

# Transcriptional control and hormonal response of thermogenic fat

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## Abstract

Obesity and its associated metabolic diseases present a major public health problem around the world. The discovery that thermogenic fat is active in adult humans has sparked a renewal of interest in the study of its development and function and in the feasibility of using modulators of thermogenesis to work against obesity. In recent years, it has been shown that there are at least two distinct types of thermogenic fat cells: brown and beige fat. In this review, we discuss the transcriptional mediators of thermogenesis and the signaling molecules that regulate thermogenic cells. We also review the effects of thermogenic fat activation on whole-body metabolic parameters and evaluate the increasing evidence that activating thermogenesis in humans can be a viable method of ameliorating obesity. In these discussions, we highlight targets that can potentially be stimulated or modified in anti-obesity treatments.

## Key Words

- ▶ obesity
- ▶ diabetes
- ▶ brown adipocyte
- ▶ beige adipocyte
- ▶ adaptive thermogenesis

*Journal of Endocrinology*  
(2015) 225, R35–R47

## Introduction

Obesity is a major health problem in the United States and around the world. Over one-third of adults in the United States (Ogden *et al.* 2014) and 11% of adults worldwide are obese (WHO: [www.who.int/mediacentre/factsheets/fs311/en](http://www.who.int/mediacentre/factsheets/fs311/en)). A number of conditions including heart disease, type 2 diabetes, and some cancers are more prevalent in obese individuals, and worldwide ~3.4 million deaths each year are related to obesity (WHO: [www.who.int/mediacentre/factsheets/fs311/en](http://www.who.int/mediacentre/factsheets/fs311/en)). Obesity is characterized by an excessive amount of lipid accumulation in fat cells; as a result there has been a continual effort to find cellular processes and molecular targets in fat that can be manipulated in anti-obesity treatments.

Three types of fat cells have been identified to date (Rosen & Spiegelman 2014). White adipocytes are primarily used for energy storage; they contain a large

lipid droplet and few cellular organelles. In contrast, brown adipocytes are primarily a site for adaptive thermogenesis which, unlike the obligatory thermogenesis that is a natural byproduct of metabolic processes, is activated in response to cold stimulation (Himms-Hagen 1989). Brown adipocytes contain multiple small lipid droplets and a number of mitochondria, which express uncoupling protein 1 (UCP1), a major component of the thermogenic program and a specific marker of thermogenic adipocytes (Cannon & Nedergaard 2004). UCP1 is activated by long-chain fatty acids and increases the conductance of the inner mitochondrial membrane, causing the mitochondria to produce heat at the expense of ATP production efficiency (Fedorenko *et al.* 2012). While overabundance of white fat is the defining characteristic of obesity and contributes to the

development of metabolic disease, brown fat in fact works to counteract obesity by converting chemical energy into heat as opposed to storing it as lipid (Rosen & Spiegelman 2014). Brown fat is primarily found interscapularly in rodents and arises from MYF5<sup>+</sup> stem cells that can also differentiate into skeletal myocytes (Seale *et al.* 2008). 'Beige' or 'brite' fat is a newly identified type of fat that is located in white adipose tissue and arises from MYF5<sup>-</sup> stem cells but has an inducible thermogenic program (Petrovic *et al.* 2010, Wu *et al.* 2012). We will refer to thermogenic cells in white fat depots as beige cells and to the process of the activation of thermogenic fat cells in white fat depots as 'beiging' in this review. It is conceivable that other types of fat exist in addition to white, brown, and beige fat. Recent work on marrow adipose tissue, for example, has begun to characterize the distinctive functions of these unique fat cells that are not seen in currently identified types of fat (Cawthorn *et al.* 2014, Scheller & Rosen 2014).

Adaptive thermogenesis in fat is activated by cold exposure mainly through the signaling of catecholamines secreted by the sympathetic nervous system (Cannon & Nedergaard 2004). The sympathetic nervous system primarily signals through the  $\alpha$  and  $\beta$ 1–3 adrenergic receptors (AR), consequently in  $\beta$ -less mice, which lack all isoforms of the  $\beta$ -adrenergic receptor, the brown fat depot comprises cells with large lipid droplets and blunted UCP1 expression (Bachman *et al.* 2002). The  $\beta$ -ARs are G-protein-coupled receptors that activate adenylyl cyclase by stimulation, and increase the levels of intracellular cAMP. This leads to the phosphorylation of protein kinase A (PKA), which in turn activates the p38 MAP kinase (MAPK) pathway and induces cAMP response-element binding protein (CREB)-mediated upregulation of UCP1 (Cannon & Nedergaard 2004). Studies have begun to show that therapeutics that act centrally can affect thermogenesis, for example a glucagon-like peptide-1 (GLP1) receptor agonist works in the CNS to activate brown adipose tissue and may increase resting energy expenditure in humans (Beiroa *et al.* 2014).  $\beta$ -adrenergic signaling may not be the only pathway for cold-induced activation of thermogenic fat. Ye *et al.* (2013) showed that cultured, mature, adipocytes can upregulate thermogenesis in response to cold exposure, suggesting that there is also a cell autonomous cold-sensing mechanism in fat cells. This implies that there may be unexplored pathways that function in parallel with  $\beta$ -AR signaling to activate cold-induced thermogenesis.

This review focuses on the transcriptional control of thermogenic genes and the signaling beyond the

sympathetic nervous system that regulates both brown and beige fat function. We also discuss the effects that thermogenic fat activation may have on systemic metabolism in humans, and highlight molecules that have begun to be tested as drug targets.

## Transcriptional control of thermogenic fat

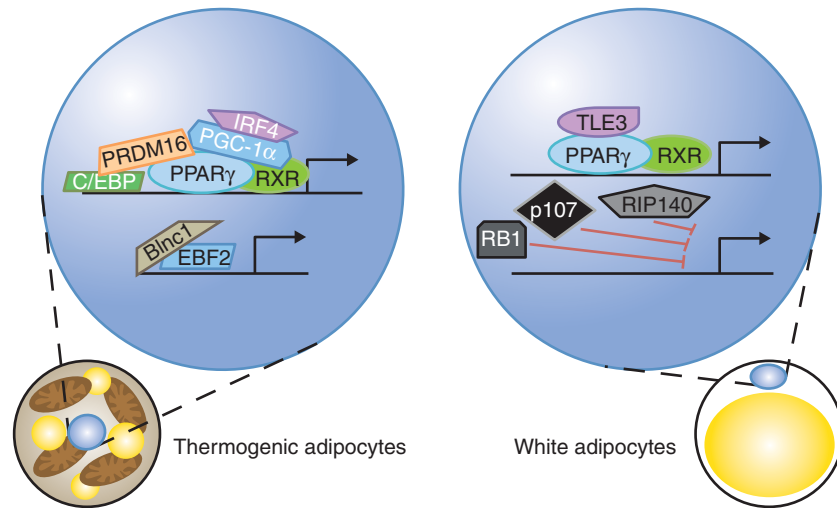
The cascades mediated by a number of transcriptional factors and cofactors tightly control the adipogenic process. The unique mechanisms that regulate thermogenic fat are much less well understood. In the following section, we discuss the factors that contribute to the development of thermogenic cells and the activation of the thermogenic program (Fig. 1).

### Peroxisome proliferator-activated receptor gamma

Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) is the master regulator of adipogenesis; ectopic expression of PPAR $\gamma$  stimulates the differentiation of fibroblasts into adipocytes (Tontonoz *et al.* 1994). PPAR $\gamma$  is a nuclear receptor that heterodimerizes with the retinoid X receptor (RXR) to induce transcription of genes related to the adipogenic program (Ahmadian *et al.* 2013). In addition to its central role in adipogenesis, PPAR $\gamma$  has been shown to be important in the regulation of thermogenesis. Chronic stimulation of adipocyte cultures with PPAR $\gamma$  agonists results in an induction of the thermogenic program (Fukui *et al.* 2000, Wilson-Fritch *et al.* 2004, Petrovic *et al.* 2010). Ongoing research is beginning to elucidate the molecular mechanisms by which PPAR $\gamma$  regulates thermogenesis. A mice model with a point mutation in *Ppar $\gamma$*  was found to have normal development of adipose tissue but has defective thermogenesis (Gray *et al.* 2006) and more recently it has been shown that SIRT1-dependent deacetylation of PPAR $\gamma$  plays a role in the upregulation of thermogenic genes (Qiang *et al.* 2012). It has also been proposed that stabilization of PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16) through the action of PPAR $\gamma$  agonists may contribute to the induction of thermogenesis (Ohno *et al.* 2012). These studies have begun to provide mechanistic insights into our understanding of how PPAR $\gamma$  regulates the function of thermogenic fat.

### PPAR $\gamma$ coactivator 1 alpha

The PPAR $\gamma$  coactivator 1 (PGC1) family of proteins are coactivators that are key inducers of mitochondrial

**Figure 1**

Transcriptional regulators of the thermogenic program. The PPAR $\gamma$ /RXR heterodimer plays a key role in regulating the development of all adipocytes. In thermogenic adipocytes (left), it also plays a major role in the regulation of the thermogenic program. A number of coactivators interact with PPAR $\gamma$ , including PGC1 $\alpha$  which is regulated in part by IRF4, and PRDM16, which interacts with both PPAR $\gamma$  and C/EBP $\beta$  to drive

thermogenesis. Other transcriptional regulators of thermogenesis include EBF2, which forms a ribonucleoprotein complex with *Blincl1* to upregulate thermogenic genes. In white adipocytes (right) the PPAR $\gamma$ /RXR heterodimer instead interacts with TLE3, leading to the expression of white fat-selective genes. RB1, p107, and RIP140 also work in white adipocytes to inhibit the transcription of thermogenic genes.

biogenesis (Puigserver 2005). The first PGC1 protein to be identified is PGC1 $\alpha$ , which was isolated in a yeast two-hybrid screen for PPAR $\gamma$ -interacting proteins in brown fat (Puigserver *et al.* 1998). Both PGC1 $\alpha$  and a closely related family member, PGC1 $\beta$ , are regulators of the thermogenic program. Brown fat cells from *Pgc1 $\alpha$* -knockout animals have a decrease in cAMP-induced thermogenesis, and loss of both PGC1 $\alpha$  and PGC1 $\beta$  in brown fat cells reduces basal levels of thermogenesis (Uldry *et al.* 2006). In addition, while *Pgc1 $\beta$* -knockout mice have a compensatory increase in PGC1 $\alpha$  expression, there is a reduction in thermogenic gene expression in the brown fat of those animals (Lelliott *et al.* 2006). Recently, interferon regulatory factor 4 (IRF4) has been shown to interact with PGC1 $\alpha$  to mediate thermogenesis. In the model of IRF4 overexpression in UCP1- positive cells, thermogenesis is activated in the brown fat of the transgenic animals compared with the controls. In addition, a *Ucp1*-CRE driven *Irf4*-knockout results in cold intolerance and a reduction in thermogenic gene expression (Kong *et al.* 2014). PGC1 $\alpha$  has also been shown to induce the expression of cell death-inducing DFFA-like effector (CIDEA), a regulator of UCP1 function. This interaction is inhibited through direct interaction of PGC1 $\alpha$  with the corepressor receptor-interacting protein 140 (RIP140) (Hallberg *et al.* 2008). RIP140 had previously been reported to inhibit mitochondrial biogenesis and the expression of

thermogenic genes such as *Ucp1* (Christian *et al.* 2005, Powelka *et al.* 2006). PGC1 $\alpha$  is also negatively regulated by retinoblastoma protein (RB1) and its closely related family member p107. RB1 has been shown to suppress PPAR $\gamma$  signaling and promote osteogenic differentiation, and *p107*-knockout mice have increased expression of PGC1A and UCP1 in both brown and white fat depots (Scime *et al.* 2005, Calo *et al.* 2010). These studies show that various interacting proteins can regulate PGC1A function in the mediation of thermogenic gene expression.

#### PRDM1-BF1-RIZ1 homologous domain containing 16

PRDM16 is a zinc finger protein that is an important regulator of thermogenic fat. Ectopic expression of PRDM16 in cultured fibroblasts and *in vivo* results in thermogenic adipocyte differentiation (Seale *et al.* 2007). It was subsequently found that PRDM16 expression in precursor cells determines cell fate, *Prdm16*-knockdown in primary brown fat precursor cells results in the differentiation of those cells into skeletal myotubes while overexpression of PRDM16 in skeletal muscle precursor cells results in brown adipocyte differentiation (Seale *et al.* 2008). The mechanism by which PRDM16 determines precursor cell fate was later shown to be controlled in part by a transcription complex consisting of PRDM16 and CCAAT/enhancer-binding protein  $\beta$  (C/EBP $\beta$ ) (Kajimura

*et al.* 2009). Further *in vivo* models showed that fat specific PRDM16 overexpression results in improved metabolic function and less weight gain in high fat diet-fed mice (Seale *et al.* 2011) and knocking out *Prdm16* results in a loss of the thermogenic program in both brown and beige fat (Cohen *et al.* 2014, Harms *et al.* 2014). Ongoing work is beginning to characterize the transcriptional complex that interacts with PRDM16 to promote thermogenesis (Dempersmier *et al.* 2015).

### Early B-cell factor 2

Early B-cell factor 2 (EBF2) is a helix-loop-helix transcription factor that regulates B lymphocytes and neuronal genes (Hagman *et al.* 1993), and has been shown to regulate adipogenesis (Akerblad *et al.* 2002, Jimenez *et al.* 2007). More recently, EBF2 has also been shown to play a role in the regulation of the thermogenic program in brown and beige adipocytes. A 2013 study used models of *Ebf2*-knockout and overexpression to demonstrate that EBF2 helps to recruit PPAR $\gamma$  to the promoter regions of thermogenic target genes and, with PPAR $\gamma$ , activates the transcription of PRDM16. *Ebf2*-knockout mice present defective brown fat development (Rajakumari *et al.* 2013) and recent studies have indicated that EBF2 may also be involved in the regulation of beige fat function (Wang *et al.* 2014a). The identification of brown fat long noncoding RNA 1 (*Blnc1*) has provided insights into the mechanisms of EBF2 action. *Blnc1* was shown to be an important component of the EBF2 ribonucleoprotein complex (Zhao *et al.* 2014). The adipocytes that over-express BLNC1 express thermogenic genes at a higher level than controls at both basal and stimulated states, underlining the role of the *Ebf2* transcription complex in the regulation of thermogenesis (Zhao *et al.* 2014).

### Transducin-like enhancer of split 3

Transducin-like enhancer of split 3 (TLE3) is a groucho family co-repressor that was identified as a modulator of adipogenesis in a high throughput cDNA screen (Villanueva *et al.* 2011). It was found that PPAR $\gamma$  directly drives *Tle3* expression and that the TLE3 protein binds to PPAR $\gamma$  and uncharacteristically acts as a co-activator to promote differentiation (Villanueva *et al.* 2011). Subsequent work has shown that TLE3 is actually a white fat selective protein; overexpression of TLE3 in fat leads to a decrease in thermogenic gene expression, and a fat-specific *Tle3*-knockout has an increase in the thermogenic response to cold exposure (Villanueva *et al.* 2013).

Co-expression experiments revealed that TLE3 and PRDM16 'compete' to bind to PPAR $\gamma$  and the resulting distinct transcription complexes determine the expression of either lipid storage 'white fat-specific' genes or thermogenic fat specific genes (Villanueva *et al.* 2013).

### SMAD

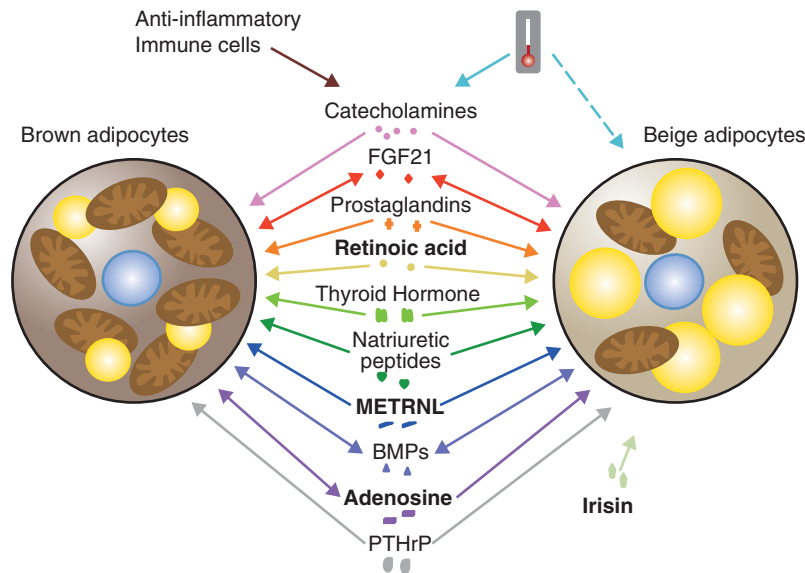
Transforming growth factor  $\beta$  (TGF $\beta$ ) signaling through SMAD proteins has been shown to negatively regulate adipogenesis (Zamani & Brown 2011). A *Smad3*-global knockout mouse is resistant to diet-induced obesity, and there is increased UCP1 expression in the adipose tissue of these animals compared with controls (Yadav *et al.* 2011). Similarly, treating WT animals with exogenous TGF $\beta$ 1 reduced thermogenic gene expression in fat (Yadav *et al.* 2011). Zinc finger protein 423, a transcriptional regulator of SMAD proteins, has been identified as playing a key role in preadipocyte fate commitment (Gupta *et al.* 2010). The therapeutic potential of targeting TGF $\beta$  signaling has been explored using a dominant-negative activin receptor type IIB fusion protein that promotes thermogenesis through binding of TGF $\beta$  and inhibition of downstream signaling (Koncarevic *et al.* 2012).

### Secreted molecules and signaling in thermogenic fat

While sympathetic signaling is the most understood pathway for the activation of adaptive thermogenesis, new research has focused on identifying other secreted factors that can activate thermogenic fat, both to gain a greater understanding of the regulation of the thermogenic program and to identify potential targets for drug discovery (Fig. 2).

### FGF family

Fibroblast growth factor (FGF) family members, such as FGF1 and FGF15/19, have been found to be contributing to the regulation of glucose homeostasis and the beiging of white fat (Fu *et al.* 2004, Jonker *et al.* 2012). FGF21 is the family member most well studied in metabolism; it has been shown to regulate glucose homeostasis, lipid metabolism, insulin sensitivity, ketogenesis, and the prevention of cardiovascular disease (Kharitonov *et al.* 2005, Badman *et al.* 2009, Lin *et al.* 2013, Itoh 2014, Patel *et al.* 2014). FGF21 is mostly produced in and released from the liver; however, thermogenic activation also increases FGF21 expression in subcutaneous and brown adipose tissue (Hondares *et al.* 2011, Fisher *et al.* 2012).

**Figure 2**

The regulation of thermogenic fat cells by secreted factors. A number of secreted factors regulate the thermogenic program in brown and beige cells. Cold exposure induces production of catecholamines from the sympathetic nervous system (solid teal line) and may also activate beige fat through a cell autonomous mechanism (dashed teal line). Anti-inflammatory macrophages can also produce catecholamines. FGF21, PG, RA, NPs, thyroid hormone, METRN, BMPs, adenosine, and PTHrP regulate

thermogenesis in brown and beige cells. In addition, the myokine irisin regulates thermogenesis in beige fat. FGF21, some BMPs, and adenosine have also been shown to be secreted by thermogenic adipocytes. To differentiate the factors that work in concert with central activation of thermogenesis and through  $\beta$ -adrenergic signaling from those that work through independent pathways, these independent factors, RA, adenosine, Irisin, and METRN, have been bolded.

Ongoing research is investigating the differential roles of liver- and adipose tissue-derived FGF21 in the regulation of energy homeostasis (Markan *et al.* 2014). Systemic administration of FGF21 increases the expression of UCP1 (Coskun *et al.* 2008) and genetic ablation of *Fgf21* impairs the ability of animals to adapt to cold exposure (Fisher *et al.* 2012). Though FGF21 is not expressed in the CNS, it can cross the blood–brain barrier to induce sympathetic nerve activity and thus centrally increase thermogenic gene expression and energy expenditure (Owen *et al.* 2014). Recent studies have revealed that adiponectin at least partially mediates the effects of FGF21 on energy expenditure and insulin action (Holland *et al.* 2013, Lin *et al.* 2013). Due to the beneficial effects of FGF21 in metabolism, there has been considerable interest in the development of an FGF21 analog drug, the successful production of which could potentially provide new strategies to improve metabolic health in humans (Kharitonov & Adams 2014).

### COX2 and prostaglandins

Cyclooxygenase-2 (COX2) is an enzyme that synthesizes prostaglandins (PGs) in response to stimuli such as

inflammatory signaling (Ricciotti & FitzGerald 2011). In 2010, two independent studies reported the induction of COX2 in white fat depots upon cold-exposure or  $\beta$ -adrenergic stimulation (Madsen *et al.* 2010, Vegiopoulos *et al.* 2010). Pharmacological inhibition or genetic ablation of COX2 diminishes the cold or  $\beta$ -AR activation-induced beiging of white adipose tissue (Madsen *et al.* 2010, Vegiopoulos *et al.* 2010). Overexpression of COX2 has been shown to increase UCP1 expression in adipose tissue, elevate energy expenditure, and reduce weight gain (Vegiopoulos *et al.* 2010). More recently, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and the enzyme that synthesizes it, microsomal synthase-1 (mPGES1), were shown to play a role in the development of beige fat (Garcia-Alonso *et al.* 2013).

### Retinoic acid

Retinoic acid (RA), the active derivative form of vitamin A, is mainly synthesized intracellularly from retinaldehyde (Rald) by retinaldehyde dehydrogenases (RALDHs) (Niederreither & Dolle 2008). Studies have suggested that RA, Rald, and RALDHs, all play functional roles in the regulation of thermogenic gene expression. It has long been recognized that RA induces UCP1 expression both in

cultured brown adipocytes and in brown adipose tissue (Puigserver *et al.* 1996). In mouse embryonic fibroblast-derived adipocytes, UCP1 expression is highly elevated upon all-trans RA stimulation in a p38 MAPK-dependent manner (Mercader *et al.* 2010). Rald has also been implicated in the induction of the thermogenic program; Plutzky and colleagues have shown that *Raldh1*-knockout mice have elevated levels of Rald and exhibit increased energy expenditure, improved insulin sensitivity, and resistance to diet-induced obesity (Ziouzenkova *et al.* 2007). Later studies demonstrated that the beneficial metabolic effects of Rald signaling likely act through a PGC1 $\alpha$ -mediated pathway (Kiefer *et al.* 2012).

### Thyroid hormone

Thyroid hormones are produced by the thyroid and bind to thyroid hormone receptors (TR)  $\alpha$ 1–2 and  $\beta$ 1–2 to affect to growth and metabolism in target tissues throughout the body, including bone, liver, heart, and fat (Yen 2001). Thyroxine, or T<sub>4</sub> thyroid hormone, comprises the majority of thyroid output and deiodinases at peripheral tissues, such as the liver and kidney, removes the 5' iodine on T<sub>4</sub> to form the metabolically active form of thyroid hormone, triiodothyronine (T<sub>3</sub>) (Yen 2001). T<sub>3</sub> is responsible for increasing the metabolic rate and it has been shown to work in concert with norepinephrine to induce transcription of *Ucp1* in the brown adipose tissue of rats *in vivo* (Bianco *et al.* 1988). Later studies showed that the treatment of primary fetal brown adipocytes from rats with T<sub>3</sub> increases *Ucp1* transcription and stabilizes *Ucp1* mRNA (Guerra *et al.* 1996). The ability of T<sub>3</sub> to induce thermogenesis was shown to be dependent on the TR isoform that it signals through; the TR $\beta$ 1-specific agonist GC1 stimulates UCP1 expression but a TR $\alpha$ 1 agonist does not (Ribeiro *et al.* 2001, Martinez de Mena *et al.* 2010). While thyroid hormone can directly activate the thermogenic program in fat cells, T<sub>3</sub> signaling in the hypothalamus through AMPK also works to activate the CNS to induce thermogenesis via  $\beta$ 3-AR signaling (Lopez *et al.* 2010).

### Natriuretic peptides

The natriuretic peptides (NPs) are a family of cardiac and vascular-derived hormones that regulate sodium homeostasis in blood and urine. There are three main types of NPs; atrial natriuretic peptide (ANP), and B- and C-type natriuretic peptides (BNP and CNP) (Zois *et al.* 2014). In addition, there are two major classes of NP receptors:

NP receptors A and B mediate an intracellular cyclic guanosine monophosphate-dependent signaling cascade, while the NP receptor C (NPRC; NPR3) facilitates the removal of NPs from circulation (Anand-Srivastava 2005). The discovery that NP receptors are expressed in the adipose tissue of rats and humans opened an area of inquiry into the actions of NPs in fat (Sarzani *et al.* 1993, 1996), and it was subsequently shown that ANP and BNP stimulate lipolysis in human adipocytes (Lafontan *et al.* 2008). Recent work on *Nprc*-global knockout mice shows that these animals have increased ANP, reduced fat depot size, and increased thermogenic gene expression (Bordicchia *et al.* 2012). Further studies evidenced both ANP and BNP in the regulation of thermogenesis: ANP was shown to mediate the induction of thermogenic genes through the stimulation of a cGMP/p38 MAPK pathway and the constant delivery of BNP to mice resulted in increased energy expenditure and beiging of white adipose tissue (Bordicchia *et al.* 2012).

### Signaling from immune cells

One consequence of obesity is a change in the macrophage populations seen in adipose tissue from anti-inflammatory M2 macrophages to pro-inflammatory M1 macrophages (Lumeng & Saltiel 2011). This switch in macrophage populations may contribute to the decrease in thermogenesis because catecholamines produced by M2 macrophages can signal through the  $\beta$ -ARs to induce thermogenesis (Nguyen *et al.* 2011, Qiu *et al.* 2014). Recently, it has been shown that other cells associated with the type 2 immune response, specifically type 2 lymphoid cells, can also contribute to the beiging of fat (Brestoff *et al.* 2015, Lee *et al.* 2015). The development of chronic inflammation during obesity leads to upregulation of the noncanonical NF $\kappa$ B target, I $\kappa$ B kinase  $\epsilon$  (IKK $\epsilon$ ) (Chiang *et al.* 2009). The increase in IKK $\epsilon$  results in catecholamine resistance in adipose tissue, which in turn suppresses the induction of UCP1 (Mowers *et al.* 2013). This is consistent with the observation that an *Ikk $\epsilon$* -global knockout mouse has less inflammation, increased energy expenditure, and upregulation of thermogenic gene expression in the visceral depot compared with WT animals (Chiang *et al.* 2009). Treating high fat diet-fed animals with a specific inhibitor of both IKK $\epsilon$  and the related kinase TBK1 results in reduced lipid deposition in brown adipose tissue and an increase in thermogenic gene expression (Reilly *et al.* 2013). These studies provide a model in which the development of obesity leads not only to the loss of a thermogenic signal from type 2 inflammatory cells

but also to the development of chronic inflammation and subsequent resistance to thermogenic signals.

### Myokines (irisin and METRNL)

Induction of PGC1 $\alpha$  in skeletal muscle has systematic benefits including an increase in energy expenditure and prevention of age-related obesity (Puigserver & Spiegelman 2003, Wenz *et al.* 2009). Elevated expression of the protein fibronectin type III domain containing 5 (FNDC5) was seen in a model of skeletal muscle-specific overexpression of PGC1 $\alpha$ . Irisin, the cleaved form of FNDC5, is a secreted hormone released after exercise, which stimulates the beiging of white fat (Bostrom *et al.* 2012, Huh *et al.* 2012). Recently, irisin has been demonstrated to be not only a myokine but also an adipokine that can be secreted from white fat tissue under certain physiological and pathological conditions (Roca-Rivada *et al.* 2013). It has been shown that cold exposure increases the levels of circulating irisin, suggesting that shivering may result in irisin release from muscle and therefore providing another potential physiological mechanism by which irisin stimulates beiging (Lee *et al.* 2014a). In addition to irisin, meteorin-like (METRNL) has also recently been implicated as playing a role in metabolism. METRNL is a hormone that is released from muscle after exercise and from adipose tissue upon cold exposure. Intravenous injections of an adenoviral METRNL construct or direct injection of recombinant protein into mice induces thermogenesis in fat, increases whole-body energy expenditure, and improves glucose tolerance through eosinophil-dependent IL4/IL13 signaling (Rao *et al.* 2014). The identification and characterization of irisin and METRNL provide a model in which myokines released during exercise influence metabolism. The extent to which myokine signaling contributes to the overall metabolic benefits of exercise and how these signals interact with other exercise-regulated pathways await further study.

### Bone morphogenetic proteins

Bone morphogenetic proteins (BMPs) are members of the TGF $\beta$  superfamily and are involved in multiple biological processes in adipose tissue, including enhancing preadipocyte proliferation (Stewart *et al.* 2010), inducing adipogenesis (Jin *et al.* 2006), influencing adipocyte lineage commitment (Huang *et al.* 2009), and regulating thermogenesis (Tseng *et al.* 2008, Whittle *et al.* 2012). BMP7 has been shown to promote brown adipogenesis through the induction of PRDM16 and PGC1 $\alpha$  (Tseng *et al.* 2008).

Another family member, BMP8b, functions in the CNS to increase sympathetic output and therefore increases the response of thermogenic fat to cold exposure through p38 MAPK and CREB signaling (Whittle *et al.* 2012). A *Bmp8b*-global knockout mouse has reduced thermogenic gene expression in adipose tissue compared with controls (Whittle *et al.* 2012).

### Newly discovered factors

More recently, additional secreted factors important to thermogenic fat biology have been reported (Gnad *et al.* 2014, Kir *et al.* 2014, Wang *et al.* 2014b, Crane *et al.* 2015, Fang *et al.* 2015). For want of space, we have briefly discussed only a few of them here.

It has been observed that cachexia, the wasting of adipose and skeletal muscle tissues seen in diseases such as cancer, is associated with the activation of brown fat (Shellock *et al.* 1986). A recent study has found that the thermogenic program is activated in fat cells treated with the conditioned medium from Lewis lung carcinoma (LLC) cells, a well-characterized model of cachexia (Kir *et al.* 2014). Global gene expression analysis of LLC cells identified parathyroid hormone-related protein (PTHrP) as regulating the activation of thermogenesis, probably through the cAMP/PKA pathway (Kir *et al.* 2014). This discovery has begun to provide mechanistic insights into the etiology of the development of cachexia and further studies may suggest treatments that can prevent tissue wasting during disease.

Adenosine has recently been shown to increase lipolysis in primary human and mouse adipocytes (Gnad *et al.* 2014). Adenosine is released from brown fat upon sympathetic stimulation and can signal brown adipocytes to stimulate thermogenesis. The adenosine receptor A<sub>2A</sub> is not highly expressed in white adipose tissue; however, pharmacological activation of A<sub>2A</sub> and viral delivery of A<sub>2A</sub> into the subcutaneous depot of mice both significantly increase beiging (Gnad *et al.* 2014).

Neuregulin 4 (NRG4), a member of the epidermal growth factor (EGF) family, has been recently discovered to be a secreted factor that is released from brown fat (Wang *et al.* 2014b). NRG4 binds to ERBB receptors in the liver and its activation inhibits the SREBP1c-lipogenic pathway through trans-repression of the liver X receptor by STAT5. *In vivo* gain- and loss-of-function studies have shown that NRG4 helps to ameliorate diet-induced obesity and insulin resistance (Wang *et al.* 2014b). These studies suggest that factors released from brown fat can play a role in the regulation of energy expenditure and systemic metabolism.

## Human thermogenic fat

While originally it was believed that the only brown fat in humans was found in newborns and was rapidly lost postnatally, analysis of  $^{18}\text{F}$ -fluorodeoxyglucose positron-emission tomographic and computed tomographic (PET-CT) scans showed that there is active thermogenic fat in some adults (Nedergaard *et al.* 2007). Biopsies of 'hot' areas indicated by PET-CT scans reveal that the fat tissue in the supraclavicular region, as well as in the neck and paraspinal regions, expresses UCP1 (Cypess *et al.* 2009, van Marken Lichtenbelt *et al.* 2009, Saito *et al.* 2009, Virtanen *et al.* 2009). The identity of the thermogenic fat in adults remains uncertain. While the thermogenic fat found in babies has the characteristics of classical brown fat, gene expression analyses performed on adult thermogenic fat have shown the presence of genes that are thought to be beige specific (Sharp *et al.* 2012, Wu *et al.* 2012, Lidell *et al.* 2013, Lee *et al.* 2014b), suggesting that they might be beige fat. Other studies have suggested that human thermogenic fat tissue may be a mixture of brown and beige cells (Cypess *et al.* 2013, Jespersen *et al.* 2013).

Recent work has indicated that thermogenic fat may play an important, active, metabolic role in humans (Table 1). Multiple studies have shown that the presence of thermogenic fat is negatively correlated with age and BMI (Cypess *et al.* 2009, van Marken Lichtenbelt *et al.* 2009, Saito *et al.* 2009, Lee *et al.* 2010, Ouellet *et al.* 2011). There is an increase in the amount of detectable thermogenic fat in patients who underwent significant weight loss after gastric bypass surgery, suggesting that the decrease in the amount of thermogenic fat seen during obesity can be reversed (Vijgen *et al.* 2012). It has also been shown that environmental temperature modulates the amount of detectable thermogenic fat in adults (van Marken Lichtenbelt *et al.* 2009, Saito *et al.* 2009, Virtanen *et al.* 2009). Furthermore, acute cold exposure increases resting metabolic rate more in individuals who have thermogenic fat visible on PET-CT scans compared with individuals without detectable thermogenic fat (Yoneshiro *et al.* 2011a, 2013, Ouellet *et al.* 2012, Chen *et al.* 2013, van der Lans *et al.* 2013), this is in line with reports that there is increased glucose and fatty acid uptake in supraclavicular fat depots in response to cold

**Table 1** Thermogenic fat in humans correlates with physiological and environmental factors and influences metabolic parameters

|   | Thermogenic fat                            | Sample size (max) | Age (range of means) | Sex | References  |
|---|--|-------------------|----------------------|-----|---|
| Age   | Negatively correlated                      | 4842              | 28–62                | ♀♂  | Au-Yong <i>et al.</i> (2009), Cypess <i>et al.</i> (2009), Saito <i>et al.</i> (2009), Lee <i>et al.</i> (2010), Pfannenber <i>et al.</i> (2010), Ouellet <i>et al.</i> (2011), Yoneshiro <i>et al.</i> (2011b), Chen <i>et al.</i> (2013) and Orava <i>et al.</i> (2013)   |
| BMI   | Negatively correlated                      | 4842              | 24–62                | ♀♂  | Au-Yong <i>et al.</i> (2009), Cypess <i>et al.</i> (2009), van Marken Lichtenbelt <i>et al.</i> (2009), Saito <i>et al.</i> (2009), Lee <i>et al.</i> (2010), Pfannenber <i>et al.</i> (2010), Ouellet <i>et al.</i> (2011), Yoneshiro <i>et al.</i> (2011b), Vijgen <i>et al.</i> (2012), Orava <i>et al.</i> (2013), Yoneshiro <i>et al.</i> (2013) and Matsushita <i>et al.</i> (2014) |
| Cold exposure and environmental temperature               | Positively correlated                      | 4842              | 23–62                | ♀♂  | Au-Yong <i>et al.</i> (2009), Cypess <i>et al.</i> (2009), van Marken Lichtenbelt <i>et al.</i> (2009), Saito <i>et al.</i> (2009), Virtanen <i>et al.</i> (2009), Orava <i>et al.</i> (2011), Ouellet <i>et al.</i> (2011, 2012), Yoneshiro <i>et al.</i> (2011a), Cypess <i>et al.</i> (2012), Vosselman <i>et al.</i> (2012) and Chen <i>et al.</i> (2013)                             |
| Respiratory exchange ratio and nonshivering thermogenesis | Increased by activation of thermogenic fat | 27                | 23–40                | ♀♂  | van Marken Lichtenbelt <i>et al.</i> (2009), Orava <i>et al.</i> (2011), Yoneshiro <i>et al.</i> (2011a), Vijgen <i>et al.</i> (2012) and van der Lans <i>et al.</i> (2013)   |
| Glucose homeostasis and insulin sensitivity               | Improved by activation of thermogenic fat  | 12                | 21–45                | ♂   | Chondronikola <i>et al.</i> (2014) and Lee <i>et al.</i> (2014c)  |



exposure (Orava *et al.* 2011, Ouellet *et al.* 2012). Most excitingly, it has recently been shown that the accumulation of thermogenic fat in response to cold exposure results in improvements in insulin sensitivity and glucose homeostasis (Chondronikola *et al.* 2014, Lee *et al.* 2014c) as well as a decrease in body weight (Yoneshiro *et al.* 2013). These studies indicate that human thermogenic fat is a viable target for anti-obesity and anti-diabetic treatments.

## Concluding remarks

In the long pursuit of better understanding and more effective therapeutics for metabolic disease, we have become aware that many of these disorders are polygenic and multifactorial, suggesting that the ultimate solution demands a thorough knowledge of all cell types involved, and of both cell autonomous regulation and intercellular communication. Increasing appreciation has been directed toward the role of adipose tissue in this complicated network. For two decades, since the cloning of leptin (Zhang *et al.* 1994) and the discovery that fat tissue can generate inflammatory cytokines in obesity (Hotamisligil *et al.* 1993), the endocrine function of adipose tissue has been studied in detail and is relatively well understood. It has only been in the last decade that researchers and clinicians in the metabolic field have begun to recognize the potential influence of thermogenic fat cells on whole-body metabolism. We have made great advances in our understanding of how these cells are regulated both transcriptionally and by circulating factors and our knowledge of how these cells contribute to human metabolism is growing. Ongoing research is continually uncovering new methods to target these cells and recent studies have begun to show that some therapeutics already in clinical use, such as the mineralocorticoid receptor antagonist spiro lactone and the GLP1 receptor agonist liraglutide, may also be able to stimulate thermogenic fat (Armani *et al.* 2014, Beiroa *et al.* 2014). With this knowledge, we can hopefully soon develop treatments that target thermogenic fat to fight against obesity and associated conditions.

### Declaration of interest

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

### Funding

Work in the Wu laboratory is supported by grants K01DK094824 and R03DK100698 from the National Institute of Diabetes and Digestive and

Kidney Diseases of the National Institutes of Health, a Young Investigator Grant RGY0082/14 from the Human Frontier Science Program, and a Pilot and Feasibility Grant from the Michigan Diabetes Research Center (NIH Grant 2P30-DK020572).

### Acknowledgements

The authors thank the members of the Wu lab for valuable discussions and apologize to those whose work we were not able to cite due to space limitations.

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Received in final form 9 March 2015

Accepted 23 March 2015

Accepted Preprint published online 24 March 2015