort has been made to understand adipose tissue-derived factors and their physiological functions in the past two decades (Zhang et al. 1994, Friedman & Halaas 1998, Scherer 2006, Giralt et al. 2016). These bioactive factors secreted...
from adipose tissue circulate and relay information to other metabolically active organs such as muscle, liver, pancreas and brain via endocrine mechanisms, thereby modulating systemic metabolism (Scherer 2006, Rosen & Spiegelman 2014, Parimisetty et al. 2016). Among these factors, adipokines-cytokines produced by adipose tissue including leptin, adiponectin, visfatin, apelin, vaspin, hepcidin, chemerin and omentin are implicated in obesity and obesity-related metabolic disorders (Lago et al. 2009, Andrade-Oliveira et al. 2015). Adipokine action is mainly mediated by binding to their respective receptors on the membrane of target cells and triggering particular intracellular signalling pathways. An enormous amount of evidence has demonstrated that impaired biosynthesis, assembly, secretion and signalling transduction of adipokines are associated with the development of obesity and its related disorders (Deng & Scherer 2010).

Adipose tissue can be classified into two subtypes: white adipose tissue (WAT) and brown adipose tissue (BAT). BAT, different from WAT which stores extra energy as TGs, dissipates chemical energy as heat via high levels of uncoupling protein 1 (UCP1) and combats hypothermia and obesity by burning lipid. Interestingly, it has been known for several years that there is another type of WAT and obesity by burning lipid. Interestingly, it has been known for several years that there is another type of WAT and obesity by burning lipid. Interestingly, it has been known for several years that there is another type of WAT

Characteristics of adipocytes

Adipocytes, also called adipose cells or fat cells, are the predominant cell type in adipose tissue. In mammals, there are three types of adipocytes: white, brown and beige (brite). They differ in origin, morphology, abundance of mitochondria and thermogenic genes expression. White adipocytes are mainly present in WAT with variable size (25–200μm) and have a unilocular lipid droplet, few mitochondria and a low oxidative rate (Janson et al. 2015). In line with this, white adipocytes have high capacity of storing energy in the form of TGs, and protect organs such as muscle and liver from lipotoxicity (Tan & Vidal-Puig 2008). Although white adipocytes arise from resident cells of mesenchymal origin in white fat, subcutaneous adipocytes have distinct developmental origins and metabolic properties from visceral adipocytes in rodents and humans (Laviola et al. 2006, Ibrahim 2010, Berry et al. 2013, Chau et al. 2014). Visceral fat including mesenteric, gonadal, epicardial, retroperitoneal, omental and peri-renal fat pad is thought to be more deleterious, while subcutaneous WAT is protective in the development of obesity and related metabolic disease in rodents (Foster et al. 2011, Seale et al. 2011). In support of this, transplantation of subcutaneous but not visceral adipose tissue improves glucose tolerance and insulin sensitivity in rodents (Tran et al. 2008, Foster et al. 2013). Although there is still a controversy about metabolic function of subcutaneous and visceral fat in humans (Thorne et al. 2002, Fabbrini et al. 2010), the fat distribution, rather than total fat mass, most likely plays an important role in the development of obesity and its associated diseases. More studies are urgently needed to elucidate whether transplantation of subcutaneous fat in humans is as efficient as that in rodents.

Brown adipocytes were originally thought to be a skeletal muscle-like lineage arising from Myf5+ precursors (Seale et al. 2008, Lepper & Fan 2010). However, the complexity of adipocyte origin and identity has been considered recently. First, brown adipocytes are not only from Myf5+ precursors. Although interscapular and subscapular BAT are derived from Myf5+ lineage, cervical BAT are partially and peri-renal and peri-aortic BAT are completely from Myf5+ precursors (Sanchez-Gurmaches & Guertin 2014). In addition, Myf5+ precursors have been shown to be present in interscapular and retroperitoneal WAT and are capable of giving rise to some white/brite adipocytes (Sanchez-Gurmaches et al. 2012, Sanchez-Gurmaches et al. 2016). Brown adipocytes are specialized cells with multicellular morphology, abundant mitochondria and enrichment of UCP1, and dissipate stored energy in the form of heat (Aherne & Hull 1966, Cannon & Nedergaard 2004). UCP1 is located in the inner mitochondrial membrane and uncouple fuel oxidation from ATP synthesis (Efdorenko et al. 2012). Brown adipocyte clusters mainly exist in the interscapular and peri-renal regions of rodents and in abdominal sites, like peri-renal region of human infants, where they are richly innervated and vascularized (Blaza 1983, Bamshad et al. 1999, Bartness et al. 2010). The adult human BAT was recently identified in the lower neck and supraclavicular areas by 18F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) scanning (Nedergaard et al. 2007, Cypess et al. 2009, van Marken...
Adipogenesis and its regulation

Adipogenesis is a cell process of differentiation from committed preadipocytes into mature adipocytes, and plays an important role in adipose development and systemic energy homeostasis (Leiper & Lazar, 2009, Ali et al., 2013). As a result, the transcriptional regulation of adipogenesis has been well studied. Peroxisomal proliferator-activated receptor γ (PPARγ), a member of nuclear-receptor superfamily, has been shown to act as the master regulator of adipogenesis (Rosen et al., 2000). Overexpressing PPARγ is sufficient to induce adipocyte differentiation in fibroblasts, and deficiency of PPARγ fails to promote adipogenic programmes and results in lipodystrophy (Tontonoz et al., 1994, Hegele et al., 2002, Koutrinikova et al., 2003). Moreover, other factors or pathways including pro-adipogenic factors such as C/EBPs, and Krüppel-like factors (KLFs) and anti-adipogenic factors such as GATA transcription factors regulate adipogenesis via PPARγ-dependent mechanisms (Rosen et al., 2000, Farmer, 2006). Furthermore, PPARγ is not only crucial for adipogenesis but is also required for the maintenance of differentiation (Rosen et al., 2000, Farmer, 2006). In support of this, impairing PPARγ function by overexpression of a dominant-negative form downregulates the expression of key genes in lipid metabolism and insulin signalling and decreases the cell size and lipid content in 3T3-L1 differentiated adipocytes (Tamori et al., 2002). In line with this, selective ablation of PPARγ in mature white and brown adipocytes leads to adipocyte death, while having little effect on the preadipocyte differentiation (Imai et al., 2004). Therefore, PPARγ has been considered as a therapeutic target for the treatment of obesity-related disorders. To this end, extensive effort has been placed on studying the regulation of PPARγ expression and its
activity (Farmer 2005, Lee & Ge 2014). Thiazolidinediones (TZDs) as the synthetic full agonists of PPARγ have been shown to improve insulin sensitivity and glucose control by activating PPARγ. However, their use has been hampered because of severe side effects. Partial agonists have been identified as selective PPARγ modulators with great promise for the treatment of type 2 diabetes (Higgins & Depaoli 2010, Taygerly et al. 2013, Kroker & Bruning 2015). INT131 (previously AMG131) acts as one of the selective PPARγ modulators to partially activate transcriptional output, enhances insulin sensitivity with decreased side effects in preclinical models and has been tested in phase II clinic trial (Kintscher & Goebel 2009, Higgins & Depaoli 2010, Taygerly et al. 2013, DePaoli et al. 2014).

C/EBPs are a type of transcription factors homologous to CCAAT/enhancer-binding protein including C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ and CHOP (Rosen & Spiegelman 2000). C/EBPs are induced during adipogenesis and coordinate with PPARγ to regulate adipocyte differentiation (El-Jack et al. 1999, Wu et al. 1999, Rosen et al. 2002, Zuo et al. 2006). The expression of C/EBPα is upregulated by PPARγ, which in turn promotes PPARγ transcription and substantially induces the expression of other adipogenic genes (Rosen et al. 2002, Letterova et al. 2008, Cho et al. 2009). As a result, C/EBPα deficiency leads to WAT loss and impaired development of BAT, suggesting a critical role of C/EBPα in adipogenesis (Wang et al. 1995). Two other C/EBP family members C/EBPβ and C/EBPδ also play important roles in regulating adipogenesis by activating the transcription of C/EBPα and PPARγ, particularly in early state of adipocyte differentiation (Tanaka et al. 1997, Tang et al. 2004, Zhang et al. 2004).

PRDM16, a zinc-finger transcriptional co-regulator, has been shown to drive brown adipocyte differentiation and repress myogenesis (Seale et al. 2008, Kajimura et al. 2009). Unlike PPARγ and C/EBPs, the key transcriptional factors of all types of adipocytes, PRDM16, functions as a key driver of brown adipocytes (Rosen & MacDougald 2006). PRDM16 selectively initiates the switch of myoblasts to brown adipocytes by forming a transcriptional complex with C/EBPβ. PRDM16 concurrently suppresses white adipocyte-specific genes by forming complexes with C-terminal binding proteins CTBP1 and CTBP2 (Kajimura et al. 2009). On the other hand, CTBPs can be displaced through the recruitment of PPARγ co-activators PGC1α and PGC1β, which leads to activation of brown-fat-specific genes (Kajimura et al. 2008). Although both PRDM16 and PGC1α are the main regulators of brown and beige adipocytes, PGC1α is required for the expression of mitochondrial biogenesis and thermogenic genes but not for differentiation genes, indicating that PGC1α is essential for thermogenesis rather than adipogenesis (Uldry et al. 2006). Furthermore, adipogenesis is regulated by multiple other factors including positive regulators such as BMPs and early B-cell factor (O/E1), and negative regulators such as transforming growth factor β (TGF-β) and preadipocyte factor 1 (Pref1) (Choy & Derynck 2003, Wang et al. 2006, Jimenez et al. 2007, Margoni et al. 2012). In addition, recent studies have also shown that miRNAs and long noncoding RNA (lncRNA) play important roles in regulating adipogenesis (Park et al. 2015). However, the intracellular mechanisms underlying the role of these factors in regulating adipogenesis remain to be fully understood.

**Adipose tissue as an energy storage organ**

As an energy storage organ, adipose tissue stores TGs and releases fatty acids through lipogenesis and lipolysis, respectively. Systemically, feeding stimulates the lipogenic pathway and storage of TGs in the adipose tissue, while fasting induces the activation of lipolytic pathway and promotes the breakdown of TGs and release of fatty acids from adipose tissue. Lipogenesis is the process that encompasses *de novo* fatty acid synthesis from acetyl-coenzyme A (acetyl-CoA) and TG biosynthesis. Glucose provides its own metabolite acetyl-CoA as the substrate for *de novo* synthesis of fatty acids, induces the expression of acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of lipogenesis and stimulates the release of pancreatic insulin which promotes lipogenesis (Fig. 1). As a result, insulin stimulates glucose uptake in the adipocytes, activates glycolytic and lipogenic enzymes, and stimulates the expression of lipogenic gene sterol regulatory element-binding protein 1 (SREBP1) that controls the expression of genes required for cholesterol, fatty acids, TG and phospholipid synthesis (Assimacopoulos-Jeannet et al. 1995, Ferre & Foufelle 2007). In addition to SREBP1, another transcriptional factor carbohydrate response element-binding protein (ChREBP) promotes *de novo* lipogenesis (DNL) gene expression and has been shown to modulate both lipid and glucose metabolism in adipose tissue and substantial whole-body insulin sensitivity (Herman et al. 2012, Eissing et al. 2013). However, under normal conditions, DNL is relatively low in WAT compared with liver and BAT in rodents and even lower in humans (Swierczynski et al. 2000, Letexier et al. 2003). The fatty acids used for TGs biosynthesis in adipocytes are...
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Teglund (1999), Angiopoietin-like 4 (Angptl4) has been shown to decrease intracellular fatty acids and promote the biosynthesis of triacylglycerols (TGs). Importantly, glycerol but not fatty acids are released during lipolysis (Fig. 1). ASP has been shown to increase TGs synthesis by activating DGAT and induce subcutaneous fat storage in females (Haagsman et al. 1982, Yasruel et al. 2011). Taken together, adipose tissue as a fuel reservoir, plays a vital role in buffering fluxes of fatty acids, lipotoxicity and insulin resistance as well as regulating clearance of plasma TGs and keeping it from being deposited in other tissues (Frayn 2002). In other words, the storage capacity of lipid in adipose tissue is a determinant of systemic insulin resistance and lipid infiltration into other tissues such as liver and muscle.

Opposite to lipogenesis, lipolysis is the catabolic process leading to the breakdown of TGs stored in adipocytes and subsequently the release of free fatty acids (FFA) and glycerol that can be either oxidized or used for gluconeogenesis and free fatty acids for oxidation according to energy needs in other organs (Kuriyama et al. 2001). Lipolysis is induced by fasting and supplies glycerol for hepatic gluconeogenesis and free fatty acids for oxidation according to energy needs in other organs (Kuriyama et al. 2000). Importantly, glycerol but not fatty acids can be used as a substrate for gluconeogenesis in the liver. In the state of high fatty acid and diminished carbohydrate availability, fatty acids can be further broken down to produce a group of substances collectively known as ketone bodies providing for the brain, which is the process called ketogenesis in the liver. Several hormones have been shown to regulate lipolysis.
the lipolytic pathway. During fasting, decreased circulating levels of insulin result in suppression of lipogenesis as well as activation of the lipolytic pathway. Consistently, elevated circulating glucagon during fasting is also responsible for the activation of cAMP-dependent protein kinase A (PKA) pathway and lipolysis in adipocytes. Meanwhile, catecholamine released by sympathetic nervous system (SNS) is also stimulated by fasting, binds to β-adrenoceptor, then activates PKA and lipolytic pathways (Carmen & Victor 2006). Lipolysis consists of lipase-based breakdown of tri-, di- and monoacylglycerides (MGs) into individual fatty acids. Adipocyte triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are the first two primary lipases for lipolysis and separately responsible for the conversion of TGs to diglycerides (DGs) and hydrolysis of DGs to MGs (Haemmerle et al. 2002, Zimmermann et al. 2004). Lipid droplet-associated proteins such as perilipin is polyphosphorylated by PKA and then translocates HSL to the lipid droplets for lipolysis (Marcinkiewicz et al. 2006, Brasaemle 2007, Lafontan & Langin 2009). Furthermore, recent studies also show that cell death-inducing DNA fragmentation factor 45-like effector family proteins (Cides), including Cidea, Cideb and Cidec (Fsp27) play critical roles in the control of lipid droplet morphology and have a distinct function in adipocytes and hepatocytes (Gong et al. 2009). In line with this, both Cidea and Cidec localize on the surface of lipid droplets, particular LD-LD contact sites, and promote atypical LD fusion and growth by lipid exchange and transfer in adipocytes (Gong et al. 2011, Wu et al. 2014, Barneda et al. 2015). Although some studies show that ATGL and HSL are responsible for basal lipolysis and PKA-stimulated lipolysis, respectively, in adipocytes, the physiological function of ATGL and HSL remains controversial (Birnbaum 2003, Langin & Arnér 2006). It has been shown that global deficiency of ATGL leads to impaired lipolysis, mild obesity and cold intolerance (Haemmerle et al. 2006). Consistent with this, adipose-specific desnutrin/ATGL knockout mice display decreased lipolysis, thermogenesis and fatty acid oxidation despite increased fat mass and improved hepatic insulin sensitivity (Ahmadian et al. 2011). However, global HSL-deficient mice display slightly decreased TGs hydrolysis and increased BAT mass, while exhibiting similar WAT mass, body mass and cold tolerance, suggesting that the compensatory effects of other lipases may exist when HSL is lacking (Osuga et al. 2000). Given that adiposity-induced mobilization of fatty acids from adipose tissue to other organs is a main cause of insulin resistance, inhibition of lipolysis has been considered for the treatment of insulin resistance (Guilherme et al. 2008). However, lipolysis is also closely linked to thermogenesis and energy expenditure by supplying fatty acids for β-oxidation (Haemmerle et al. 2006, Mottillo et al. 2012, Chondronikola et al. 2016). On the other hand, inhibiting adipose lipogenesis by fatty acid synthase deficiency promotes energy expenditure and protects from diet-induced obesity and insulin resistance (Lodhi et al. 2012). These findings suggest that the balance between lipogenesis and lipolysis is critical for maintaining systemic energy homeostasis and insulin sensitivity.

The endocrine function of adipose tissue

In addition to storing energy, adipose tissue exerts an extremely active endocrine function and produces a variety of factors which circulate and regulate systemic metabolism and inflammation (Maury & Brichard 2010, Fasshauer & Bluher 2015) (Fig. 2). Among these factors, adiponectins are defined as those cytokines secreted by adipose tissue. Leptin was the first adipokine to be discovered in 1994 (Zhang et al. 1994), followed by the cloning of adiponectin in 1995 (Scherer et al. 1995). Many other adipokines including resistin, chemerin, apelin, visfatin, plasminogen activator inhibitor 1 (PAI1), monocyte chemoattractant protein 1 (MCP1), tumour necrosis factor alpha (TNFα) and interleukin 6 (IL6) were later discovered (Fasshauer & Bluher 2015). Leptin as well as adiponectin is the adipokine mainly secreted from adipocytes, known as adipocyte hormone, and plays an important role in regulating energy homeostasis (Bluher & Mantzoros 2015). Leptin is also produced or present in other non-adipose organs like stomach, muscle and intestine (Bado et al. 1998, Wang et al. 1998, Sobhani et al. 2000, Hansen et al. 2008). Although resistin was originally described as adipocyte-specific hormone linking obesity and insulin resistance, increasing evidence indicates that resistin is also expressed moderately in mononuclear leucocytes, macrophages and bone marrow cells in humans (Jamaluddin et al. 2012). Similar to resistin, chemerin was thought to be an adipocyte hormone and regulate adipocyte differentiation and lipolysis. However, it is also found in other cell types such as endothelial cells (Goralski et al. 2007, Mattern et al. 2014). Adipose-resident immune cells and endothelial cells are the main sources of other adipokines including apelin, visfatin, PAI1, MCP1, TNFα and IL6 (Loskutoff &
Dysregulated production or secretion of these adipokines causes adipose tissue dysfunction and is implicated in obesity-induced inflammation and insulin resistance.

**Leptin**

Leptin, a satiety hormone of 16-kDa peptide encoded by the obesity (ob) gene regulates energy balance by inhibiting hunger (Zhang et al. 1994, Halaas et al. 1995, Caro et al. 1996). The satiety effect of leptin is achieved by passing the blood–brain barrier and targeting the hypothalamus, a primary hunger centre regulating food intake and body weight to regulate adipose tissue mass by decreasing food intake and modulating glucose and fat metabolism (Zhang et al. 1994, Trayhurn et al. 1998, Dieguez et al. 2011, Morton & Schwartz 2011). In support of this, mice with deficiency of leptin expression (ob/ob) or receptor function (db/db) display increased food intake/hyperplasia, decreased energy expenditure and severe early onset obesity (Coleman 1978, Zhang et al. 1994, Lee et al. 1996). Along this line, administration of recombinant leptin in rodents suppresses food intake and promotes energy expenditure and weight loss, despite the fact that humans do not demonstrate the same dramatic results (Halaas et al. 1995, Pelleymounter et al. 1995, Heymsfield et al. 1999, Westerterp-Plantenga et al. 2001).

Leptin action in hypothalamus is mediated by leptin receptor and downstream signalling pathways including JAK2/STAT3 pathway (Sahu 2003). By binding to its receptors, leptin inhibits orexigenic neurons such as neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons (Schwartz et al. 1996, Arvaniti et al. 2001). Moreover, leptin controls feeding by regulating multiple orexigenic neuropeptides including NPY, AgRP, melanin-concentrating hormone (MCH), galanin, orexin and galanin-like peptide (Schwartz et al. 1996, Sahu 1998, Lopez et al. 2000, Meister 2000, Arvaniti et al. 2001, Kumano et al. 2003). In addition, the satiety effect of leptin is also mediated by regulation of anorexigenic peptides such as POMC, cocaine- and amphetamine-regulated transcript, neurotensin, corticotropin-releasing hormone and brain-derived neurotrophic factor (BDNF) (Golden et al. 1997, Sahu 1998, Meister 2000, Liao et al. 2012). Although the central effect of leptin has been well studied, accumulating studies show that leptin also plays an important role in directly regulating the function of peripheral organs like reproductive organs (Moschos et al. 2002, Perez-Perez et al. 2015). Leptin targets pancreatic β-cell to modulate glucose homeostasis through producing effects on β-cell mass and insulin expression and secretion (Marroqui et al. 2012). Moreover, leptin regulates the immune response including both adaptive and innate immune cells that links to metabolic adaption (Naylor & Petri 2016). Furthermore, some studies show that leptin increases energy expenditure given that ob/ob mice display hypo-metabolic rate and decreased oxygen.
consumption, and leptin administration increased energy expenditure when normalized to body weight (Trayhurn et al. 1977, Pelleymounter et al. 1995). However, some of later studies do not support this and argue that the hypometabolism in leptin-deficient mice is only the effect of normalization (Himms-Hagen 1997, Kaiyala et al. 2015, Fischer et al. 2016). When the energy expenditure is not normalized, leptin deficiency leads to increase of oxygen consumption and hyper-metabolism (Himms-Hagen 1997, Kaiyala et al. 2015). However, some groups found that leptin administration has little effect on the energy expenditure without normalization (Ukropec et al. 2006, Fischer et al. 2016). Moreover, other two studies show that leptin treatment has little effect on energy expenditure even when the data is normalized with body mass (Doring et al. 1998, Hogberg et al. 2006). This controversy needs to be further clarified.

As leptin inhibits appetite and promotes weight loss, it has been widely considered as a therapeutic target for the treatment of obesity and its associated disorders. The recombinant leptin has been developed and applied in clinical treatment. However, majority of obese patients exhibit leptin resistance. Thus, treatment with classical leptin may not be of therapeutic potential in the general population (Farooqi et al. 1999, Savage & O’Rahilly 2002, Chou & Perry 2013). Alternatively, the drugs improving leptin sensitivity offer a new way to treat obese patients. Amylin (pramlintide), a potential leptin sensitizer, has been shown to exert a weight-lowering effect together with leptin or leptin analog metreleptin. However, a recent clinical trial suggests that amylin administration causes energy homeostasis (Kaiyala et al. 2004). Adiponectin is present primarily in three species: a low-molecular-weight (LMW) trimer of approximately 67 kDa, a hexamer of ~120kDa and a high-molecular-weight (HMW) multimer of >300 kDa. The HMW adiponectin has been shown to possess the most potent insulin sensitizing activity (Tsao et al. 2002, Waki et al. 2003, Pajvani et al. 2004).

Circulating adiponectin is capable of targeting multiple tissues and regulating insulin sensitivity as well as energy homeostasis. Liver is a primary target tissue of adiponectin, and the action of adiponectin in liver contributes predominantly in its insulin sensitizing effect. It has been shown that adiponectin activates AMP-activated protein kinase (AMPK) and reduces the expression of gluconeogenic enzymes such as phosphoenolpyruvate carboxylase and glucose-6-phosphatase, leading to the suppression of gluconeogenesis (Combs et al. 2004, Nawrocki et al. 2006). In addition, adiponectin is able to enhance the ceramidase activity and suppress hepatic ceramide content by which it improves hepatic and whole-body insulin sensitivity independent of AMPK (Holland et al. 2011). Moreover, adiponectin also exerts its insulin sensitizing effects through modulating the biological actions of growth factors such as platelet-derived growth factor, fibroblast growth factor (FGF) and heparin-binding epidermal growth factor-like growth factor (HB EGF) (Wang et al. 2005). In addition to targeting liver, the globular form of adiponectin (gAd) has been shown to activate the AMPK pathway in skeletal muscle, leading to increased phosphorylation of ACC, fatty acid oxidation and glucose uptake (Tomas et al. 2002, Yamauchi et al. 2002). Besides insulin sensitizing, another functional role of adiponectin is to regulate thermogenesis and energy homeostasis (Masaki et al. 2003, Qi et al. 2004, Kubota et al. 2007, Kajimura et al. 2013, Hui et al. 2015). However, it remains controversial whether adiponectin promotes or suppresses energy homeostasis and thermogenesis. The physiological function and underlying mechanisms of adiponectin in the regulation of energy expenditure need to be clarified in the future.

Adiponectin exerts multiple beneficial effects by binding to its receptors, adiponectin receptor 1 and receptor 2 (AdipoR1 and AdipoR2) (Yamauchi et al. 2003, Kadowaki et al. 2006, Yamauchi et al. 2007). In addition, T-cadherin, a cell surface-anchored glycoprotein, is effective in binding adiponectin and mediates adiponectin signalling, while it falls short of being a receptor that binds to both ligands and transduces intracellular signalling pathways (Denzel et al. 2010). There is accumulating evidence showing that adiponectin exhibits insulin sensitizing effect through multiple signalling pathways downstream of adiponectin receptors, such as AMPK, Ca²⁺, PPARα, ceramide and S1P.
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Savage Patel Chen). Besides, several animal studies, Hotta, ... circulating levels of insulin or glucose (Le Lay et al. 2001, Milan et al. 2002, Lee et al. 2005). Thus, the physiological function of resistin needs to be further clarified in the future.

**FGF21**

Fibroblast growth factor 21 (FGF21) has been defined as a hepatokine, adipokine and myokine and exerts diverse biological functions in metabolism (Hotta et al. 2009, Fisher et al. 2012, Kim et al. 2013). As an adipokine, FGF21 is induced by cold exposure, and in turn promotes thermogenic gene expression in BAT and inguinal WAT (IWAT) (Hondares et al. 2010, Fisher et al. 2012, Adams et al. 2013, Emanuelli et al. 2014, Lee et al. 2014a). FGF receptor 1c and a coreceptor β-klotho have been suggested to mediate the action of FGF21 in BAT and IWAT (Itah 2010, Foltz et al. 2012). Moreover, FGF21 induces browning effect and thermogenic gene expression by upregulating PGC1α through paracrine and/or autocrine mechanisms (Hondares et al. 2011, 2014, Fisher et al. 2012, Lee et al. 2014b). However, it remains unknown whether FGF21 regulates metabolic pathways solely dependent on its action in adipose tissue. Some studies suggest that FGF21 action in WAT mediates its beneficial effect on metabolic parameters such as body weight, glucose homeostasis and plasma TGs (Wu et al. 2011, Veniant et al. 2012). Whereas another study shows that FGF21 requires neither UCP1 nor brite adipocytes to elicit weight loss and improve glucose homeostasis (Veniant et al. 2015). In addition, the circulating levels of FGF21 are upregulated in obese and type 2 diabetic patients, suggesting that this paradoxical increase of FGF21 may be a compensatory response or a result from FGF21 resistance (Chen et al. 2008, Zhang et al. 2008). Although FGF21 has drawn growing attention for its anti-obesogenic and antidiabetic actions, the mechanisms underlying FGF21 action need to be established (Emanuelli et al. 2014).

**Thermogenic function of brown and beige adipose tissue**

Brown and beige fat dissipate energy in the form of heat and offer a new way to battle obesity and its associated...
disorders (Lowell & Spiegelman 2000, Cannon & Nedergaard 2004). Different from brown fat with relatively high thermogenic activity and enrichment of UCP1 under thermoneutral condition in rodents, the expression of UCP1 in beige fat is very low under this condition, which may be due to low number of beige adipocytes as well as low expression level of UCP1 in individual beige adipocytes (Wu et al. 2012). However, the UCP1 expression in beige fat is markedly induced upon cold exposure or treatment of agonists of the β3-adrenoceptor or PPARγ (Young et al. 1984, Cousin et al. 1992, Petrovic et al. 2010, Wu et al. 2012). Regardless of cold stimulation, the expression levels of UCP1 protein in beige fat are still relatively lower compared with brown fat where the UCP1 gene is also induced at certain extent (Nedergaard & Cannon 2013). Moreover, the mRNA and protein of UCP1 are differentially altered at some particular conditions, suggesting the importance of protein analysis of UCP1 as a thermogenic marker in the future studies (Nedergaard & Cannon 2013). On the other hand, the thermogenic activity and physical location of brown and beige fat vary in different species. It has been shown that the basal levels of UCP1 in human brown fat is not as enriched as that in rodents under thermoneutral conditions (Wu et al. 2012). Moreover, rodent brown and beige fat are physically apart from each other, while they are mixed in humans (Xue et al. 2005, Xue et al. 2007, Sharp et al. 2012, Cypess et al. 2013). The SNS plays a predominant role in cold-induced thermogenesis and browning of white fat by releasing norepinephrine (NE). Upon cold, feeding or stress exposure, NE is produced and released from sympathetic fibres innervated in adipose tissue to bind to β3-adrenoceptor on the cell surface of adipocytes (Ueta et al. 2012). This binding results in activation of cAMP/protein kinase A (PKA) pathway which induces lipolysis and thermogenic genes expression and substantially activates brown and beige adipocytes (Cao et al. 2004b, Ye et al. 2013). In adult humans, BAT activation by prolonged cold exposure appears to increase lipid mobilization from other fat depots to BAT and promotes lipid burning through heat production in mitochondria (Chondronikola et al. 2016). Brown and beige fat not only maintains energy balance through non-shivering thermogenesis but also promotes glucose uptake and TGs clearance from the circulation (Bartelt et al. 2011). In line with this, ablation of UCP1 results in disruption of diet-induced thermogenesis and exacerbation of diet-induced obesity in mice exempt from thermal stress by living at thermoneutrality (Feldmann et al. 2009). Furthermore, the implantation of human beige adipocyte acquired from beige progenitors into high-fat diet (HFD)-fed mice improves systemic glucose tolerance (Min et al. 2016).

The therapeutic potential of brown and beige fat for the treatment of obesity and diabetes

Accumulating data has shown that activation of brown and beige adipocytes protects against obesity and related metabolic diseases in rodents (Koppey et al. 1995, Cederberg et al. 2001, Seale et al. 2011). Consistent with this, administration of β3-adrenergic agonist, CL 316,243 that mimics cold stress induces WAT browning and improves obesity-induced metabolic dysregulation in rodents (Ghorbani & Himms-Hagen 1997, Guerra et al. 1998). Although BAT was primarily found in infants (Lidell et al. 2013), it was unknown whether adult humans possess brown fat until Dr Spiegelman, Kahn, Teule and Nuuttila groups identified brown fat in adult humans with PET-CT in 2009 (Cypess et al. 2009, van Marken Lichtenbelt et al. 2009, Virtanen et al. 2009). Nowadays, the BAT has been shown to exist in humans at various ages (Gilsanz et al. 2013). The following studies show that BAT is inversely correlated with body mass index, decreased during ageing and induced upon cold stimulation (Cypess et al. 2009, Lee et al. 2010, Pfannenberg et al. 2010, Jacene et al. 2011, Cypess et al. 2014). Unlike rodent beige adipocytes physically separated from brown adipocytes, adult human brown adipocytes are found together with beige and white adipocytes (Sharp et al. 2012, Wu et al. 2012, Lidell et al. 2013). Despite this, the activated human brown and beige adipocytes have a therapeutic potential against obesity and diabetes.

The inducible characteristics of human brown adipocytes under cold exposure make them the promising therapeutic targets for the treatment of obesity and type 2 diabetes. Cold training has been studied as a viable anti-obesity therapeutic. It has been shown that mild cold exposure is sufficient to increase human BAT activity and energy expenditure, and prolonged (5–8 h) cold exposure in BAT-positive individuals significantly increases resting energy expenditure, whole-body glucose metabolism and insulin sensitivity (Chen et al. 2013, Chondronikola et al. 2014). Moreover, BAT recruitment by repeated cold exposure or daily ingestion of capsinoids increases energy expenditure and decreases body fat mass in healthy individuals who have lower BAT activity originally compared with control subjects (Yoneshiro et al. 2013). These findings again suggest the potential role of human BAT in the prevention and treatment of obesity.
In addition, administration of β3-adrenoceptor agonists also activates BAT and promotes energy expenditure both in humans and rodents despite low efficacy in humans compared with rodents (Cannon & Nedergaard 2004, Cypress et al. 2012, Carey et al. 2013, van der Lans et al. 2013). However, traditional β3-adrenoceptor agonists such as CL316,243, L-796568 and TAK-677 were not approved for use in clinical trials due to multiple side effects including the increased heart rate (Arch 2011, Cypress et al. 2015). Mirabegron, a new class of β3-adrenoceptor agonists approved for the treatment of overactive bladder, has been shown to promote human BAT thermogenesis with great therapeutic potential (Cypress et al. 2015). Recent studies have also shown that BAT transplantation corrects metabolic phenotypes, and improves type 1 diabetes in streptozotocin-treated mice as well as HFD-induced insulin resistance in mice (Gunawardana & Piston 2012, Stanford et al. 2013). Consistently, implantation of human beige adipocytes improves systemic glucose tolerance in HFD-induced glucose-intolerant mice (Min et al. 2016). Given that preadipocytes or stem cells are also able to be differentiated into mature brown or beige adipocytes, brown or beige adipocytes transplantation may offer a practicable means for the treatment of obesity and diabetes (Bayindir et al. 2015). Therefore, understanding the nature and expansion of human brown or beige adipocytes is urgently needed for BAT-based therapeutics in the future.

Regulation of non-shivering thermogenesis
Thermogenesis and browning of WAT are predominately controlled through SNS (Himms-Hagen et al. 1994, Murano et al. 2009, Richard et al. 2012, Bi & Li 2013) (Fig. 3). Central nervous system (CNS), especially hypothalamic neurons project to the SNS and drive sympathetic outflows to adipose tissue to modulate adaptive thermogenesis (Bamshad et al. 1999, Cano et al. 2003). Several specific hypothalamic areas or neural circuits within the hypothalamus have been shown to exert thermoregulation through modulating the activity of SNS (Oldfield et al. 2002, Cao et al. 2004b, Contreras et al. 2015). In line with this, NPY/AgRP signalling derived from the arcuate nucleus (Arc) inhibits sympathetically innervated BAT thermogenesis through regulation of tyrosine hydroxylase neurons in the hypothalamic paraventricular nucleus (PVN), while alpha-melanocyte-stimulating hormone (α-MSH) from POMC neurons increases SNS activity and BAT thermogenesis (Yasuda et al. 2004, Shi et al. 2013). Furthermore, Ruan et al. reported that O-GlcNAc transferase (OGT), a rate-limiting enzyme for O-linked β-N-acetylglucosamine (O-GlcNAc) modification of cytoplasmic and nuclear proteins, suppresses browning of WAT and thermogenesis by regulating neuronal excitability in AgRP neurons (Ruan et al. 2014). In addition to sympathetic neurons, serotonin (5-HT) neurons were recently reported to recruit and activate brown and beige adipocytes and subsequently regulate glucose and lipid homeostasis (McGlashon et al. 2015). These findings strongly suggest that CNS plays a critical role in sensing cold, feeding and stress and regulating adaptive thermogenesis through SNS. However, the adverse cardiovascular effects of SNS activation have raised a concern for its therapeutic purpose for the treatment of obesity and its associated diseases.

Given that UCP1 is the key marker for browning of WAT and thermogenesis, concerted effort has been focusing on the understanding of the transcriptional regulation of UCP1 expression in the past decade. Several transcriptional factors including PRDM16, PGC1α, PPARγ, C/EBPβ and interferon regulator factor 4 (IRF4) have been discovered to promote UCP1 expression (Kelly et al. 1998, Barbera et al. 2001, Seale et al. 2007, Kajimura et al. 2009, Kong et al. 2014) (Fig. 3). PRDM16 drives the cell fate switching from skeletal myoblasts to brown adipocytes and initiates brown adipocyte differentiation by binding to PPARγ and PGC1α and β (Seale et al. 2008). Interestingly, PRDM16 is an important factor for thermogenic program, while it is dispensable for brown fat development (Seale et al. 2011, Harms et al. 2014). Kajimura and coworkers also showed that formation of PRDM16/C/EBPβ complex is required for the activation of thermogenic program and induction of PPARγ, PGC1α and UCP1 expression (Kajimura et al. 2009). In support of this, gain-and loss-of-function studies have demonstrated a cell-autonomous and determinant role of PRDM16 in regulating thermogenic programing and the browning effect in subcutaneous adipose tissues, indicating the importance of PRDM16 in driving thermogenic programing (Seale et al. 2011, Cohen et al. 2014). On the other hand, PGC1α is induced upon cold exposure and modulates the expression of thermogenic markers such as UCP1 (Kleiner et al. 2012). Consistent with this, PGC1α is upregulated by FGF21 and mediates the prompting effect of FGF21 on browning of WAT (Fisher et al. 2012). Moreover, IRF4 as the key thermogenic transcriptional partner of PGC1α promotes thermogenic genes expression and energy expenditure, suggesting the key role of PGC1α in regulating thermogenesis and WAT browning (Kong et al. 2014). Furthermore, several other intracellular pathways including p38MAPK, mTORC1,
Grb10, mineralocorticoid receptor (MR), and K(+) channel TASK1 have been shown to modulate thermogenic gene expression, browning of WAT and/or lipolysis through β3-adrenoceptor-dependent or independent mechanisms (Cao et al. 2004a, Bordicchia et al. 2012, Armani et al. 2014, Liu et al. 2014, Liu et al. 2016, Pisani et al. 2016) (Fig. 3). More studies are needed to further determine whether the modulation of WAT browning by these pathways is intrinsic or secondary effects.

In addition, several circulating factors such as insulin, leptin and GLP-1 have been identified to promote thermogenesis through targeting hypothalamus neurons and regulating activity of SNS (Shimizu et al. 1987, Rahmouni & Morgan 2007, Sanchez-Alavez et al. 2010, Harlan et al. 2011, Lockie et al. 2012, Beiroa et al. 2014, Dodd et al. 2015) (Fig. 3). Furthermore, central-derived factors also play important roles in regulating SNS activity and thermogenesis (Fig. 3). Hypothalamic BDNF enhances thermogenesis and energy expenditure by acting on PVN and VMH neurons, while NPY in the dorsomedial hypothalamic nucleus (DMH) promotes WAT browning and BAT activity leading to increase of energy expenditure (Wang et al. 2007, Wang et al. 2010, Chao et al. 2011). BMP8B as a factor derived from the hypothalamus promotes energy balance and thermogenesis through activation of AMPK in the key hypothalamic nuclei and subsequent stimulation of sympathetic tone (Whittle et al. 2012). Notably, BMP8B is also produced in BAT and enhances norepinephrine action by regulating p38MAPK/CREB pathway (Whittle et al. 2012). However, which type of cells produces BMP8B in BAT is undetermined. Myokine irisin, encoded by fndc5 gene, is induced by exercise, promotes browning of WAT by increasing beige adipocyte differentiation and UCP1 expression, and augments brown fat thermogenesis in concert with FGF21 in humans upon cold exposure (Bostrom et al. 2012, Lee et al. 2014a, Jedrychowski et al. 2015). However, the induction of irisin by exercise occurs only in a minority of human subjects (Timmons et al. 2012, Pekkala et al. 2013). Therefore, the role of irisin in regulating metabolism has been questioned. Moreover, multiple secreted factors from adipose and other tissues including Slit2, BMP7, Vitamin A derivatives, VEGF, prostaglandins, bile acids, FGF21, natriuretic peptides, catecholamine, enkephalin, and thyroid hormone (triiodothyronine, T3), and intracellular modulators such as Histone Deacetylase 1 (HDAC1) and miRNAs have been identified to regulate thermogenic program (Watanabe et al. 2006, Tseng et al. 2008, Thomas et al. 2009, Lopez et al. 2010, Nguyen et al. 2011, Bordicchia et al. 2012, Fisher et al. 2012, Kiefer et al. 2012, Bagchi et al. 2013, Sun et al. 2014, Brestoff et al. 2015, Park et al. 2015, Li et al. 2016, Svensson et al. 2016) (Fig. 3). However, whether β3-adrenoceptor/PAK signalling pathway mediates the effect of these factors on thermogenic program remains largely unknown.
Conclusions

Adipose tissue plays a major role in the regulation of systemic metabolic homeostasis via its profound effects on energy storage, endocrine function and adaptive thermogenesis. The dysfunction of adipose tissue as a causal factor is linked to obesity and its related disorders. Therefore, understanding adipose tissue biology and pathology is of great importance for the identification of novel and potential therapeutic targets for the prevention and treatment of obesity-related disorders. In particular, enormous evidence of newly discovered endocrine and thermogenic function of adipose tissue strongly suggests that selectively targeting adipose tissue as the therapeutic approach is feasible and practicable. Supportively, multiple adipokines have been found to be potential therapeutic targets. The deficient mice of adipokine leptin (ob/ob) and leptin receptor (db/db) have been widely used in the obesity and diabetes research as well as in other fields. Moreover, accumulating studies in rodents have demonstrated that leptin-based therapy may be a promising strategy for the prevention and treatment of obesity, whereas further research is needed to determine if leptin gene therapy is safe and effective in humans. Furthermore, adiponectin has great potential as a therapeutic target for a variety of obesity-associated diseases. However, preclinical manipulation of circulating adiponectin has been quite challenging due to its complicated multimeric structure and high circulating level at about roughly 3 orders of magnitude greater than most other hormones in humans. Although brown and beige fat have provided another therapeutic approach for the treatment of obesity, the efficacy of β3-adrenergocceptor agonists in humans is relatively lower than that in rodents. Therefore, future studies focusing on adipokines and thermogenesis regulation will be urgently needed for adipose tissue-related therapeutic purpose.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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