Anna Fenzl and Florian W. Kiefer*

Brown adipose tissue and thermogenesis

Abstract: The growing understanding of adipose tissue as an important endocrine organ with multiple metabolic functions has directed the attention to the (patho)physiology of distinct fat depots. Brown adipose tissue (BAT), in contrast to *bona fide* white fat, can dissipate significant amounts of chemical energy through uncoupled respiration and heat production (thermogenesis). This process is mediated by the major thermogenic factor uncoupling protein-1 and can be activated by certain stimuli, such as cold exposure, adrenergic compounds or genetic alterations. White adipose tissue (WAT) depots, however, also possess the capacity to acquire brown fat characteristics in response to thermogenic stimuli. The induction of a BAT-like cellular and molecular program in WAT has recently been termed "browning" or "beiging". Promotion of BAT activity or the browning of WAT is associated with in vivo cold tolerance, increased energy expenditure, and protection against obesity and type 2 diabetes. These preclinical observations have gained additional significance with the recent discovery that active BAT is present in adult humans and can be detected by ^{18}fluor-deoxy-glucose positron emission tomography coupled with computed tomography. As in rodents, human BAT can be activated by cold exposure and is associated with increased energy turnover and lower body fat mass. Despite the tremendous progress in brown fat research in recent years, pharmacological concepts to harness BAT function therapeutically are currently still lacking.

Keywords: brown adipose tissue; obesity; thermogenesis; uncoupling protein-1.

DOI 10.1515/hmbci-2014-0022
Received August 7, 2014; accepted August 11, 2014

Introduction

The prevalence of obesity is increasing at an alarming rate worldwide, and already affects one-third of the world population [1]. Obese people are at high risk for developing complications such as type 2 diabetes, cardiovascular disease, and the metabolic syndrome [2, 3]. Obesity is a consequence of altered energy balance and develops when energy intake exceeds total energy expenditure [4], which is dictated by the basal metabolic rate, physical activity, and thermogenesis [5]. Excess nutrient-derived energy is mainly stored in white adipose tissue (WAT) and leads to the expansion of total body mass [6]. Adipose tissue depots exert distinct local and systemic effects. Due to its unique metabolic function, brown adipose tissue (BAT) has recently been in the focus of metabolism research. BAT, in contrast to WAT, dissipates energy through a process called uncoupled respiration mediated by uncoupling protein-1 (UCP1), resulting in increased fatty acid oxidation and heat production (thermogenesis) [7, 8]. The thermogenic capacity of brown fat has been appreciated particularly in small mammals and infants, where it serves a vital role in maintaining core body temperature [8]. Notably, promoting brown fat function or the acquisition of BAT characteristics within white adipose depots (referred to as "browning" or "beiging") has been shown to protect against obesity and related metabolic complications in animal studies [9–15]. Evidence for a clinical relevance provided the recent discovery of active BAT in adult humans, detected by ^{18}fluor-deoxy-glucose positron emission tomography coupled with computed tomography (FDG-PET/CT) [16–21]. Importantly, active BAT in humans appears to be negatively correlated with body mass index, body fat mass [22–25], blood glucose levels [25, 26] and diabetes status [27].

In this review we will summarize the function of brown and beige adipocytes, their putative developmental origin and their role in energy metabolism. We will highlight recent advances in translational human studies and discuss potential therapeutic approaches for the treatment of obesity.

Body of review

Different types of fat: white, beige and brown

The functional importance of different adipose depots in energy metabolism and nutritional homeostasis mainly

***Corresponding author: Florian W. Kiefer,** Clinical Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna, Waehringer Guertel 18–20, 1090 Vienna, Austria, Phone: +0043-1-40400–43380, Fax: +0043-1-40400-77900, E-mail: florian.kiefer@meduniwien.ac.at
Anna Fenzl: Clinical Division of Endocrinology and Metabolism, Department of Medicine III, Medical University of Vienna, Waehringer Guertel 18–20, 1090, Vienna, Austria

depends on the composition of the various types of adipocytes, classified as white, beige or brown (Table 1).

WAT mainly consists of mature white adipocytes, which contain a single cytoplasmic lipid droplet (unilocular) and a peripherally-located nucleus. White fat can store excess energy in the form of triglycerides that can be released as free fatty acids into the circulation in times of high energy demand [28]. Moreover, WAT serves as a thermal insulator, protects organs against mechanical damage [29] and secretes adipokines that are implicated in inflammation, angiogenesis, and metabolism [30, 31]. WAT can be found at various anatomic locations and possesses distinct metabolic functions. For example, expansion of the visceral WAT is closely associated with inflammation, insulin resistance, and type 2 diabetes [29]; whereas subcutaneous WAT has been shown to be less inflammatory [32–35] but more susceptible to acquiring brown fat characteristics [9, 10, 14, 36–38].

BAT, the major site of non-shivering thermogenesis, was first discovered in hibernating animals and infants, where it helps maintain an adequate core body temperature [39–42]. BAT also differs morphologically from WAT, containing multiple small (multilocular) cytoplasmic lipid droplets, a central nucleus, and a large number of mitochondria [8]. In contrast to WAT, BAT lipids serve primarily as fuel for oxidative phosphorylation and heat production [43], the latter process primarily depends on UCP1 activity [7, 44]. Briefly, free fatty acids released into the cytoplasm by lipolysis of fat droplets are channeled towards the mitochondria via the general activation/carnitine shuttle system. As a result, free fatty acids are β-oxidized to generate NADH, $FADH_2$ and acetyl coenzyme A. Acetyl coenzyme A enters the tricarboxylic acid cycle (TCA) where it produces additional electron carriers (NADH and $FADH_2$) that subsequently donate electrons to the complexes of the electron transport chain, located in the inner mitochondrial membrane. The terminal electron acceptor is molecular oxygen, which is reduced to water. An electrochemical gradient is generated as protons are pumped through the mitochondrial respiratory chain complexes. Finally, ATP is synthesized by the ATP synthase complex, which is driven by the energy of the proton gradient. UCP1, located in the inner mitochondrial membrane of brown adipocytes, acts as a transmembrane protein allowing protons to re-enter the mitochondrial matrix, thereby dissipating the electrochemical gradient that drives ATP synthesis. This process results in the release of significant amounts of chemical energy in the form of heat [8, 45, 46] (see Figure 1).

In addition to classic brown adipocytes, which are typically located in distinct brown fat depots, it has previously been noted that brown adipocytes can also emerge in white adipose depots in response to prolonged cold exposure or β-adrenergic stimulation [8, 12, 13, 47–50]. This phenomenon has recently been termed "browning" or "beiging." Depending upon the stimulus, these recruitable brown adipocytes [51] display the molecular and metabolic characteristics of either white or brown adipocytes

Table 1 Characteristics of white, beige and brown adipose tissue in rodents and humans.

	White adipose tissue	Beige adipose tissue	Brown adipose tissue
Localization			
Rodents	Subcutaneous, intra-abdominal	Predominantly subcutaneous (inguinal, axillary)	Interscapular, perivascular
Humans	Subcutaneous, intra-abdominal	Cervical, parasternal, supraclavicular, para- and prevetebral	Cervical, parasternal, supraclavicular, para- and prevetebral
Developmental origin	Myf5-negative	Myf5-negative	Myf5-positive
Lipid droplets	Unilocular	Uni/multilocular	Multilocular
Mitochondrial content	Low	Low to high	High
Vascularization	Low	Basal: low Stimulated: abundant	Abundant
UCP1 expression	Low	Basal: low Stimulated: high	High
Adaptive thermogenesis	Negative	Basal: low Stimulated: high	High
Marker genes	Leptin, TCF21	CD137 (TNFRSF9), TMEM26, TBX1	LHX8, ZIC1
Energy metabolism	Energy storage	Stimulated: energy dissipation	Energy dissipation
Obesity and diabetes	Positive correlation	Negative correlation (in animal models)	Negative correlation
Activators	High fat diet	Cold, catecholamines, β-adrenergic receptor agonists, FGF21, irisin, METRLN, vitamin A derivatives, BMP4, BMP7, BMP8b, BMP9, etc.	

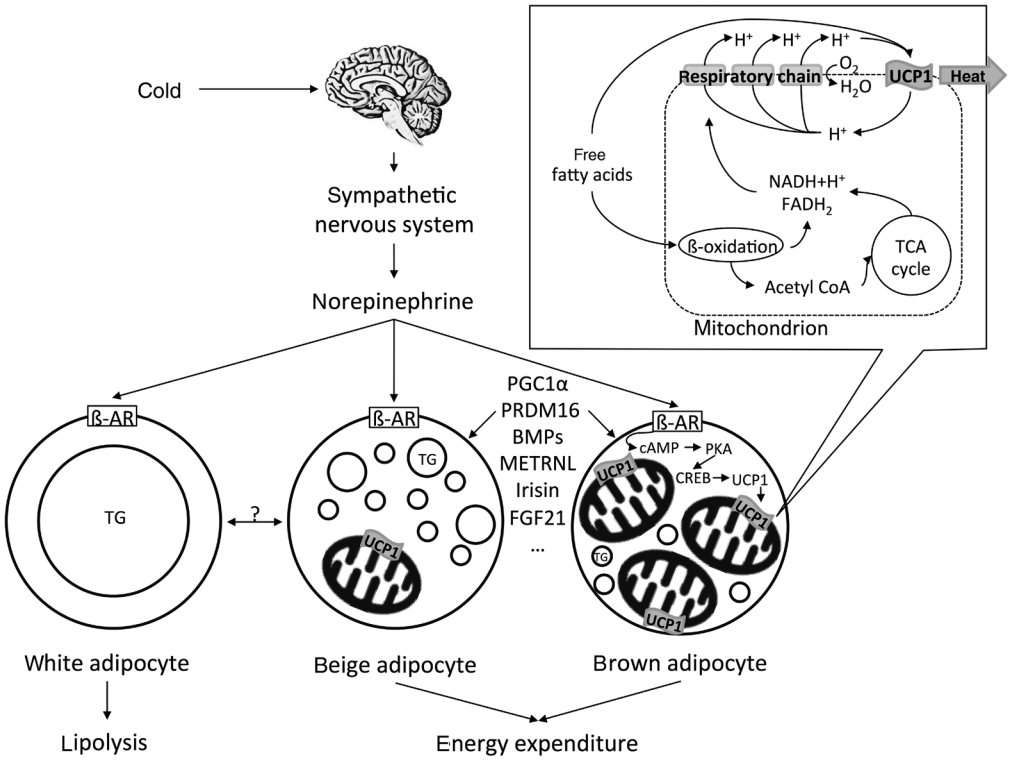

Figure 1 Brown adipose tissue physiology. The activation of the sympathetic nervous system via cold results in the release of norepinephrine. The catecholamine binds to β-adrenergic receptors (β-AR) leading to the transcription of the central thermogenic factor uncoupling protein-1 (UCP1). UCP1, located in the mitochondria, uncouples respiration from the ATP synthesis and thus induces the generation of heat (thermogenesis). In addition to cold-stimulated sympathetic activation, endogenous factors (e.g., PGC1α, PRDM16, bone morphogenetic proteins, irisin, meteorin-like and FGF21) play an important role in the activation of a thermogenic phenotype; however, β-adrenergic stimulation in white adipose tissue results primarily in lipolysis. TG: triglycerides.

and are therefore also named beige [52] or brite adipocytes [53]. These cells possess a high thermogenic capacity and thus significantly contribute to energy expenditure in various in vivo models [9, 12, 35]. In addition to cold and β-adrenergic stimulation, multiple genetic factors have recently been identified to regulate the browning of WAT, highlighting the transcriptional complexity underlying this process [32, 38, 54–58].

Developmental origin of brown and beige adipocytes

The adipogenesis of white and brown adipocytes includes the development of pre-adipocytes from mesenchymal stem cells that further differentiate to mature adipocytes. Adipose mesenchymal stem cells can be committed to either Myf5-negative cells, which differentiate to white adipocytes, or Myf5-positive cells, giving rise to brown adipocytes and skeletal myoblasts [59, 60]. The bi-directional cell fate switch between brown fat and muscle cells has been attributed to the transcriptional regulator PR domain containing 16 (PRDM16) [60]. Although, beige adipocytes derive from Myf5-positive cells, their origin remains a matter of debate and includes two major theories. One implies that brown adipocytes in WAT arise from transdifferentiation or transformation of white adipocytes [10, 48, 61–63]. This theory is supported by lineage tracing of transiently- or permanently-labeled brown and beige adipocytes in transgenic mice showing bi-directional conversion between white and beige adipocytes upon cold or warm conditions [63]. Alternatively, beige adipocytes may arise from distinct progenitor cells by de novo formation [14, 64–66]. These progenitor cells, which express the surface markers CD34, stem cell antigen 1 (SCA1), and platelet-derived growth factor receptor alpha (PDGFRα) were found to give rise to either white or beige adipocytes depending upon the stimuli [65, 67, 68]. Regardless of their exact developmental origin, beige adipocytes constitute a new competent thermogenic cell type [63].

Genetic signature of brown and beige adipocytes

In order to ease the distinction between white, beige, and brown adipocytes, researchers have set out to compare the genetic signature of these three cell types. Combined efforts have revealed a number of "marker" genes for each fat cell type. However, most of this work has been done in mice and it turned out that the expression of these marker genes was far less consistent in human adipocytes. Some genes identified from rodent studies include TCF21 and Leptin for white adipocytes [69]; CD137 (TNFRSF9), TMEM26, and TBX1 for beige [14]; and LHX8 and ZIC1 for brown adipocytes [70]. Using a combination of gene expression analysis and imaging techniques it has been shown that human infants possess classical brown fat in their interscapular and supraclavicular region [71]. However, the genetic profiling of adult BAT has been more challenging and largely depends on the anatomical location of the fat depot. Characterization of human deep neck fat biopsies (a preferred location of BAT in adults) revealed conflicting results, with some authors arguing that human BAT resembles *bona fide* interscapular brown fat in mice and others suggesting it may express the genetic signatures of both, beige and brown adipocytes [14, 21, 70–73]. Possible explanations for such inconsistent reports may be the large inter-individual variability as well as the heterogeneous distribution of brown/beige adipocytes throughout white fat depots in humans. Nonetheless, all authors agree that UCP1-expressing adipocytes do exist in distinct human fat depots, but their presence may depend on various factors including anatomical location, sex, age, environmental conditions, and metabolic state.

Metabolic implications of brown/beige fat activity

The therapeutic potential of both, brown and beige adipocyte activation in the treatment of obesity and diabetes is now essentially settled science, at least in animal models [38, 56, 57]. Numerous genetic factors have been identified that regulate a thermogenic phenotype in mice [74]. Most of these mouse models are characterized by the browning of WAT or promotion of classic BAT function and they share a number of common features: mitochondria density and/or mitochondrial activity is increased and the expression of brown fat markers including *UCP1* is augmented. Substrate utilization is shifted towards fatty acid oxidation and energy expenditure is enhanced. This phenotype can be unmasked under cold conditions or in response to high-fat diet feeding, where mice display cold tolerance and protection against diet-induced obesity, respectively. Consequently glucose metabolism is improved in these models and diabetes progression can be halted [11, 12, 14, 32, 38, 56–58, 75–80].

Transcriptional regulators of brown/beige fat function

Expression of a thermogenic program in adipose tissue is orchestrated by a sophisticated transcriptional machinery that involves a plethora of transcription factors, co-activators, and co-repressors. Several dozen transcriptional regulators have already been identified and the list is constantly growing. The following is a selection of some prototypical factors that have been demonstrated to be potent mediators of brown fat thermogenesis.

PGC1α–PPARγ complex

One of the first identified transcriptional regulators of adaptive thermogenesis was peroxisome proliferator–activated receptor gamma (PPARγ) coactivator 1-alpha (PGC1α) [81]. PGC1α is induced upon cold exposure, exercise or fasting, and exerts its function on adaptive thermogenesis mainly through the nuclear receptor PPARγ but also through the thyroid hormone receptor [81–83]. Overexpression of PGC1α in white adipocytes induces the expression of UCP1 and key mitochondrial enzymes of the respiratory chain [81]. In contrast, fat-specific deletion of PGC1α in mice results in a blunted thermogenic response following cold exposure with decreased core body temperature and attenuated thermogenic gene expression in adipose depots. Although PGC1α is not required for BAT development, it is indispensable for cold- or β-agonist-induced activation of brown adipocytes [84, 85] as well as for the browning of WAT [86]. In addition, adipose PGC1α seems to be critical for glucose and lipid metabolism under obese conditions in mice [86] and defective PGC1α may be linked to an increased susceptibility to insulin resistance and type 2 diabetes in humans [87–89]. This notion is further supported by the observation of reduced PGC1α expression in the adipose tissue of patients with type 2 diabetes [90, 91].

PRDM16

PRDM16 is a recently-identified transcriptional co-regulator that controls the fate between muscle and brown fat cell development. In an elegant study by Bruce Spiegelman's group, overexpression of PRDM16 in myogenic precursor cells was sufficient to reprogram these cells towards brown adipogenesis whereas PRDM16 knockdown forced brown preadipocytes towards muscle cell differentiation [60]. During brown fat development, PRDM16 interacts with PPARα/PPARγ and the CCAAT/enhancer-binding protein beta (C/EBPβ) family [38, 92, 93] leading to the induction of brown-fat genes as well as the repression of selective WAT or skeletal muscle markers [94–96]. Notably, PRDM16 also contributes to the browning of WAT. Transgenic expression of PRDM16 in adipose tissue strongly induces a thermogenic program in subcutaneous but not in visceral WAT, with potent effects on energy expenditure and glucose metabolism. Likewise, transplantation of PRDM16- and C/EBPβ-expressing embryonic fibroblasts into the fat pads of nude mice results in increased thermogenic activity of these fat depots as determined by FDG-PET scans [92]. PRDM16 is also required for PPARγ agonist-mediated white-to-brown fat conversion. The PPARγ agonist rosiglitazone exerts its thermogenic effects in adipocytes through increasing the protein half-life of PRDM16 [96]. Finally, PRDM16 has been shown to bind to and act in concert with PGC1α and PGC1β, both known to be powerful inducers of mitochondrial biogenesis and respiration [82, 97–101].

Retinoids

Another group of potent transcriptional activators of brown fat activation are vitamin A metabolites or retinoids. The main metabolite, retinoic acid, strongly induces UCP1 expression in adipocytes through binding and activation of the nuclear receptors retinoic acid receptor and retinoid X receptor [102–107]. Respective retinoic acid receptor response elements have been identified at the enhancer region of the UCP1 promoter. The in vivo relevance of the vitamin A pathway for energy metabolism has been supported by the observations that retinoid administration in mice is associated with increased adipose UCP1 expression and reduced body weight on a high-fat diet [102, 104, 107, 108]. We have recently shown that retinaldehyde-dehydrogenase 1, the rate-limiting enzyme of retinoid conversion, regulates a thermogenic program in WAT. Retinaldehyde-dehydrogenase 1 deficiency in mice induces a BAT-like transcriptional program, particularly in the visceral WAT which results in cold tolerance, limited body weight gain, and improved insulin sensitivity in response to high-fat diet feeding [56].

Secreted proteins and browning

Recently an interorgan crosstalk between skeletal muscle and WAT was shown to promote browning via a muscle-secreted factor called irisin. Exercise is known to increase the expression of PGC1α in skeletal muscle. Mice with transgenically-increased muscle PGC1α are resistant to diet-induced obesity and diabetes largely due to browning of various white fat depots. The observation that media from PGC1α-expressing myocytes induces the transcription of several brown-fat-specific genes in primary subcutaneous fat cells led to the assumption that a muscle-derived secreted factor, under the control of PGC1α, controls a BAT-like program in white adipocytes. Using a combination of gene expression arrays and bioinformatics approaches, Bruce Spiegelman's laboratory identified a protein called fibronectin type III domain-containing protein 5 (FNDC5) as a putative candidate driving browning in WAT. Upon cleavage, by a yet unknown protease, a secreted polypeptide, named irisin, is formed [54]. Adenoviral overexpression of hepatic FNDC5 resulted in a significant increase of plasma irisin concentrations and the induction of UCP1 expression in WAT paralleled by enhanced energy expenditure, lowered obesity, and improved glucose tolerance in mice. Although in vitro and in vivo thermogenic effects of irisin have been observed in animal studies [54, 76, 109, 110], the physiological significance in humans remains uncertain. In particular, irisin's potential role in human energy metabolism remains controversial since inconsistent results have been published regarding the exercise-dependent regulation of circulating irisin and its association with energy expenditure [111–117].

Fibroblast growth factor 21 (FGF21), another circulating peptide hormone secreted upon exercise and cold exposure in mice [117–120], has recently attracted significant attention due to its ability to regulate energy expenditure and glucose metabolism. Systemic administration and transgenic overexpression of FGF21 leads to weight loss in obese mouse models [121, 122]. Accumulating evidence suggests an important role for FGF21 in the activation of BAT thermogenesis and browning of WAT upon cold exposure involving PGC1α-dependent mechanisms [50, 120, 123]. Notably, induction of hepatic FGF21 expression in new-born mice in response to milk intake seems

to contribute to the thermogenic activation of neonatal BAT [120, 122, 124]. These preclinical observations have been further supported by reports that circulating FGF21 is increased in humans in response to cold exposure and exercise. Stimulation with recombinant FGF21 enhances a thermogenic program in human neck adipocytes and shows synergistic effects with FNDC5. It has therefore been suggested that FGF21 may act in concert with irisin to augment cold- and exercise-mediated brown fat thermogenesis [125]. In summary, FGF21 is now considered a promising new anti-obesity agent based on its beneficial effects on body weight, energy expenditure, lipid mobilization, hepatic and peripheral insulin sensitivity, and hepatic steatosis [122, 126–129].

Bone morphogenetic proteins (BMPs) are a family of secreted molecules that play a role in the differentiation of mesenchymal stem cells and drive the formation and thermogenic activation of BAT [64, 130, 131]. BMP7 activates brown pre-adipocytes in vitro and promotes brown adipocyte differentiation and thermogenesis in vivo [131]. BMP8b is a thermogenic protein that directly regulates energy balance by increasing the cellular response to noradrenaline [130]. Another BMP family member, BMP9, drives brown adipogenesis of human adipose tissue-derived stem cells. In vivo, BMP9 treatment results in browning of subcutaneous WAT, improved glucose tolerance and reduced weight gain [132]. Finally, BMP4 transgenic mice display BAT-like changes in WAT and are therefore protected against diet-induced obesity and diabetes [133].

Immune cells and browning

Adipose tissue is a rich source of immune cells, which play important roles in the metabolic function of distinct fat depots. Depending upon their immunological and metabolic function, two broad classes of immune cells can be distinguished in fat. Neutrophils, mast cells, M1 macrophages, B lymphocytes, CD8+ and CD4+ T-lymphocytes are predominantly pro-inflammatory and thus have detrimental effects on insulin sensitivity and glucose metabolism. The other group includes regulatory T-cells, alternatively activated (M2) macrophages, eosinophil and innate lymphoid cells, which act to contain the inflammatory response, contribute to tissue repair, and ultimately have beneficial effects on insulin sensitivity and the metabolic state [134]. Recently, two cell types among the latter group have been implicated in the regulation of thermogenic processes in WAT, thus linking immune responses to adaptive thermogenesis and energy expenditure. These two cell types are eosinophils and M2 macrophages. Eosinophils are the major source of interleukin-4 (IL-4) in adipose tissue. IL-4 helps polarize macrophages towards the alternatively activated type (M2). Interestingly, cold exposure has recently been shown to promote M2 macrophage activation in adipose tissue in an IL-4/IL-13 dependent manner. Cold-activated macrophages then release catecholamines and induce a local BAT-like phenotype in WAT with effects on whole-body energy metabolism [35]. In contrast, mouse models lacking alternatively activated macrophages or macrophage recruitment due to disrupted chemokine receptor signaling, show a blunted thermogenic response as a result of impaired browning of WAT [33, 35, 135, 136]. The involvement of eosinophil-derived signals in the browning of WAT is further supported by the identification of the circulating factor meteorin-like that is induced in adipose tissue upon cold exposure and in muscle after exercise [33]. Overexpression or the administration of recombinant meteorin-like in mice drives expression of IL-4/IL-13 in adipose tissue together with alternative activation of adipose tissue macrophages, which promote a thermogenic and anti-inflammatory gene program in fat.

Brown adipose tissue in humans

While BAT has long been appreciated as an important player in cold defense in human infants, BAT thermogenesis was thought to be completely blunted during adulthood. This view changed in 2009 when three groups reported independently that active BAT was present in adults and could be activated via cold exposure [16, 18, 20]. Only moderate cold stimulation (14–17°C) for a short period of time (1–2 h) is sufficient to augment glucose uptake in BAT accompanied by enhanced fatty acid oxidation and increased energy expenditure [22, 137, 138]. The observations that BAT activity is negatively associated with human obesity and type 2 diabetes have further propelled clinical research in this field [16, 17, 20, 22, 25–27]. FDG-PET/CT is currently considered the gold standard for measuring BAT activity in humans, which correlates positively with intracellular glucose uptake. However, other techniques including magnetic resonance imaging (MRI) are being investigated as alternative approaches for BAT detection [16, 17, 71, 137, 139–142]. Using FDG-PET/CT scans a typical anatomical pattern of BAT distribution has been identified in humans. Most active BAT is located in the cervical, parasternal, para- and pre-vertebral region [16,

18, 20, 70], (Figure 2). Reports that short-term cold exposure can trigger glucose uptake in distinct fat depots provided the first evidence that thermogenically-active BAT pre-exists in adult humans and that the observed cold-mediated alterations are not dependent on de novo brown fat formation. However, several case studies suggest that the recruitment of brown adipocytes into white fat depots can occur in humans like in rodents. In patients with untreated pheochromocytoma, significant amounts of active brown/beige adipocytes have been found in visceral adipose tissue, which is most likely due to a chronic over-stimulation with catecholamines [143, 144]. A few months after resection of the tumor, FDG positivity of the visceral fat depot was no longer present, consistent with a regression of thermogenically-active brown/beige fat [144]. Some controversy exists regarding the molecular signature of human BAT, with reports demonstrating a gene expression pattern that resembles the classic interscapular brown fat in mice, while others argue that human BAT also displays characteristics of inducible beige fat cells [14, 15, 70–72, 145]. Nonetheless, all authors agree that the promotion of BAT activity drives energy expenditure in humans and thus may counteract obesity. Despite such evidence, recent attempts to harness BAT thermogenesis therapeutically have had little success. Administration of β-adrenergic compounds such as ephedrine or isoproterenol has only shown modest effects on glucose uptake in BAT as determined by FDG-PET scans [144, 146–149]. Irrespectively, the use of such substances is of very limited therapeutic value, given their known side effects on the cardiovascular system. In contrast, moderate cold exposure (17°C) for 6 weeks, 2 h daily significantly enhanced BAT activity and energy expenditure accompanied by a decrease in body fat mass [22]. Nonetheless, effective pharmacological approaches to counteract obesity are still lacking, therefore the search for novel therapeutic strategies targeting brown fat continues.

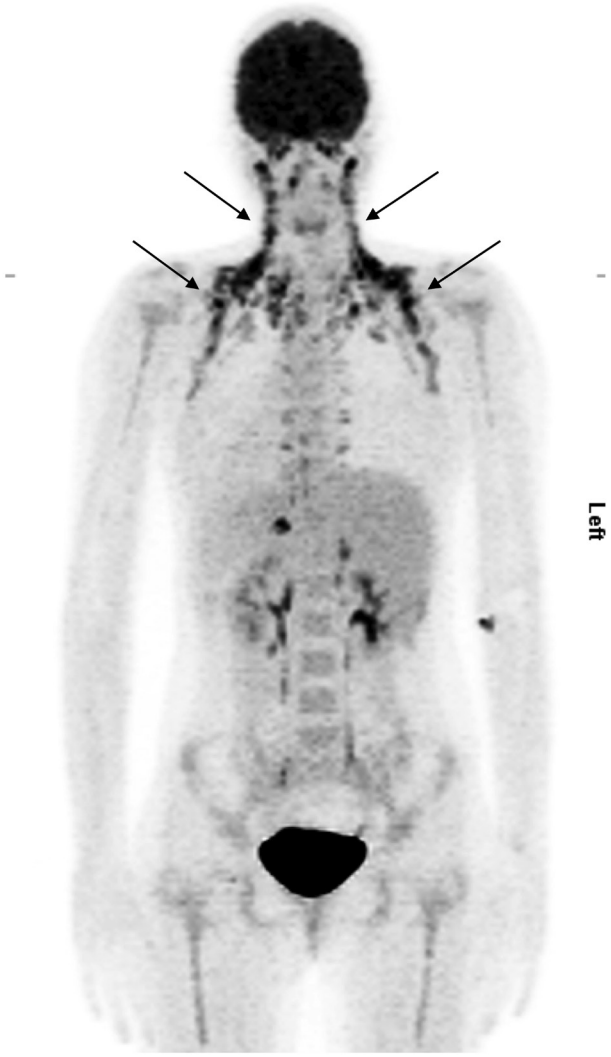

Figure 2 Active brown adipose tissue visualized by ^{18}fluor-deoxy-glucose positron emission tomography coupled with computed tomography (arrows). Provided by the Division of Nuclear Medicine, Department of Radiology and Nuclear Medicine, Medical University of Vienna.

In contrast to such efforts, two recent studies suggest that beige fat thermogenesis may also have deleterious effects under certain circumstances. Increased browning of white fat seems to be a major trigger of tumor cachexia in some cancer models. The pro-inflammatory state (particularly IL-6) along with tumor-derived factors such as parathyroid-hormone-related protein have been discussed as possible mediators of adipose tissue browning that contributes to tumor cachexia [150, 151]. It remains to be shown whether the induction of WAT thermogenesis also plays a part in cachexia associated with other catabolic diseases, such as congestive heart failure, chronic kidney disease or critically ill patients.

Expert opinion

The metabolic effects of thermogenically-active brown fat are indisputable. The important role that brown/beige fat cells play in the regulation of total energy expenditure has now been evidenced in countless preclinical and an increasing number of human studies. With the rise of the obesity and diabetes prevalence, new scientific challenges have evolved and a new era of research, focused on the regulation of energy pathways, has begun. Less than 20 years ago the major thermogenic factor UCP1 was yet to be discovered. A lot has happened since and the entire

field of metabolism research has been heavily influenced by the tremendous progress that has been made over the last decade. Brown/beige fat has lately been the center of attention and due to its metabolic properties it is a legitimate candidate for novel anti-obesity concepts. The impact that brown fat thermogenesis will have on future therapeutic regimens in metabolic diseases is still up in the air but we dare to offer a potential outlook.

Outlook

The incredible developments in brown fat research in the past years are a strong indicator of what we can expect in the near future. The primary goal of many researchers (and of pharmaceutical companies) is the discovery of novel drug candidates that promote brown/beige fat function, energy expenditure, and body weight loss in humans. Despite the fact that a lot more studies will be needed to gain a better understanding of how beige fat conversion and induction of thermogenic responses are regulated in human fat depots, it would not be surprising if the first clincial trials evaluating the effects of novel substances that promote BAT activity will soon be presented. It will be interesting to see whether increased energy expenditure due to enhanced thermogenesis will be associated with increased food intake that may partially counteract the effects on body weight. In such a case, a combination of BAT activity enhancers together with appetite regulators might be the most powerful pharmacological approach to target obesity. In terms of BAT detection in humans, significant progress can be expected involving new imaging techniques. In particular, functional MRI, PET/MRI, dual-energy computed tomography and single-photon emission computed tomography are currently being investigated as alternative approaches to FDG-PET/CT [139, 141, 152]. Also new radiotracers could be developed to improve the sensitivity of PET-based imaging strategies.

Key points

- Brown adipocytes are capable of dissipating energy in the form of heat (thermogenesis) and thus exhibit anti-obesity and anti-diabetic properties.
- Uncoupling protein-1 is the central regulator of thermogenic processes.
- Beige adipocytes can emerge in white fat depots upon certain stimuli (genetic factors, cold or adrenergic stimulation, and exercise).
- Active brown adipose tissue is present in humans, it can be activated by cold, and correlates negatively with obesity.
- Currently no therapeutic agents are available for the activation of brown/beige fat in humans.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA, Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang SX, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014; pii: S0140-67361460460–8.
2. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J. Heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation 2010;121:e46–215.
3. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metab 2011;14:575–85.
4. Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation 2012;126:126–32.
5. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature 2000;404:652–60.
6. Schutz Y. Macronutrients and energy balance in obesity. Metabolism 1995;44(9 Suppl 3):7–11.
7. Klingenberg M. Uncoupling protein – a useful energy dissipator. J Bioenerg Biomembr 1999;31:419–30.
8. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev 2004;84:277–359.

9. Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev 2013;27:234–50.
10. Vitali A, Murano I, Zingaretti MC, Frontini A, Ricquier D, Cinti S. The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. J Lipid Res 2012;53:619–29.
11. Qiang L, Wang L, Kon N, Zhao W, Lee S, Zhang Y, Rosenbaum M, Zhao Y, Gu W, Farmer SR, Accili D. Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Ppar-gamma. Cell 2012;150:620–32.
12. Lim S, Honek J, Xue Y, Seki T, Cao Z, Andersson P, Yang X, Hosaka K, Cao Y. Cold-induced activation of brown adipose tissue and adipose angiogenesis in mice. Nat Protoc 2012;7:606–15.
13. Klingenspor M. Cold-induced recruitment of brown adipose tissue thermogenesis. Exp Physiol 2003;88:141–8.
14. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366–76.
15. Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP. Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. J Clin Invest 1998;102:412–20.
16. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009;360:1509–17.
17. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009;58:1526–31.
18. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P. Functional brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518–25.
19. Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, Scheinin M, Taittonen M, Niemi T, Enerback S, Virtanen KA. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab 2011;14:272–9.
20. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500–8.
21. Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. FASEB J 2009;23:3113–20.
22. Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M. Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest 2013;123:3404–8.
23. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Hoeks J, Schrauwen P, van Marken Lichtenbelt WD. Increase in brown adipose tissue activity after weight loss in morbidly obese subjects. J Clin Encocrinol Metab 2012;97:E1229–33.
24. Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD. Brown adipose tissue in morbidly obese subjects. PLoS One 2011;6:e17247.
25. Lee P, Greenfield JR, Ho KK, Fulham MJ. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2010;299:E601–6.
26. Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M. Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. Int J Obes (London) 2014;38:812–7.
27. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J Clin Encocrinol Metab 2011;96:192–9.
28. Attie AD, Scherer PE. Adipocyte metabolism and obesity. J Lipid Res 2009;50 (Suppl):S395–9.
29. Trayhurn P. Adipocyte biology. Obes Rev 2007;8 (Suppl 1):41–4.
30. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–32.
31. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847–53.
32. Ye L, Kleiner S, Wu J, Sah R, Gupta RK, Banks AS, Cohen P, Khandekar MJ, Bostrom P, Mepani RJ, Laznik D, Kamenecka TM, Song X, Liedtke W, Mootha VK, Puigserver P, Griffin PR, Clapham DE, Spiegelman BM. TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. Cell 2012;151:96–110.
33. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM. Meteorin-like Is a Hormone that Regulates Immune-Adipose Interactions to Increase Beige Fat Thermogenesis. Cell 2014;157:1279–91.
34. Ghoshal S, Trivedi DB, Graf GA, Loftin CD. Cyclooxygenase-2 deficiency attenuates adipose tissue differentiation and inflammation in mice. J Biol Chem 2011;286:889–98.
35. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 2011;480:104–8.
36. Auffret J, Viengchareun S, Carre N, Denis RG, Magnan C, Marie PY, Muscat A, Feve B, Lombes M, Binart N. Beige differentiation of adipose depots in mice lacking prolactin receptor protects against high-fat-diet-induced obesity. FASEB J 2012;26:3728–37.
37. Cousin B, Cinti S, Morroni M, Raimbault S, Ricquier D, Penicaud L, Casteilla L. Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. J Cell Sci 1992;103(Pt 4):931–42.
38. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 2011;121:96–105.
39. Rasmussen JM, Entringer S, Nguyen A, van Erp TG, Guijarro A, Oveisi F, Swanson JM, Piomelli D, Wadhwa PD, Buss C, Potkin SG. Brown adipose tissue quantification in human neonates using water-fat separated MRI. PLoS One 2013;8:e77907.
40. Rasmussen AT. The so-called hibernating gland. J Morphol 1923;38:147–205.
41. Rylander E, Pribylova H, Lind J. A thermographic study of infants exposed to cold. Acta Paediatr Scand 1972;61:42–8.

42. Carter BW, Schucany WG. Brown adipose tissue in a newborn. Proc (Bayl Univ Med Cent) 2008;21:328–30.
43. Cinti S. The adipose organ. Prostaglandins, leukotrienes, and essential fatty acids. 2005;73:9–15.
44. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab 2009;9:203–9.
45. Klingenberg M, Huang SG. Structure and function of the uncoupling protein from brown adipose tissue. Biochim Biophys Acta 1999;1415:271–96.
46. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 1997;387:90–4.
47. Collins S, Daniel KW, Petro AE, Surwit RS. Strain-specific response to beta 3-adrenergic receptor agonist treatment of diet-induced obesity in mice. Endocrinology 1997;138:405–13.
48. Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S. The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 2010;298:E1244–53.
49. Geloen A, Collet AJ, Guay G, Bukowiecki LJ. Beta-adrenergic stimulation of brown adipocyte proliferation. Am J Physiol 1988;254(1 Pt 1):C175–82.
50. Chartoumpekis DV, Habeos IG, Ziros PG, Psyrogiannis AI, Kyriazopoulou VE, Papavassiliou AG. Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. Mol Med 2011;17:736–40.
51. Enerback S. The origins of brown adipose tissue. N Engl J Med 2009;360:2021–3.
52. Ishibashi J, Seale P. Medicine. Beige can be slimming. Science 2010;328:1113–4.
53. Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 2010;285:7153–64.
54. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463–8.
55. Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang C, Takahashi N, Sarzani R, Collins S. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Invest 2012;122:1022–36.
56. Kiefer FW, Vernochet C, O'Brien P, Spoerl S, Brown JD, Nallamshetty S, Zeyda M, Stulnig TM, Cohen DE, Kahn CR, Plutzky J. Retinaldehyde dehydrogenase 1 regulates a thermogenic program in white adipose tissue. Nat Med 2012;18:918–25.
57. Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Berriel Diaz M, Rozman J, Hrabe de Angelis M, Nusing RM, Meyer CW, Wahli W, Klingenspor M, Herzig S. Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 2010;328:1158–61.
58. Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, Enerback S. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 2001;106:563–73.
59. Timmons JA, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, Hamilton DL, Gimeno RE, Wahlestedt C, Baar K, Nedergaard J, Cannon B. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. Proc Natl Acad Sci USA 2007;104:4401–6.
60. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. Nature 2008;454:961–7.
61. Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G, Cinti S. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. Am J Physiol Cell Physiol 2000;279:C670–81.
62. Cinti S. Reversible physiological transdifferentiation in the adipose organ. Proc Nutr Soc 2009;68:340–9.
63. Rosenwald M, Perdikari A, Rulicke T, Wolfrum C. Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 2013;15:659–67.
64. Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, Cerletti M, McDougall LE, Giorgadze N, Tchkonia T, Schrier D, Falb D, Kirkland JL, Wagers AJ, Tseng YH. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. Proc Natl Acad Sci USA 2011;108:143–8.
65. Lee YH, Petkova AP, Mottillo EP, Granneman JG. In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. Cell Metab 2012;15:480–91.
66. Lee YH, Petkova AP, Granneman JG. Identification of an adipogenic niche for adipose tissue remodeling and restoration. Cell Metab 2013;18:355–67.
67. Uezumi A, Fukada S, Yamamoto N, Takeda S, Tsuchida K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 2010;12:143–52.
68. Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA, Rossi FM. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 2010;12:153–63.
69. Walden TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am J Physiol Endocrinol Metab 2012;302:E19–31.
70. Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrila A, Hasselgren PO, Mori MA, Molla M, Tseng YH. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 2013;19:635–9.
71. Lidell ME, Betz MJ, Dahlqvist Leinhard O, Heglind M, Elander L, Slawik M, Mussack T, Nilsson D, Romu T, Nuutila P, Virtanen KA, Beuschlein F, Persson A, Borga M, Enerback S. Evidence for two types of brown adipose tissue in humans. Nat Med 2013;19:631–4.
72. Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V, Kajimura S. Human BAT

possesses molecular signatures that resemble beige/brite cells. PLoS One 2012;7:e49452.
73. Nedergaard J, Cannon B. How brown is brown fat? It depends where you look. Nature medicine. 2013;19:540–1. PubMed PMID: 23652104. Epub 2013/05/09. eng.
74. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med 2013;19:1252–63.
75. Zhou Z, Yon Toh S, Chen Z, Guo K, Ng CP, Ponniah S, Lin SC, Hong W, Li P. Cidea-deficient mice have lean phenotype and are resistant to obesity. Nat Genet 2003;35:49–56.
76. Zhang C, McFarlane C, Lokireddy S, Masuda S, Ge X, Gluckman PD, Sharma M, Kambadur R. Inhibition of myostatin protects against diet-induced obesity by enhancing fatty acid oxidation and promoting a brown adipose phenotype in mice. Diabetologia 2012;55:183–93.
77. Yadav H, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, Zerfas P, Zhigang D, Wright EC, Stuelten C, Sun P, Lonning S, Skarulis M, Sumner AE, Finkel T, Rane SG. Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. Cell Metab 2011;14:67–79.
78. Picard F, Gehin M, Annicotte J, Rocchi S, Champy MF, O'Malley BW, Chambon P, Auwerx J. SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 2002;111:931–41.
79. Patwari P, Emilsson V, Schadt EE, Chutkow WA, Lee S, Marsili A, Zhang Y, Dobrin R, Cohen DE, Larsen PR, Zavacki AM, Fong LG, Young SG, Lee RT. The arrestin domain-containing 3 protein regulates body mass and energy expenditure. Cell Metab 2011;14:671–83.
80. Kopecky J, Clarke G, Enerback S, Spiegelman B, Kozak LP. Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. J Clin Invest 1995;96:2914–23.
81. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998;92:829–39.
82. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Entocr Rev 2006;27:728–35.
83. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115–24.
84. Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM. Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. Cell Metab 2006;3:333–41.
85. Leone TC, Lehman JJ, Finck BN, Schaeffer PJ, Wende AR, Boudina S, Courtois M, Wozniak DF, Sambandam N, Bernal-Mizrachi C, Chen Z, Holloszy JO, Medeiros DM, Schmidt RE, Saffitz JE, Abel ED, Semenkovich CF, Kelly DP. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. PLoS Biol 2005;3:e101.
86. Kleiner S, Mepani RJ, Laznik D, Ye L, Jurczak MJ, Jornayvaz FR, Estall JL, Chatterjee Bhowmick D, Shulman GI, Spiegelman BM. Development of insulin resistance in mice lacking PGC-1alpha in adipose tissues. Proc Natl Acad Sci USA 2012;109:9635–40.
87. Ek J, Andersen G, Urhammer SA, Gaede PH, Drivsholm T, Borch-Johnsen K, Hansen T, Pedersen O. Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus. Diabetologia 2001;44:2220–6.
88. Fanelli M, Filippi E, Sentinelli F, Romeo S, Fallarino M, Buzzetti R, Leonetti F, Baroni MG. The Gly482Ser missense mutation of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) gene associates with reduced insulin sensitivity in normal and glucose-intolerant obese subjects. Dis Markers 2005;21:175–80.
89. Vimaleswaran KS, Radha V, Ghosh S, Majumder PP, Deepa R, Babu HN, Rao MR, Mohan V. Peroxisome proliferator-activated receptor-gamma co-activator-1alpha (PGC-1alpha) gene polymorphisms and their relationship to Type 2 diabetes in Asian Indians. Diabet Med 2005;22:1516–21.
90. Hammarstedt A, Jansson PA, Wesslau C, Yang X, Smith U. Reduced expression of PGC-1 and insulin-signaling molecules in adipose tissue is associated with insulin resistance. Biochem Biophys Res Commun 2003;301:578–82.
91. Semple RK, Crowley VC, Sewter CP, Laudes M, Christodoulides C, Considine RV, Vidal-Puig A, O'Rahilly S. Expression of the thermogenic nuclear hormone receptor coactivator PGC-1alpha is reduced in the adipose tissue of morbidly obese subjects. Int J Obes Relat Metabol Disord 2004;28:176–9.
92. Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. Nature 2009;460:1154–8.
93. Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM. Transcriptional control of brown fat determination by PRDM16. Cell Metab 2007;6:38–54.
94. Kajimura S, Seale P, Tomaru T, Erdjument-Bromage H, Cooper MP, Ruas JL, Chin S, Tempst P, Lazar MA, Spiegelman BM. Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes Dev 2008;22:1397–409.
95. Vernochet C, Peres SB, Davis KE, McDonald ME, Qiang L, Wang H, Scherer PE, Farmer SR. C/EBPalpha and the corepressors CtBP1 and CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. Mol Cell Biol 2009;29:4714–28.
96. Ohno H, Shinoda K, Spiegelman BM, Kajimura S. PPARgamma agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. Cell Metab 2012;15:395–404.
97. Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M. PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. Cardiovasc Res 2005;66:562–73.
98. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. J Biol Chem 2003;278:26597–603.
99. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 2006;127:397–408.

100. Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 2002;277:1645–8.
101. Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 2005;1:361–70.
102. Puigserver P, Vazquez F, Bonet ML, Pico C, Palou A. In vitro and in vivo induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. Biochem J 1996;317(Pt 3):827–33.
103. Mercader J, Palou A, Bonet ML. Induction of uncoupling protein-1 in mouse embryonic fibroblast-derived adipocytes by retinoic acid. Obesity (Silver Spring) 2010;18:655–62.
104. Berry DC, DeSantis D, Soltanian H, Croniger CM, Noy N. Retinoic acid upregulates preadipocyte genes to block adipogenesis and suppress diet-induced obesity. Diabetes 2012;61:1112–21.
105. Alvarez R, de Andres J, Yubero P, Vinas O, Mampel T, Iglesias R, Giralt M, Villarroya F. A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. J Biol Chem 1995;270:5666–73.
106. Rabelo R, Reyes C, Schifman A, Silva JE. A complex retinoic acid response element in the uncoupling protein gene defines a novel role for retinoids in thermogenesis. Endocrinology 1996;137:3488–96.
107. Alvarez R, Checa M, Brun S, Vinas O, Mampel T, Iglesias R, Giralt M, Villarroya F. Both retinoic-acid-receptor- and retinoid-X-receptor-dependent signalling pathways mediate the induction of the brown-adipose-tissue-uncoupling-protein-1 gene by retinoids. Biochem J 2000;345(Pt 1):91–7.
108. Mercader J, Ribot J, Murano I, Felipe F, Cinti S, Bonet ML, Palou A. Remodeling of white adipose tissue after retinoic acid administration in mice. Endocrinology 2006;147:5325–32.
109. Shan T, Liang X, Bi P, Kuang S. Myostatin knockout drives browning of white adipose tissue through activating the AMPK-PGC1alpha-Fndc5 pathway in muscle. FASEB J 2013;27:1981–9.
110. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, Greene NP, Wu J, Estall JL, Irving BA, Lanza IR, Rasbach KA, Okutsu M, Nair KS, Yan Z, Leinwand LA, Spiegelman BM. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell 2012;151:1319–31.
111. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? Nature 2012;488:E9–10; discussion E–1.
112. Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, Jung R, Wisloff U, Tjonna AE, Raastad T, Hallen J, Norheim F, Drevon CA, Romacho T, Eckardt K, Eckel J. Evidence against a beneficial effect of irisin in humans. PLoS One 2013;8:e73680.
113. Lecker SH, Zavin A, Cao P, Arena R, Allsup K, Daniels KM, Joseph J, Schulze PC, Forman DE. Expression of the irisin precursor FNDC5 in skeletal muscle correlates with aerobic exercise performance in patients with heart failure. Circ Heart Fail 2012;5:812–8.
114. Besse-Patin A, Montastier E, Vinel C, Castan-Laurell I, Louche K, Dray C, Daviaud D, Mir L, Marques MA, Thalamas C, Valet P, Langin D, Moro C, Viguerie N. Effect of endurance training on skeletal muscle myokine expression in obese men: identification of apelin as a novel myokine. Int J Obes 2014;38:707–13.
115. Hecksteden A, Wegmann M, Steffen A, Kraushaar J, Morsch A, Ruppenthal S, Kaestner L, Meyer T. Irisin and exercise training in humans-results from a randomized controlled training trial. BMC Med 2013;11:235.
116. Scharhag-Rosenberger F, Morsch A, Wegmann M, Ruppenthal S, Kaestner L, Meyer T, Hecksteden A. Irisin Does Not Mediate Resistance Training-Induced Alterations in RMR. Med Sci Sports Exerc 2014;46:1736–43.
117. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab 2014;19:302–9.
118. Cuevas-Ramos D, Almeda-Valdes P, Meza-Arana CE, Brito-Cordova G, Gomez-Perez FJ, Mehta R, Oseguera-Moguel J, Aguilar-Salinas CA. Exercise increases serum fibroblast growth factor 21 (FGF21) levels. PLoS One 2012;7:e38022.
119. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS. Acute exercise induces FGF21 expression in mice and in healthy humans. PLoS One 2013;8:e63517.
120. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM. FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 2012;26:271–81.
121. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627–35.
122. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 2008;149:6018–27.
123. Hondares E, Iglesias R, Giralt A, Gonzalez FJ, Giralt M, Mampel T, Villarroya F. Thermogenic activation induces FGF21 expression and release in brown adipose tissue. J Biol Chem 2011;286:12983–90.
124. Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F. Hepatic FGF21 expression is induced at birth via PPARalpha in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metab 2010;11:206–12.
125. Lee P, Werner CD, Kebebew E, Celi FS. Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (2005). 2014;38:170–6.
126. Xu J, Stanislaus S, Chinookoswong N, Lau YY, Hager T, Patel J, Ge H, Weiszmann J, Lu SC, Graham M, Busby J, Hecht R, Li YS, Li Y, Lindberg R, Veniant MM. Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models – association with liver and adipose tissue effects. Am J Physiol Endocrinol Metab 2009;297:E1105–14.
127. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, Vonderfecht S, Hecht R, Li YS, Lindberg RA, Chen JL, Jung DY, Zhang Z, Ko HJ, Kim JK, Veniant MM. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 2009;58:250–9.
128. Gao M, Ma Y, Cui R, Liu D. Hydrodynamic delivery of FGF21 gene alleviates obesity and fatty liver in mice fed a high-fat diet. J Control Release 2014;185:1–11.
129. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by

PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 2007;5:426–37.
130. Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vazquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, Lopez M, Vidal-Puig A. BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 2012;149:871–85.
131. Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN, Kahn CR. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 2008;454:1000–4.
132. Kuo MM, Kim S, Tseng CY, Jeon YH, Choe S, Lee DK. BMP-9 as a potent brown adipogenic inducer with anti-obesity capacity. Biomaterials 2014;35:3172–9.
133. Qian SW, Tang Y, Li X, Liu Y, Zhang YY, Huang HY, Xue RD, Yu HY, Guo L, Gao HD, Liu Y, Sun X, Li YM, Jia WP, Tang QQ. BMP4-mediated brown fat-like changes in white adipose tissue alter glucose and energy homeostasis. Proc Natl Acad Sci USA 2013;110:E798–807.
134. Mathis D. Immunological goings-on in visceral adipose tissue. Cell Metab 2013;17:851–9.
135. Yao L, Heuser-Baker J, Herlea-Pana O, Zhang N, Szweda LI, Griffin TM, Barlic-Dicen J. Deficiency in adipocyte chemokine receptor CXCR4 exacerbates obesity and compromises thermoregulatory responses of brown adipose tissue in a mouse model of diet-induced obesity. FASEB J 2014 Jul 11. pii: fj.14–249797. [Epub ahead of print].
136. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 2014;157:1292–308.
137. Chen YC, Cypess AM, Chen YC, Palmer M, Kolodny G, Kahn CR, Kwong KK. Measurement of human brown adipose tissue volume and activity using anatomic MR imaging and functional MR imaging. J Nucl Med 2013;54:1584–7.
138. van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 2013;123:3395–403.
139. Heeren J, Bruns O. Nanocrystals, a new tool to study lipoprotein metabolism and atherosclerosis. Curr Pharm Biotechnol 2012;13:365–72.
140. Chen YI, Cypess AM, Sass CA, Brownell AL, Jokivarsi KT, Kahn CR, Kwong KK. Anatomical and functional assessment of brown adipose tissue by magnetic resonance imaging. Obesity (Silver Spring) 2012;20:1519–26.
141. Bruns OT, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Lauterwasser J, Nikolic MS, Mollwitz B, Merkel M, Bigall NC, Sapra S, Reimer R, Hohenberg H, Weller H, Eychmuller A, Adam G, Beisiegel U, Heeren J. Real-time magnetic resonance imaging and quantification of lipoprotein metabolism in vivo using nanocrystals. Nat Nanotech 2009;4:193–201.
142. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J. Brown adipose tissue activity controls triglyceride clearance. Nat Med 2011;17:200–5.
143. Di Franco A, Guasti D, Mazzanti B, Ercolino T, Francalanci M, Nesi G, Bani D, Forti G, Mannelli M, Valeri A, Luconi M. Dissecting the origin of inducible brown fat in adult humans through a novel adipose stem cell model from adipose tissue surrounding pheochromocytoma. J Clin Endocrin Metab 2014 Jun 27:jc20141431. [epub ahead of print].
144. Wang Q, Zhang M, Ning G, Gu W, Su T, Xu M, Li B, Wang W. Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. PLoS One 2011;6:e21006.
145. Jespersen NZ, Larsen TJ, Peijs L, Daugaard S, Homoe P, Loft A, de Jong J, Mathur N, Cannon B, Nedergaard J, Pedersen BK, Moller K, Scheele C. A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. Cell Metab 2013;17:798–805.
146. Cypess AM, Chen YC, Sze C, Wang K, English J, Chan O, Holman AR, Tal I, Palmer MR, Kolodny GM, Kahn CR. Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc Natl Acad Sci USA 2012;109:10001–5.
147. Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M. Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. Obesity (Silver Spring) 2011;19:13–6.
148. Carey AL, Formosa MF, Van Every B, Bertovic D, Eikelis N, Lambert GW, Kalff V, Duffy SJ, Cherk MH, Kingwell BA. Ephedrine activates brown adipose tissue in lean but not obese humans. Diabetologia 2013;56:147–55.
149. Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P, van Marken Lichtenbelt WD. Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. Diabetes 2012;61:3106–13.
150. Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, Swarbrick M, Rose-John S, Rincon M, Robertson G, Zechner R, Wagner EF. A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab 2014 Jul 15. [epub ahead of print].
151. Kir S, White JP, Kleiner S, Kazak L, Cohen P, Baracos VE, Spiegelman BM. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature 2014 Jul 13. [epub ahead of print].
152. Borga M, Virtanen KA, Romu T, Leinhard OD, Persson A, Nuutila P, Enerback S. Brown adipose tissue in humans: detection and functional analysis using PET (positron emission tomography), MRI (magnetic resonance imaging), and DECT (dual energy computed tomography). Methods Enzymol 2014;537:141–59.