YEARBOOK OF PHYSICAL ANTHROPOLOGY 156:98–115 (2015)

The "Skinny" on Brown Fat, Obesity, and Bone

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KEY WORDS brown adipose tissue; obesity; bone; human

ABSTRACT The discovery that metabolically active brown fat is present in humans throughout ontogeny raises new questions about the interactions between thermoregulatory, metabolic, and skeletal homeostasis. Brown adipose tissue (BAT) is distinct from white adipose tissue (WAT) for its ability to burn, rather than store, energy. BAT uniquely expresses uncoupling protein-1 (abbreviated as UCP1), which diverts the energy produced by cellular respiration to generate heat. While BAT is found in small mammals, hibernators, and newborns, this depot was thought to regress in humans during early postnatal life. Recent studies revealed that human BAT remains metabolically active throughout childhood and even in adulthood, particularly in response to cold exposure. In addition to the constitutive BAT depots present at birth, BAT cells can be induced within WAT depots under specific metabolic and climatic conditions. These cells, called inducible brown fat, "brite," or beige fat, are currently the focus of intense investigation as a possible treatment for obesity. Inducible brown fat is associated with higher bone mineral density, suggesting that brown fat interacts with bone growth in previously unrecognized ways. Finally, BAT may have contributed to climatic adaptation in hominins. Here, I review current findings on the role of BAT in thermoregulation, bone growth, and metabolism, describe the potential role of BAT in moderating the obesity epidemic, and outline possible functions of BAT across hominin evolutionary history. Yrbk Phys Anthropol 156:98–115, 2015.

Humans have the largest ecogeographic flexibility of any terrestrial mammal, thanks to a combination of cultural buffering, such as clothing and shelter, and biological mechanisms common to all mammals. Thermoregulatory adaptations include changes in body size and limb proportions (Bergmann's Law and Allen's Rule), insulation via subcutaneous adipose tissue, modulation of metabolic rate, perspiration, shivering, and exercise (Steegmann et al., 2002). However, until recently, it was widely believed that our lineage had all but lost one key adaptation to life in the cold, namely nonshivering thermogenesis in brown adipose tissue (BAT, also known as brown fat).

Nonshivering thermogenesis in BAT is crucial for maintaining body temperature in neonates, including human infants, and in small-bodied mammals throughout life (Aherne and Hull, 1966; Rowlatt et al., 1971; Heaton, 1972; Symonds and Lomax, 1992; Klingenspor, 2003). BAT also plays an essential role in arousal and rewarming following hibernation or torpor in species including rodents, bats, and bears (Nedergaard and Cannon, 1990; Oelkrug et al., 2011), and at least one primate, the mouse lemur (Genin et al., 2003). In contrast to white adipose tissue (WAT), which stores fat for use as energy, BAT burns fat and glucose to generate heat (Lowell and Spiegelman, 2000; Townsend and Tseng, 2014). Sympathetic tone increases expression of a protein unique to brown adipocytes called uncoupling protein-1 (UCP1) (see Box 1) which is expressed in the mitochondrial inner cell membrane (Cooney and Newsholme, 1982; Williamson, 1986; Arbuthnott, 1989; Ricquier et al., 1991; Nicholls and Rial, 1999). Active UCP-1 creates an alternative pathway through the mitochondrial inner cell membrane. As a result, hydrogen atoms produced in the cellular respiration of glucose and triglycerides are not channeled through ATP synthase, and the proton motive force is released as heat rather than being used to generate ATP (Nicholls and Rial, 1999) (Fig. 1).

Brown adipocytes are easily identified morphologically. While white adipocytes are unilocular, consisting of a single, large lipid droplet and few mitochondria, brown adipocytes are multilocular, containing many small lipid droplets and the dense mitochondria that give the tissue its characteristic brown or tan color (Fig. 2). Classical BAT depots consist of bilateral nodules in the interscapular region, which persist throughout the lifespan in small mammals but regress in human neonates soon after birth (Heaton, 1972; Lidell et al., 2013). As a result, BAT was thought to have no role in human thermogenesis beyond infancy, despite periodic descriptions of adult brown fat in the literature (Aherne and Hull, 1966; Heaton, 1973; Doniach, 1975). For example, autopsy data showed persistence of multilocular fat cells resembling BAT near the kidneys, aorta, neck, and mediastinum in subjects up to 60–80 years of age (Heaton, 1972), and a study of outdoor workers exposed to cold found tissue that appeared to be BAT in the neck and pericardium (Huttunen et al., 1981). There were even early suggestions of a link between decreased BAT thermogenesis and obesity (Jung et al., 1979; James and Trayhurn, 1981). However, the overall consensus was that the quantities of BAT in adults were too small to have significant metabolic effects (Astrup et al., 1985; Cunningham et al., 1985).

Recent studies have transformed our understanding of human brown fat by demonstrating that while the

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DOI: 10.1002/ajpa.22661
Published online 11 November 2014 in Wiley Online Library (wileyonlinelibrary.com).

Box 1. Evolutionary origins of brown fat

The prevailing hypothesis for the origin of brown adipose tissue is that BAT arose with eutherian endothermy, to maintain body temperature in the cold, particularly in neonates and small-bodied taxa (Smith and Horwitz, 1969; Foster and Frydman, 1978; Nicholls and Locke, 1984; Casteilla et al., 1989). Mammals exhibit BAT at birth, primarily in the periclavicular and interscapular regions, and the appearance of BAT precedes WAT in fetal development (Cannon and Nedergaard, 2004). However, the protein that allows heat production in BAT is uncoupling protein-1 (UCP1), and the uncoupling protein family is far older than brown fat. UCP1 was the first of the uncoupling proteins to be identified, which focused attention on the role of uncoupling proteins in mammalian nonshivering thermogenesis. Subsequent studies revealed that there are multiple UCP homologues in eutherian and noneutherian vertebrates, protozoa, and even plants (Nedergaard and Cannon, 1990). Thus, the uncoupling protein has a deep evolutionary origin, predating the divergence of plants and animals, and likely originally evolved as a mechanism for stabilizing the relationship between respiration and ATP production in the cell by moderating proton availability (Bing et al., 1998). Within early vertebrates, there were two duplication events of the UCP gene, leading to the paralogues UCP2 and UCP3, which are less potent uncouplers than UCP1 and likely not thermogenic in function (Hughes and Criscuolo, 2008). UCP1 and UCP2 were subsequently lost in the avian lineage (Hughes and Criscuolo, 2008). Given this variability of function, it is interesting to consider an alternative hypothesis, that BAT evolved not for generalized temperature homeostasis, but for incubation during reproduction, and only later became an essential component of migration to colder climates (Oelkrug et al., 2013). Data on brown fat morphology in a greater diversity of taxa should help to clarify BAT's original function, which also may have included reducing oxidative stress, reducing aging phenotypes, and increasing lifespan, as seen in other members of the UCP family.

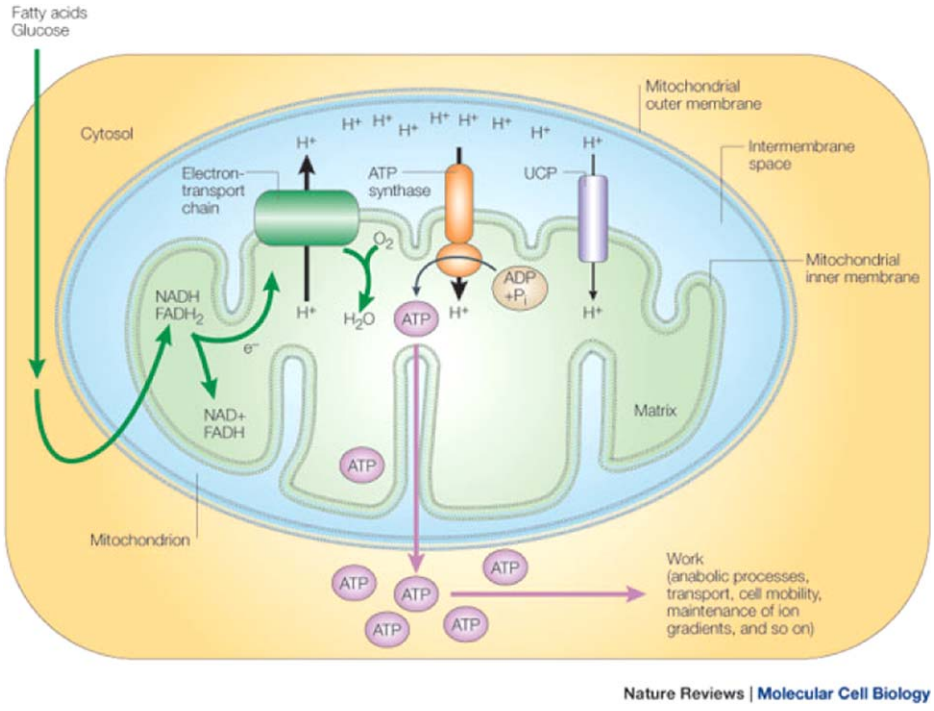

Fig. 1. Uncoupling protein (UCP-1) creates a leak across the mitochondrial inner membrane. Instead of being channeled through ATP synthase (**A**), protons pass through UCP-1 (**B**), causing the proton motive force to be released as heat. Republished by permission from Macmillan Publishers Ltd: Fig. 1 in Krauss et al., Nat Rev Mol Cell Biol 6:248–261, copyright 2005. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

neonatal interscapular depot does regress in early postnatal life, metabolically relevant quantities of brown adipocytes can persist into adulthood and/or can be induced to form within white adipose depots throughout life. These cells, variously termed beige fat, inducible BAT (iBAT), recruitable, or brite fat, are found interspersed within white fat depots in humans and in rodents (Sharp et al., 2012; Vitali et al., 2012; Wu et al., 2012). In humans, early reports identified tissue morphologically resembling brown or beige fat in supraclavicular, paravertebral, pericardial, and perirenal depots, among others (Heaton, 1972; Sacks and Symonds, 2013), but thus far gene expression has only confirmed UCP1 expression in supraclavicular and pericardial depots. Although recent evidence suggests that beige fat derives from a different stem cell precursor than constitutive brown fat, both types of BAT engage in nonshivering thermogenesis in response to cold exposure or sympathetic activation.

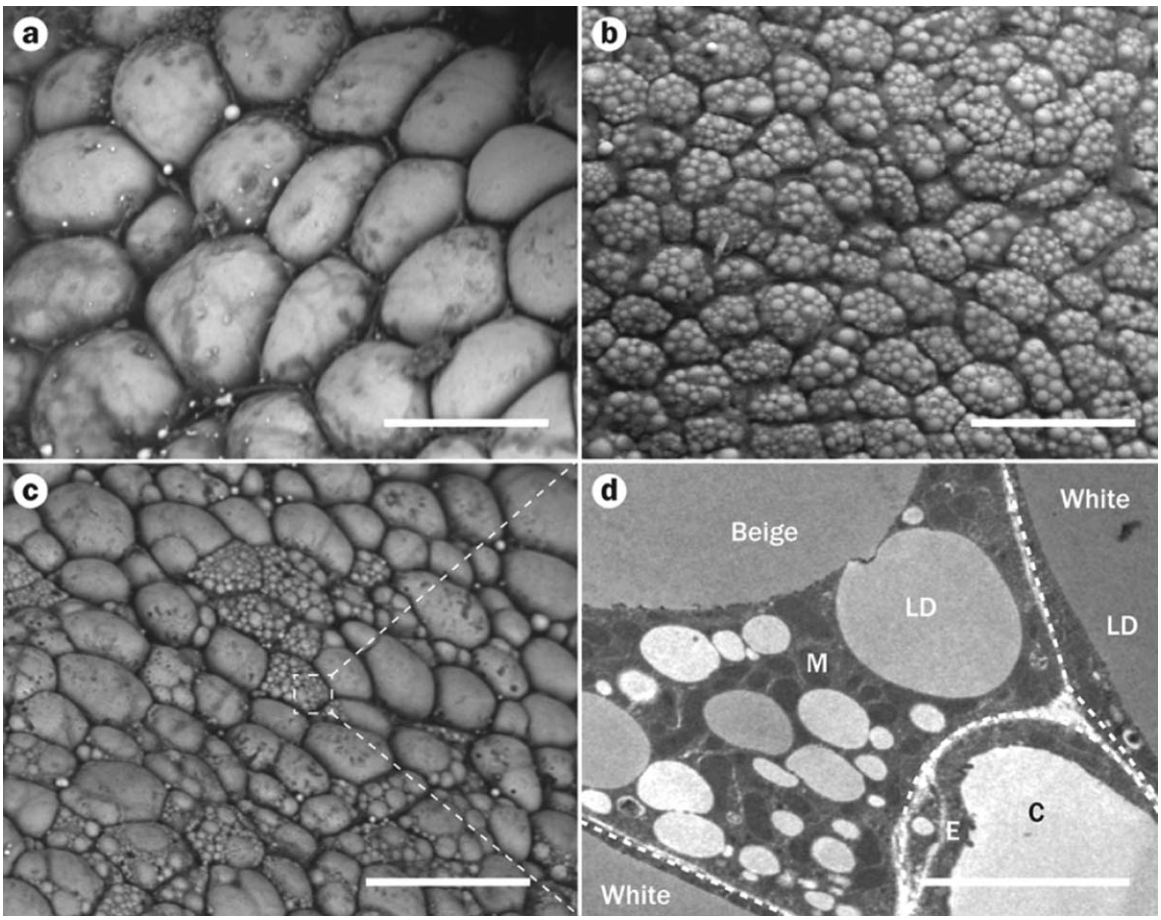

Fig. 2. Environmental scanning electron micrographs demonstrate the characteristic appearance of mouse adipose tissue. **A**: White adipocytes in inguinal WAT contain a single lipid droplet. **B**: Brown adipocytes in interscapular brown adipose tissue contain multilocular lipid droplets. **C**: Browning of WAT (induced by sustained pharmacological activation of β3-adrenergic receptors) leads to formation of islets of multilocular beige adipocytes within inguinal WAT. **D**: Transmission electron micrograph of beige adipocytes within WAT, showing their high mitochondrial content. Scale bars: a–c, 50 μm; d, 5 μm. Abbreviations: C, capillary; E, endothelial cell; LD, lipid droplet; M, mitochondria; WAT, white adipose tissue. Republished by permission from Macmillan Publishers Ltd: Fig. 1 in Bartelt & Heeren, Nat Rev Endocrinol 10:24–36, copyright 2014.

The discovery that humans have active BAT throughout the life course has generated intense interest both in the role of BAT in human physiology and in the potential for harnessing its energy-burning properties to combat the obesity epidemic. Recent studies identified several unexpected aspects of BAT function, including stimulation of BAT by exercise and a positive correlation of brown and beige fat and bone mass. From an evolutionary perspective, these discoveries raise numerous interesting questions, including whether BAT has contributed to climatic adaptation in humans and other primates, and whether BAT upregulation by exercise and BAT moderation of bone mass may be adaptive. In this article I explore the role of brown and beige fat in human evolutionary biology. After reviewing current findings on the role of brown and beige fat in thermoregulation, bone growth, and energy balance and metabolism, I will describe the potential contribution of reduced BAT activity to the obesity epidemic. I will then outline some outstanding questions about how brown and beige fat may have functioned over the course of hominin evolution, particularly in adaptations to new environments. I will conclude by proposing some hypotheses for beginning to test the role of BAT in human climatic adaptation and skeletal homeostasis.

BAT activity in adult humans

Adult human BAT was rediscovered inadvertently, via use of integrated ^{18}F-fluorodeoxyglucose positron-emission tomography and computed tomography (^{18}F-FDG PET-CT) imaging for cancer surveillance. Briefly, ^{18}F-FDG PET-CT uses uptake of a short-lived radionuclide tracer, in this case the glucose analogue ^{18}F-fluorodeoxyglucose, to label areas of high metabolic activity, such as rapidly dividing cancer cells. As ^{18}F-FDG PET-CT scanning became widespread, anecdotal reports emerged of patients exhibiting noncancerous, bilaterally symmetric areas of high metabolic activity in the supraclavicular and paravertebral regions. While early reports dismissed this signal as artifactual high glucose uptake by the tensed muscles of anxious patients, subsequent studies showed that the tissue was not muscle, but fat. The authors hypothesized that the fat contained brown adipocytes and showed that the metabolic activity was higher in colder months, suggesting a relationship to

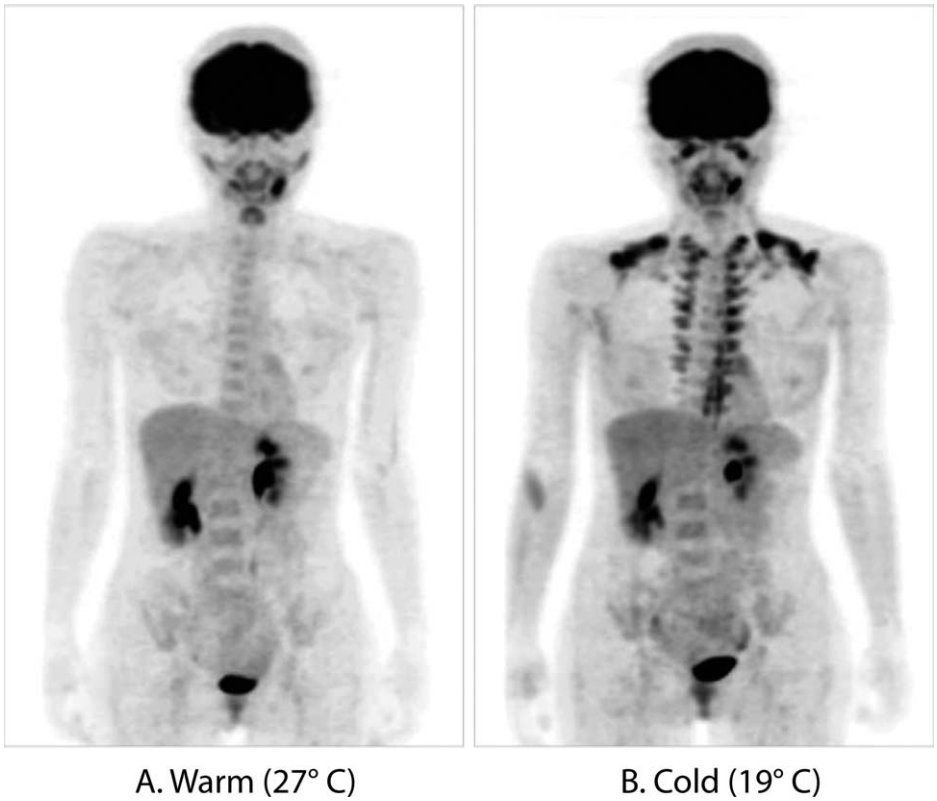

Fig. 3. Human brown adipose tissue detected by fluorodeoxyglucose (FDG)-positron emission tomography (PET). FDG uptake into adipose tissue at the supraclavicular and paraspinal regions is detected by PET. The FDG uptake into adipose tissues is negligible under a warm condition at 27°C (**A**), but increases greatly after exposure to cold at 19°C (**B**) for 2 h. Reprinted from Fig. 2 in Saito M, Diabetes Metab J. 2013 Feb;37(1):22–29. Copyright © 2013 Korean Diabetes Association, Open Access under the terms of the Creative Commons Attribution Non-Commercial License.

temperature homeostasis (Hany et al., 2002; Cohade et al., 2003a,b; Yeung et al., 2003; Truong et al., 2004).

The definitive breakthrough came in 2009, when multiple groups combined ^{18}F-FDG PET-CT with tissue histology to confirm that the unidentified tissue was metabolically active BAT (Fig. 3). In the largest study, a retrospective review of 3,640 ^{18}F-FDG PET-CT scans on 1972 patients, metabolically active BAT was evident in 7.5% of the women and 3.1% of the men, primarily in the neck and supraclavicular regions (Cypess et al., 2009). This and subsequent studies showed that BAT is more common in younger than in older individuals, that women have more BAT with higher metabolic activity compared with men, that BAT is inversely correlated with BMI, particularly in men, and that BAT function is lower in diabetes (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Pfannenberg et al., 2010; Ouellet et al., 2011; Yoneshiro et al., 2011; Wang et al., 2011). In studies conducted at thermoneutrality, about 20–21°C (68–69.8°F) for clothed humans (Kingma et al., 2012), the prevalence of active BAT on a single scan has been low, generally under 10%, which would seemingly cast doubt on its metabolic relevance. However, extending the analysis to serial scans of the same individual showed that the likelihood of detecting active BAT is higher when outdoor temperature is lower and when the individual has more BAT tissue (Pace et al., 2011), and increases with each sequential scan, suggesting that BAT may be present in as many as 65% of adults (Lee et al., 2010). Furthermore, exposure to cold challenge (16–19°C for 2 h) increased the proportion of subjects with active BAT to 50–90% in those under age 35, although only to 8% in older subjects (Saito et al., 2009; van Marken Lichtenbelt et al., 2009). Combining ^{18}F-FDG PET-CT with tissue biopsies and quantitative PCR showed that cold exposure increased glucose uptake 15-fold and increased *UCP1* gene expression 1,000-fold in BAT vs. WAT, and confirmed that the cells were histologically consistent with BAT (Virtanen et al., 2009). To quantify metabolic activity following cold exposure, Ouellet et al. (2012) combined ^{18}FDG PET-CT with an additional tracer for fatty acids, and found that cold increased glucose and fatty acid uptake and oxidative metabolism in BAT, but not in adjacent tissues.

Consistent with its thermogenic function, BAT function varied seasonally, with active BAT in 7.2% of winter PET-CT scans vs. 2.5% of summer scans (Au-Yong et al., 2009). Interestingly, although the variation was highly correlated with ambient temperature, it was even more highly correlated with photoperiod ($r^2 \sim 0.7$ and 0.9, respectively). Subjects for whom consecutive scans were available showed pronounced differences in BAT glucose uptake between seasons, and active BAT was about three times more common in women vs. men (7.2% vs. 2.8%) (Au-Yong et al., 2009), as seen in other studies (Cypess et al., 2009; Ouellet et al., 2011).

These studies showed that many adults have brown adipose tissue, and that this tissue can be stimulated by cold exposure to produce heat. However, it was unknown whether the brown adipocytes seen in adults were

constitutive brown fat that had been retained since infancy, as seen in small mammals and neonatal humans, or beige fat that had been induced to form in WAT depots, or a combination of the two. Since beige fat is often found near the clavicles and sternum, not far from the interscapular region, one hypothesis was that these cells represented remnants of the original interscapular depot. Alternatively, the discovery that adults have inducible beige fat raised the possibility that humans may have lost the ability to form constitutive BAT at all, and that even neonatal BAT might consist of inducible beige adipocytes. Studies of the distribution and developmental origin of BAT in humans at birth have helped to resolve these questions.

BAT in neonates and children

Like other mammals, human neonates rely on BAT for temperature homeostasis as they adjust to postnatal life. Historically, neonatal constitutive BAT has been estimated at 1–3% of birthweight (Aherne and Hull, 1966; Hull, 1969; Prechtl, 1986), or about 1.5–4 oz (36–109 g) for an 8 lb (3,629 g) infant. As Kuzawa (1998) points out, this quantity of neonatal BAT is typical for a mammal of our size, while the 12–14% of body mass consisting of WAT (15–18 oz, 435–508 g) is higher than in other species. Early work using thermographic imaging of the skin surface demonstrated apparent responsiveness to cold in neonatal interscapular BAT (Rylander et al., 1972), but more comprehensive imaging and quantification of BAT in neonates by PET-CT has been challenging due to the risk from radiation exposure. More recent studies have relied on a new technique to quantify BAT using magnetic resonance imaging (MRI). Given that BAT is more highly vascularized than WAT and each cell contains multiple lipid droplets, it has a higher water:fat ratio compared to WAT, and thus the depots can be differentiated spectroscopically (Ma, 2008; Hamilton et al., 2011; Hu and Kan, 2013; Hu et al., 2013).

A recent study of sleeping neonates used the MRI technique to identify BAT deposits near the spine, supraclavicular region, and axilla (Rasmussen et al., 2013). Lidell et al. (2013) also used this approach in postmortem infants, combining MRI imaging with immunohistochemistry of biopsies taken from the imaged regions. The data confirmed that the tissue was BAT and showed that the average BAT volume in neonates is 3.6 ± 2.4 mL, which the authors note is actually proportional to the relative BAT volume found in one study of adults (Virtanen et al., 2009). Thus, at least in this study, infants did not have proportionally more BAT tissue compared with adults, although there could be differential activation of brown adipocytes, irrespective of the volume of cells, at different stages of ontogeny. Interscapular biopsies identified multilocular adipocytes, with a high density of mitochondria expressing *UCP1* by immunohistochemistry, but few white adipocytes. In contrast, supraclavicular biopsies were consistent with beige fat, or BAT mixed with WAT. The most important finding from these data was that human neonates have histologically distinct BAT depots in multiple locations, including constitutive BAT, at least transiently, as well as inducible BAT within the same white adipose depots where it is found in adults (Lidell et al., 2013).

Although the perinatal interscapular depot regresses after birth, cells morphologically consistent with BAT can be found throughout the thorax and abdomen in children and adolescents (Aherne and Hull, 1966; Heaton, 1972; Emery and Dinsdale, 1978). Assessing the metabolic activity of these adipocytes is difficult in children (Cypess and Kahn, 2010), but a recent retrospective study showed that BAT activity is seen in 43–45% of boys and girls, and is inversely correlated with body mass (Drubach et al., 2011). It is particularly interesting to note that BAT activity peaks around puberty. When subjects are divided by Tanner stage, only 15% of boys and girls in Tanner Stage 1 exhibit active BAT, compared with 75% of those in Tanner Stages 2–5, with the biggest increase in BAT volume occurring during Tanner Stages 4–5 (Gilsanz et al., 2012). The increase in BAT is greater in boys and is positively correlated with muscle volume, both of which imply a role for sex steroids, particularly testosterone, in this pattern (Gilsanz et al., 2011, 2012; Ponrartana et al., 2013). Other hormones, including the growth hormone/insulin-like growth factor axis, may also contribute to this pattern.

BAT in nonhuman primates

Many nonhuman primate neonates have multilocular adipose cells consistent with BAT, including strepsirhines and both new and old world monkeys, although data from apes are lacking (Rowlatt et al., 1971). Multilocular adipocytes have also been reported in fetal and adult *Macaca mulatta* (Chaffee et al., 1970; Strieleman et al., 1985; Swick et al., 1986), adult *M. fascicularis* (Kates et al., 1990; Meyers et al., 1997), and adult *Saimiri sciurea* (Chaffee et al., 1966). In *S. sciurea*, tissue resembling BAT was described in the axilla, which appeared to have increased mitochondrial density following cold exposure (Chaffee et al., 1966). In *M. mulatta* exposed to 5°C or 35°C for 12–24 months, the cold-exposed group had higher RMR (Chaffee and Allen, 1973) and a three-fold increase in BAT postmortem (Chaffee et al., 1975).

Beige or brown?

Taken together, the data from infants, children, and adults show that there are two distinct types of human brown fat: a constitutive interscapular depot present at birth, and inducible brown fat cells (beige fat) resident in white fat, primarily in the supraclavicular region (Harms and Seale, 2013). Detailed studies in rodents, which also have both brown and beige fat, revealed that these adipocytes arise from independent lineages (Cousin et al., 1993; Guerra et al., 1998). Lineage tracing showed that while both brown and beige fat cells derive from mesenchymal stem cell (MSC) populations, constitutive brown fat comes from a myoblast lineage, specifically myf5+ dermomyotome cells (Harms and Seale, 2013; Rajakumari et al., 2013), while inducible beige fat likely derives from a distinct myf5- mesenchymal progenitor pool derived from vascular and endothelial cells found within WAT depots (Seale et al., 2011). In mice, several bone morphogenetic proteins induce browning of WAT, including BMP4 (Qian et al., 2013), BMP2 (Olmsted-Davis et al., 2007), and BMP7 (Tseng et al., 2008; Schulz et al., 2013), although a recent study suggested BMP7 induction does not occur at or above thermoneutrality (Boon et al., 2013). In humans, adipocyte precursors from the supraclavicular depot can be induced in vitro to form beige adipocytes expressing abundant *UCP1* (Lee et al., 2011b, 2014b). Key browning factors include cardiac natriuretic peptides (Bordicchia

et al., 2012), fibroblast growth factor-21 (FGF21) (Fisher et al., 2012), and the hormone irisin (Bostrom et al., 2012), all of which can induce browning in human as well as murine adipocytes in vitro (Bordicchia et al., 2012; Lee et al., 2014a). While some studies have reported transdifferentiation of white adipocytes into beige under certain conditions (Himms-Hagen et al., 2000; Cinti, 2005), Wang et al. (2013) recently labeled mature WAT cells prior to beige adipocyte induction and showed that induced beige adipocytes were unlabeled, meaning that beige adipocytes in white fat arose from a separate precursor population within the depot.

On a molecular level, there are several key differences between brown and beige adipocytes (Harms and Seale, 2013). Both types of BAT express *UCP1* mRNA, the definitive marker of brown adipocytes and the protein required for nonshivering thermogenesis. The most important distinction from the perspective of human biology is that brown adipocytes express *UCP1* constitutively, including at thermoneutrality, while in beige adipocytes *UCP1* expression and thermogenesis occur only in response to stimulus, such as climatic or pharmacological upregulation of sympathetic tone (Wu et al., 2013). Both brown and beige adipocytes also express cell death-inducing DNA fragmentation factor alpha-like effector A (*CIDEA)*, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PGC1α*), and PRD1-BF1-RIZ1 homologous domain containing 16 (*PRDM16*) (Gesta et al., 2007; Kajimura et al., 2010; Harms and Seale, 2013). These genes are markers of BAT and also play a role in "browning" of white fat, meaning increased differentiation of BAT cells within WAT depots, rather than white adipocytes transforming into brown ones (Seale et al., 2011; Ohno et al., 2012). The gene expression profile of human *UCP1*-expressing cells has been variously reported to be similar to murine beige fat (Sharp et al., 2012; Wu et al., 2012), brown fat (Cypess et al., 2013), or both types (Jespersen et al., 2013). A recent comprehensive study of the human BAT transcriptome, based on supraclavicular fibroblast-like precursors induced to form beige adipocytes, indicates that beige adipocytes express some classic brown fat markers (e.g., *UCP1, PRDM16*) as well as some novel markers (e.g., *TMEM26*, *HOXC9*) (Lee et al., 2014b).

When do brown and beige fat turn on?

Whether brown adipocytes are constitutive or induced, they must be activated in order to generate heat (Shabalina et al., 2010). Multiple pathways have been identified to date, with additional mechanisms likely to be found. These include exogenous stimuli such as cold exposure and exercise, and endogenous factors such as thyroid hormone and cardiac natriuretic peptides. In most if not all of these cases, the final common pathway for upregulation in both brown and beige fat is canonical activation by sympathetic tone (Cannon and Nedergaard, 2004).

Sympathetic tone

BAT depots respond rapidly to sympathetic activation, thanks to extensive vascularization and sympathetic innervation (Aherne and Hull, 1966; Symonds and Lomax, 1992; Bartness et al., 2010). Brown and beige adipocytes express β-adrenergic receptors in the cell membrane, which can bind norepinephrine to initiate a signaling cascade that includes activation of adenylate cyclase subtype III (AC-III) and conversion of ATP to cyclic AMP (cAMP), increasing transcription of the *UCP1* gene, and hormone-sensitive lipase, an enzyme that hydrolyzes stored triglycerides into free fatty acids that provide the fuel source for thermogenesis (Granneman, 1995; Pecqueur et al., 2001; Cannon and Nedergaard, 2004). The presence of these free fatty acids activates the UCP1 protein in the inner cell membrane, allowing protons to leak across the cell membrane and uncoupling oxidative phosphorylation, such that the energy is released as heat rather than used to synthesize ATP (Nicholls and Locke, 1984; Cannon and Nedergaard, 2004) (Fig. 1). One important nuance is that the β3 adrenergic receptor in fat is reportedly less responsive to sympathetic stimulation in humans compared to other mammals (Lafontan and Berlan, 1993), which may help to explain why pharmacological β3 agonism in obese humans has not produced the weight loss or other metabolic benefits seen in rodents and dogs (Grujic et al., 1997; Weyer et al., 1999; Robidoux et al., 2004; Kozak, 2010).

Cold exposure

The primary environmental stimulus for BAT thermogenesis is cold exposure, via afferent signals from thermoreceptors in body tissues that increase sympathetic tone and trigger hypothalamic norepinephrine release, initiating nonshivering thermogenesis as described above (Wilkerson et al., 1974; Klingenspor, 2003; Stocks et al., 2004; Morrison et al., 2014). Human thermoneutrality without clothing occurs at a temperature range of about 27–31°C (80.6–87.8°F), and with clothing at about 20–21°C (68–69.8°F) (Erikson et al., 1956; Kingma et al., 2012). Interestingly, human dorsal and pedal cutaneous nerve fibers begin to sense cold at 29.4 ± 2°C (85°F), approximately the temperature of unclothed thermoneutrality (Campero et al., 2001). In addition to this central relay, recent studies have identified at least two direct pathways by which cells can sense cold. First, murine brown adipocytes have a cold-sensing ion channel, transient receptor potential cation channel subfamily M member 8 (*Trpm8*), which is activated by temperatures below ~26°C (79°F) and stimulates thermogenesis via *Ucp1* (Bautista et al., 2007; Ma et al., 2012). In addition, subcutaneous white and beige fat cells can sense temperatures of 27–33°C (80.6–91.4°F) and respond via norepinephrine-independent thermogenesis (Ye et al., 2013). As well as triggering heat production in existing brown or beige adipocytes, cold exposure also can stimulate hyperplasia of BAT tissue, increasing future thermogenic capacity (Bukowiecki et al., 1982; Vitali et al., 2012). One of the genes upregulated by cold exposure in mice is PPAR-γ-coactivator-1α (*Pgc1α*), which in turn upregulates *Ucp1* expression and mitochondrial biogenesis, increasing the capacity for future heat production (Puigserver et al., 1998). In mice, beige cells persist after rewarming but lose *Ucp1* expression, although expression can return on cold challenge (Rosenwald et al., 2013). In both mice and humans, cold induces production of FGF21 and irisin, both of which contribute to browning of WAT by increasing UCP1 expression {Lee, 2014; Fisher, 2012}.

Thyroid hormone

In addition to directly mediating nonshivering thermogenesis, BAT mass, and *UCP1* expression, cold exposure can indirectly increase BAT activity via upregulation of

thyroid hormone. Thyroid hormone raises sympathetic tone, as seen in hyperthyroid Graves' disease, and cold-adapted humans exhibit seasonal increases in triiodothyronine (T3) levels (see discussion below). The effects of hypothyroidism on BAT are more complex. While one study suggested hypothyroidism impairs adrenergic-mediated cAMP production, attenuating BAT thermogenesis (Carvalho et al., 1996), a recent case study reported abundant BAT in a severely hypothyroid girl, which decreased with thyroid hormone replacement (Kim et al., 2014).

Food intake

One of the controversies about BAT is whether it contributes to diet-induced thermogenesis (DIT), the heat produced by metabolizing food. The idea that brown fat might play a role in body mass homeostasis originated with the observation that rodents fed an obesogenic cafeteria diet ate 80% more calories but became only 27% heavier than controls (Rothwell and Stock, 1979). Cafeteria-fed animals also exhibited a 30% increase in energy expenditure as measured by resting oxygen consumption, which was blocked by propranolol, an adrenergic inhibitor, and which returned to normal after 2 weeks of recovery on a normal diet. These data led the authors to conclude that the experimental caloric excess had stimulated DIT in brown fat, mediated by sympathetic tone, and that this increased energy expenditure had partially blunted the rats' weight gain. This hypothesis was consistent with studies demonstrating that rodents with ablated BAT and the *Ucp1* knockout (KO) mouse were susceptible to high fat diet-induced obesity and diabetes, particularly with age (Lowell et al., 1993; Hamann et al., 1998; Kontani et al., 2005). However, by tracking blood flow using microspheres and by measuring arteriovenous O_2 in brown fat, Ma et al. (1988) showed that cafeteria diet in rats increased whole body resting O_2 consumption, but did not increase O_2 consumption in brown fat. Furthermore, at thermoneutrality, wildtype and *Ucp1* KO mice exhibited similar increases in O_2 consumption when placed on an obesogenic diet, and similar weight gain, with *Ucp1* KO mice exhibiting resistance to diet-induced obesity only below thermoneutrality (Golozoubova et al., 2001; Liu et al., 2003; Anunciado-Koza et al., 2008; Feldmann et al., 2009). More generally, the idea that BAT thermogenesis evolved to maintain body mass by using up excess energy has been criticized on evolutionary grounds, since energy excess is unlikely to have been a common mammalian problem in the premodern environment (Kozak, 2010).

Exercise upregulation of the newly identified protein irisin

One of the most surprising findings to emerge about brown and beige fat is that exercise can trigger BAT thermogenesis. Bostrom et al. (2012) demonstrated in a mouse model that exercise causes production of a previously unknown peptide hormone called irisin, which is made via upregulation of *Pgc1α* in muscle and cleavage of the prohormone fibronectin type III domain-containing protein 5 (FNDC5). *Pgc1α* is a central regulator of mitochondrial abundance, glucose metabolism, and adaptive thermogenesis (Handschin and Spiegelman, 2006), and prior studies had shown a role for this gene in brown and beige fat thermogenesis, along with other factors (Puigserver et al., 1998; Cao et al., 2004). In mice, irisin was shown to induce browning of subcutaneous WAT depots to produce beige fat, leading to an increase in energy expenditure and improved glucose homeostasis. In both mice and humans, plasma irisin levels increased following exercise, but anti-FNDC5 antibodies blunted the exercise-induced increase in *Ucp1* expression in mice (Bostrom et al., 2012). Irisin also increased energy expenditure and improved glucose metabolism in mice with diet-induced obesity (Bostrom et al., 2012). These data led the authors to hypothesize that many of the health effects of exercise are mediated via increased irisin production.

The finding that irisin could mediate browning of WAT to beige fat, at least in mice, generated particular excitement, as it suggested a functional link between exercise and BAT activation. However, while irisin is still being characterized, it should be noted that not all data are consistent with the model that irisin is an exercise mimetic. For example, irisin is higher in obese vs. normal weight individuals, and rises and falls in parallel with gain and loss of body mass (Crujeiras et al., 2014). Also, exercise does stimulate norepinephrine release in proportion to its intensity, and given that β-adrenergic receptors in BAT are responsive to sympathetic tone, it follows that high intensity exercise, in particular, could stimulate BAT. However, catecholamine release with exercise is blunted with training, such that spikes in norepinephrine release may be greater in untrained or sedentary individuals.

It remains to be seen how the data from obese humans can be integrated into the concept of irisin upregulation by exercise. More problematically from an evolutionary perspective, it is entirely unclear why it would be beneficial for exercise to stimulate BAT thermogenesis, thereby increasing both the energetic cost and the heat produced by activity. One hypothesis is that irisin upregulation of BAT may have evolved as an alternative to shivering (Bostrom et al., 2012; Lee et al., 2014a), but that this mechanism is also triggered by the muscle contractions of exercise. This hypothesis is consistent with a human study showing an inverse correlation of shivering and BAT upregulation (Ouellet et al., 2012). However, cold already stimulates BAT thermogenesis via multiple mechanisms, including direct sensation by cells, sympathetic tone, and thyroid hormone levels (discussed above), so it remains to be explained why an additional pathway that also causes increased BAT thermogenesis during exercise would be adaptive, unless this mechanism has some other unknown function.

Cardiac natriuretic peptides

Another stimulus for BAT is cardiac natriuretic peptides, hormones involved in fluid homeostasis that also stimulate lipolysis in primates, particularly in response to cold, exercise, and food intake (Sengenes et al., 2002; Birkenfeld et al., 2005; Birkenfeld et al., 2006; Birkenfeld et al., 2008). Bordicchia et al. (2012) recently showed that in a mouse model, cardiac natriuretic peptides and β-adrenergic signaling have additive stimulatory effects on BAT, and induce browning in WAT. Therefore, exercise has the potential to increase BAT activity not only through β-adrenergic signaling and irisin, but also through the heart muscle. In the short term, this mechanism increases the availability of fatty acids to the heart muscle during exertion, but constant

elevation of cardiac natriuretic peptides levels is associated with high BMI and the metabolic syndrome (Sugisawa et al., 2010; Khan et al., 2011). Thus, as with irisin, the relationship between elevated cardiac natriuretic peptides and exercise is complex, and such elevations may not always be beneficial.

Psychological factors

If increased sympathetic tone is the final common pathway for BAT activation, then it is possible that psychological stress responses could also trigger nonshivering thermogenesis. It is clear that sympathetic agonists can cause pharmacological BAT activation. Some studies have shown experimentally induced psychological stress stimulates BAT thermogenesis in rats (Mohammed et al., 2014), while others have not (Marks et al., 2009). The effect of psychological stress on human BAT merits further study.

Suppressors of BAT

Although current clinical interest is heavily focused on how to upregulate BAT for weight loss, it is equally relevant to understand what downregulates or suppresses BAT. While suppressors of BAT function are not yet well studied, there is some evidence that BAT is downregulated in lactation (Krol et al., 2011), and by the hormones ghrelin (Mano-Otagiri et al., 2009), neuropeptide Y (NPY) (Shi et al., 2013), and insulin (Klein et al., 2000), although insulin may also upregulate BAT under some conditions (Valverde et al., 2003).

The finding that orexigenic hormones such as ghrelin and NPY suppress BAT is consistent with data suggesting BAT function is suppressed in women with active or previous anorexia, even following weight recovery (Bredella et al., 2012; Pasanisi et al., 2013). This pattern likely reflects NPY function, which is to increase the drive to eat but decrease sympathetic outflow to BAT (Shi et al., 2013). However, NPY does not change in response to cold exposure and thus is not the mediator of cold-induced hyperphagia (Bing et al., 1998).

Assessing ecogeographic variation in human BAT thermogenesis

While BAT function has not yet been directly assessed in cold-adapted human populations, a number of other physiological factors have been assessed that are known to interact with BAT function. It is well documented that in populations indigenous to the circumpolar region, basal metabolic rate (BMR) is seasonally elevated in males (+7–19%) and females (+3–17%) (Leonard et al., 2002; Leonard et al., 2005). For example, in the Yakut (Sakha) people of Siberia, BMR in both males and females is about 6.5% higher than would be expected based on body mass and 20.8% higher than would be expected based on fat-free mass, and these differences cannot be explained by high protein intake (Snodgrass et al., 2005). Cold-induced increases in BMR are mediated in part by thyroid hormones, and prior work has shown correlations of free thyroxine (T4) and elevated BMR in another indigenous Siberian population, the Evenki (Leonard et al., 1999). Yakut thyroid function follows a seasonal pattern, with lower free T3 and free T4 and higher thyroid stimulating hormone (TSH) in the winter as compared to the summer months (Levy et al., 2013). While this pattern may seem counterintuitive, it is thought to result from both greater production and greater uptake of T3 in the cold, such that circulating levels of the hormone fall. This phenomenon is known as the "polar T3 syndrome" (Reed et al., 1990), and is seen in other circumpolar populations, including the Inuit (Andersen et al., 2012), naval officers serving in Antarctica (Reed et al., 1986; Reed et al., 1990), and Finns (Hassi et al., 2001), who also showed higher winter TSH. The pattern is less clear for T4, with various studies reporting increased (Levine et al., 1995), decreased (Plasqui et al., 2003), and unchanged winter levels (Reed et al., 1986; Reed et al., 1990; Hassi et al., 2001). It is worth noting that T3 rather than T4 is the primary regulator of BMR, and BAT tissue is capable of converting T4 to T3 (Symonds and Lomax, 1992), so the level of T3 rather than T4 is of primary interest. It remains unclear whether TSH has effects on BAT independent of its modulation of thyroid hormone production.

These data support the premise that circumpolar modern humans exhibit cold-mediated increases in sympathetic tone and thyroid function that are known to stimulate BAT in other mammals. Thus, it is reasonable to hypothesize that some of the higher energy expenditure observed in circumpolar populations such as the Yakut reflects BAT thermogenesis, particularly since the changes in both energy expenditure and thyroid function are more pronounced in individuals living a more traditional vs. more urban lifestyle (Snodgrass et al., 2006; Levy et al., 2013). However, quantifying BAT activity in the field is challenging. As described above, 18FDG-PET-CT and MRI provide precise data about BAT volume and activity *in vivo*, but these modalities are expensive and unwieldy in field settings. A practical alternative is thermal imaging, which has been used since the 1970s to measure changes in skin temperature from BAT thermogenesis (Rylander, 1972; Rylander et al., 1972; Rothwell and Stock, 1979). Recent studies have shown the feasibility of detecting BAT activation inexpensively and noninvasively in animal models and in humans, with the caveat that this technique can only detect temperature changes fairly close to the skin surface.

In rodents, Crane et al. (2014) recently measured dorsal skin temperature in wildtype and *Ucp1* KO mice after injection with vehicle or with a β3-adrenergic agonist, CL-316,243. Infrared imaging revealed agonist-induced increases oxygen uptake and dorsal interscapular skin temperature in wildtype but not *Ucp1* KO mice, demonstrating this technique can detect BAT-mediated changes in skin temperature in mice. Although humans lack extensive interscapular BAT depots beyond infancy, the supraclavicular BAT depot can be imaged. In studies including children, teens, and adults, infrared thermography showed a supraclavicular temperature increase following a standard cool challenge (Lee et al., 2011a; Symonds et al., 2012). Taken together, the human and animal studies support the utility of infrared thermography to measure BAT activity noninvasively in field settings, and such studies are currently underway.

Potential evolutionary variation in BAT thermogenesis

Beyond variation in modern humans, it is interesting to consider the possible contribution of BAT upregulation to hominin expansion outside the temperate zone. African climates encompass a wide range of diurnal

temperature variation, but beginning with *H. erectus*/*ergaster*, hominins venturing out of Africa would have been exposed to more extreme seasonal temperatures. Both Neanderthals and circumpolar modern humans successfully colonized the arctic zone thanks to a range of ecogeographic adaptations to cold, including broad bodies with shortened distal limb segments (Pearson, 2000). Steegmann (2002) has proposed that Neanderthals would have relied on BAT for warmth, along with fire, shelter, and clothing (Wales, 2012). While this hypothesis is reasonable given the data on BAT function in living populations, it is difficult to test directly. Data on genomic variation in hominin cold genes are currently limited, and thus far differences in *UCP1* have not been reported. Sazzini et al. (2014) studied variation in 28 genes involved in nonshivering thermogenesis in living populations from varying climate zones, as well as in one Neandertal and one Denisovan, both from Siberia. Overlap between modern humans and the archaic samples was seen at particular loci. For example, a derived allele of SNP rs1137101 on the leptin receptor gene (*LEPR*), which is critical for energy homeostasis and fat metabolism as well as mitochondrial heat dissipation, is common to the Neandertal, the Denisovan, and 87% of East Asians, while a derived allele for *PPARGC1B* is common to the two hominins sampled and >84% of modern humans. Overall, however, BAT-derived alleles showed only 2.9% overlap between archaic and modern populations, suggesting largely independent mechanisms of cold adaptation. Consistent with these findings, a recent study of Neanderthal introgression in modern humans identified Neanderthal-derived alleles associated with skin and hair color and with diseases including Crohn's disease, lupus, and Type 2 diabetes, but did not report introgression of BAT-related genes (Sankararaman et al., 2014). However, the recent finding that BAT activity is linked to higher bone mass raises the possibility that signatures of BAT thermogenesis might be detectable in the bones of cold-adapted hominins.

Brown fat and bone

In humans and in animal models, there is a consistent positive association between metabolically active BAT and bone mass, although it remains unclear whether BAT has direct osteogenic effects, or whether bone and BAT are positively correlated with a third factor, such as lean body mass. In humans, the quantity of cold-activated BAT was positively related to bone mineral density (BMD) in young, lean women (Bredella et al., 2012), and to femoral total area, cross-sectional area, and thigh muscle area in men and women across wide ranges of age and BMI (Bredella et al., 2014). Lee et al. (2013a) found a positive correlation of active BAT and BMD in female subjects, but not in males, and BAT volume was also a significant predictor of femoral total cross-sectional area and cortical bone area in children and adolescents (Ponrartana et al., 2012). In both younger and older subjects, the positive relationship between BAT and bone mass may be mediated by positive effects of muscle tissue on both BAT mass and bone mass (Ponrartana et al., 2012; Bredella et al., 2014).

The data demonstrating positive correlations of BAT and bone mass were all obtained from US populations living at artificial thermoneutrality. In contrast, several studies in indigenous cold-adapted populations have shown accelerated bone loss compared with nonindigenous groups beginning in the 5th decade of life (Mazess and Mather, 1974; Mazess and Mather, 1975; Thompson and Gunness-Hey, 1981; Harper et al., 1984). In the past, this pattern of bone loss was attributed to high protein diet, but this hypothesis did not fit the ontogenetic pattern that bone density appeared to be normal until about age 40. Lazenby (1997) showed the Inuit exhibit accelerated age-related bone loss and suggested the cause could be cold-induced hyperthyroidism, which in clinical studies has been associated with dose-dependent bone loss in both the trabecular and cortical compartments due to increased bone turnover (Allain and McGregor, 1993; Schneider et al., 1994; Schneider et al., 1995). Thus, while the data from US populations linked active BAT with higher bone mass, data from cold-adapted humans, who would presumably have even more active BAT, suggested increased bone loss, at least in older individuals. It is difficult to reconcile these data without more detailed measurements of BAT and BMD from circumpolar individuals, but data from animal models with loss of function of *Ucp1* offer some insight into potential mechanisms.

Animal models for impaired BAT function include the Misty mouse, which has a mutation in the gene *Dock7* that leads to delayed BAT function. Lower *Ucp1* expression and thus impaired BAT thermogenesis during early postnatal life was linked to elevated sympathetic tone, bone loss due to an imbalance between formation and resorption, and higher marrow fat, with females more severely affected than males (Motyl et al., 2013). Bone loss was reduced by the β-blocker propranolol, implicating increased sympathetic tone as the mechanism. Other intriguing observations have come from heterotopic ossification, a condition of pathological bone formation within muscle. In this disorder, brown fat cells initiate local hypoxia, leading to a cascade of osteogenic activity including blood vessel formation, chondrogenic activity, and finally osteogenesis (Olmsted-Davis et al., 2007). These data led Motyl and Rosen (2011) to propose that cold-induced elevation of sympathetic tone could cause bone loss, consistent with earlier studies showing that sympathetic activation and β-adrenergic signaling can decrease bone mass (Takeda et al., 2002), and that brown adipose tissue may protect against this bone loss.

A second potential mechanism by which BAT could affect bone mass was recently identified in the $FoxC2_{AD}^{+/Tg}$ mouse, which overexpresses forkhead box C2 (*Foxc2*) in adipocytes (Rahman, 2013). *Foxc2* is a transcription factor that mediates BAT development and mitochondrial function, and $FoxC2_{AD}^{+/Tg}$ mice were known to have high beige fat. However, these mice also exhibited high bone mass due to high bone turnover, with formation outpacing resorption. In vitro, exposure to conditioned media from $FoxC2_{AD}^{+/Tg}$ adipocytes increased the activity of osteoblasts and decreased levels of the anti-osteogenic protein sclerostin in osteocytes, suggesting the presence of an unidentified factor produced by these adipocytes that was anabolic to bone (Rahman et al., 2013).

Although more work is needed, the animal studies suggest two mechanisms by which BAT can increase or maintain bone mass. First, BAT thermogenesis may protect bone mass by blunting cold-induced increases in sympathetic tone. BAT senesces in humans with aging, and sympathetic tone increases, both of which may contribute to age-related bone loss (Motyl and Rosen, 2011). Cold-dwelling individuals might experience greater

increases in sympathetic tone and/or thyroid hormone levels as BAT regresses with age, leading to accelerated bone loss relative to individuals in more temperate climates. A second potential mechanism involves brown adipocyte secretion of a thus far unidentified endocrine or paracrine factor that increases osteogenic activity and bone turnover (Rahman et al., 2013).

WAT, BAT, or shivering?

The fact that active BAT is relatively common in adult humans raises the question of how its advantages and disadvantages compare to other thermogenic mechanisms, including exercise, shivering, subcutaneous WAT insulation, and increasing BMR. A comparison of the costs and benefits of each of these strategies suggests humans have an increased reliance on WAT and decreased reliance on BAT thermogenesis or other thermogenic mechanisms. The heat produced by the body is usually expressed in watts (W), equivalent to joules per second (J/s), with 1 J/s equal to 0.00024 kilocalories/s (kCal/s). Humans at thermoneutrality generate an average of about 0.7–1.5 W/kg body mass (Henry, 2005), corresponding to about 1,450 kCal/day for a 70 kg human. As noted above, BMR can increase by up to 17–19% during seasonal cold exposure (Leonard et al., 2002, 2005), which would cost about 250 extra kCal/day. Shivering increases heat production by 3–5 times resting metabolic rate (Eyolfson et al., 2001), corresponding to 2.1–7.5 W/kg body mass, or 125–450 extra kCal per *hour*, and thus is only a short term strategy. Exercise is more thermogenic than shivering, with low and high intensity exercise producing ~20–35 W/kg muscle, respectively, but energy is also being expended in mechanical work (Krustrup et al., 2003). In contrast, maximum BAT heat production in rodents is reportedly on the order of 300–400 W/kg (Foster and Frydman, 1978, 1979; Heldmaier and Buchberger, 1985). If the heat production of human BAT were equivalent to that of a rodent, 40–50 g of (maximally stimulated) BAT would burn 290–340 kCal/day, leading to the commonly cited estimate that BAT could consume up to 20% of an individual's daily caloric expenditure (Rothwell and Stock, 1979). However, this estimate is based on the mouse, which is potentially problematic since total body metabolic rate scales with negative allometry in mammals (Kleiber's law), and tissue-specific energy expenditure likely does as well (van Marken Lichtenbelt and Schrauwen, 2011). According to van Marken Lichtenbelt and Schrauwen (2011), 45–70 W/kg, or 50–75 kCal/day, would be a more realistic estimate for heat production by human BAT. Such energy expenditure would not induce rapid weight loss, but might be enough to stave off the gradual weight gain that most adults exhibit (Mozaffarian et al., 2011). Unfortunately from a weight loss perspective, a recent study that quantified the energy expenditure from mild cold exposure found that active BAT consumed only 15–25 extra kCal/day (Muzik et al., 2013). Thus, while BAT is a more efficient strategy for thermogenesis compared with shivering and exercise, it does not appear to burn many calories in humans under typical conditions.

This finding is consistent with data suggesting that compared with other primates, humans have shifted their thermogenic strategy away from nonshivering thermogenesis in BAT and toward passive insulation by subcutaneous WAT. Humans are among the fattest mammals both at birth and throughout life, particularly compared with other primates and other tropical mammals (Altmann et al., 1993; Wells, 2006, 2009). Both human ability to store WAT and its ontogenetic patterning are distinct from other taxa. Although infant fatness at birth varies, humans average 15% vs. less than 2% in most other mammals (Kuzawa, 1998). The gain in fat mass continues after birth before slowing in later infancy and childhood, and then rebounding around puberty (Wells, 2006). Humans are also highly encephalized, and our greater ability to store WAT compared with other taxa provides a stable energy source to buffer our large, energetically expensive brains during fluctuations in food availability (Leonard et al., 2003). Indeed, females having higher relative body fat than males at every life stage, and periods of particular vulnerability, including pregnancy, lactation, and rapid neonatal brain growth, are associated with especially substantial fat reserves (Wells, 2006). This pattern of greater reliance on WAT to buffer the energy demands of the brain has the additional consequence of reducing the need for inducible brown fat in humans as compared with other mammals, since the additional WAT carried on our bodies provides insulation as well as stored energy.

Clinical data from humans are consistent with this hypothesis. In studies comparing lean and obese subjects, cold upregulated BAT activity in both groups, but the response was blunted in individuals with higher body mass and %body fat (Himms-Hagen, 1984; Nicholls and Rial, 1999; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). In the morbidly obese (BMI > 35 kg/m^2), BAT activity was markedly low, seen in only 20% of subjects (Vijgen et al., 2011). BMI and %body fat explained 64% and 56% of the variation in BAT response to cold, respectively, and BAT activity declined steeply as BMI increased from 22 to 27 kg/m^2 and body composition increased from 10 to 20% fat, with little BAT activity at BMI > 35 kg/m^2 and body fat >30–40% (Levy et al., 2013).

This pattern is consistent with changes in overall energy expenditure in response to cold stress in lean vs. obese humans, although these measurements reflect nonexercise activity thermogenesis and/or heat produced in muscle tissue as well as by BAT (Levine et al., 1999; Wijers et al., 2008). As little as one hour of mild cold stress (15°C) elicited a three-fold difference in energy expenditure between lean and obese subjects (Claessens-van Ooijen et al., 2006). A similar pattern was seen after 48 h at 16°C, with obese subjects exhibiting smaller and more variable increases in energy expenditure compared with lean subjects, particularly at the highest BMIs (Wijers et al., 2010). The authors concluded that WAT insulation protected obese subjects from heat loss, reducing the need for cold-induced energy expenditure, as seen in other studies (Hayward and Keatinge, 1981; Savastano et al., 2009). In support of this relationship, Pasanisi et al. (2013) found that 7 of 7 constitutively lean subjects (mean BMI 16.2) had active BAT, while only 3 of 24 normal weight controls (mean BMI 22.2) did, at room temperature. In contrast, Bredella et al. (2012) found 4 of 5 healthy controls (mean BMI 21.9) had active BAT after experimental cooling. This difference suggests constitutively lean subjects began relying on BAT at higher temperatures compared to subjects with normal BMI.

It is not yet clear whether reduced BAT function causes individuals to gain weight, and/or whether high WAT mass makes nonshivering thermogenesis

unnecessary and prevents individuals from becoming cold enough to initiate the "browning" needed to create beige fat. Comparative data on adipose distribution in cold-dwelling humans do not strongly support WAT accumulation as a thermogenic strategy (Kuzawa, 1998). However, the pattern of excessive WAT and low BAT activity seen in overnourished humans at thermoneutrality is likely not representative of the functions of these depots at normal body mass and particularly in cold temperatures. Data from animal models suggest several direct links between obesity and impaired BAT function. One recent study found that mouse visceral WAT contains a precursor population with the potential to become WAT or BAT, and that caloric excess drives these preadipocytes to the WAT vs. the BAT lineage, providing a mechanism by which weight gain could be a cause rather than a consequence of BAT senescence (Lee et al., 2012). Another study showed that cells from a mouse beige adipocyte precursor population can function as white adipocytes in caloric excess (Wu et al., 2012). Finally, overnutrition in rat pups during lactation increased WAT gain, as expected, but also reduced BAT function, such that overnourished rats had lower *Ucp1* expression and a lower thermogenic response to cold challenge later in life (Xiao et al., 2007). These data suggest mechanistic tradeoffs between WAT and BAT in mice and perhaps in humans, supporting a possible role of BAT dysfunction in the obesity epidemic.

Brown fat and the obesity epidemic

From a biomedical perspective, the fact that brown and beige fat burns rather than stores energy represents a therapeutic opportunity to use BAT agonists in the treatment of obesity. In other words, increasing the number of inducible brown adipocytes and/or increasing their metabolic activity would promote weight loss by increasing energy expenditure. However, there is no such thing as a free lunch, and efforts to induce BAT thermogenesis pharmacologically face at least three major challenges.

First, there is no reason to expect that the negative energy balance generated by pharmacological activation of BAT would be treated differently by the body than any other caloric deficit. Cold exposure, exercise, and hyperthyroidism, which are physiological upregulators of BAT thermogenesis, all stimulate hunger and hyperphagia, and it is likely that pharmacological BAT stimulation would similarly increase caloric consumption, negating any caloric deficit. In humans, there does appear to be an inverse correlation of BAT and WAT volume, which might mean that people with more BAT are better able to burn off excess caloric intake. However, BAT activity is also inversely related to age, Type 2 diabetes and obesity, and it is thus far unclear whether low BAT activity is a cause or consequence of these diseases (Pfannenberg et al., 2010; Ouellet et al., 2011; Lee et al., 2013b).

Second, the way BAT burns calories is by generating heat, and it is unclear how pharmacological approaches would avoid this side effect. Unstimulated BAT does not generate much heat or burn many calories. Individuals with rare benign brown adipose tissue tumors called hibernomas and pheochromocytomas have significant quantities of brown fat, but may or may not exhibit significant weight loss (Ricquier et al., 1982; Lean et al., 1986; Fukuchi et al., 2004; Manieri et al., 2010; Petrak et al., 2013). Hyperthyroidism involves both high sympathetic tone and higher BAT metabolism as measured by glucose uptake, but no reported increase in the number of brown adipocytes (Lahesmaa et al., 2014). Consequently, obesity intervention requires both an increase in brown or beige fat tissue volume and increased activation of nonshivering thermogenesis in this tissue.

Finally, the long term side effects of using BAT upregulation to treat obesity are unknown. Data from animal models have shown that cold causes oxidative stress in multiple tissues, potentially due to hyperthyroidism (Venditti et al., 2010), which could lead to long-term cell damage. Cold-induced activation of epicardial BAT has been linked to deposition of atherosclerotic plaque, potentially leading to heart disease. Epicardial fat contains brown adipocytes, which might help protect the heart muscle and coronary arteries from temperature drops (Heaton, 1972; Sacks, 2009). While there is some evidence that the loss of these perivascular fat cells could increase progression of atherosclerosis (Chang et al., 2012), there are also data suggesting perivascular BAT contributes to heart disease. Specifically, Dong et al. (2013) recently studied cold-induced BAT upregulation in two genetic knockout mouse strains used to model atherosclerosis, the apolipoprotein $E^{-/-}$ ($ApoE^{-/-}$) and LDL $receptor^{-/-}$ ($Ldlr^{-/-}$) mice. In both strains, 4–8 weeks of cold exposure caused increases in plasma low density lipoproteins, arterial plaque deposition, inflammation, and microvascular growth, and in the $ApoE^{-/-}$ mouse, knockout of the *Ucp1* gene prevented plaque accumulation (Dong et al., 2013). This mechanism might be a contributing factor to the epidemiological link between cold temperature and increased incidence of heart attacks and strokes (Bhaskaran et al., 2010; Palmer, 2010). However, these studies also raise the possibility that pharmacological *UCP1* activation in humans could increase the risk of heart disease, particularly in the population who would be likely candidates for a BAT-based antiobesity drug. The approach of weight loss via increased sympathetic activity has already been tried in the weight loss drug sibutramine (Meridia), a norepinephrine reuptake inhibitor that gained FDA approval in 1997 but was withdrawn in 2010 following observed increases in heart attack and stroke. In rats, sibutramine dose-dependently increased oxygen consumption and body temperature, along with an 18-fold increase in glucose uptake by BAT (Connoley et al., 1999), suggesting it reduced obesity at least in part via BAT activation.

While pharmacological BAT activation could have deleterious side effects, physiologically mediated BAT stimulation via exposure to cooler temperatures might provide the same benefits with less risk. Several recent studies have suggested that indoor heating has contributed to obesity by reducing cold-induced thermogenesis (Keith et al., 2006; Johnson et al., 2011; Moellering and Smith, 2012). In a recent study in humans, 10 days of mild cold exposure was sufficient to increase BAT activity and improve subjects' comfort in the cold (van der Lans et al., 2013). Even short-term lowering of indoor temperature from 24°C to 19°C increased BAT thermogenesis by an average of 10%, although individual responses were highly variable (Chen et al., 2013). However, as noted above, mild cold exposure only burns an extra 15–25 kCal/day, suggesting much lower temperatures would be needed to change body mass (Muzik et al., 2013).

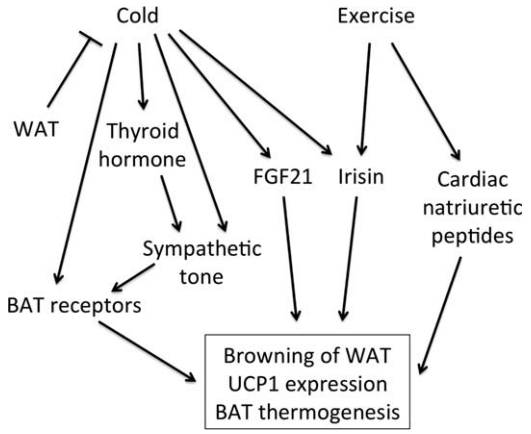

Fig. 4. Model for BAT activation. BAT is upregulated following cold or exercise and suppressed in obesity. Key hormonal upregulators include thyroid hormone, irisin, and cardiac natriuretic peptides.

Discussion and future directions

The rediscovery of functional brown adipose tissue in adult humans raises a host of interesting questions for human evolutionary biology and for biomedical approaches to the obesity epidemic. It is now clear that there are two distinct brown adipose depots in humans, a constitutive interscapular depot in neonates that regresses after birth, and inducible brown adipocytes that are found within white adipose depots throughout life, but have impaired function in obesity, aging, and diabetes. Although both brown and beige adipocytes express *UCP1* and produce heat, they derive from different precursors, and are induced and activated by partially independent mechanisms. The primary mechanism for beige fat induction is cold exposure that induces browning within WAT depots, either directly or via increased thyroid hormone levels. Absent a need for nonshivering thermogenesis, as in warm climate or obesity, beige fat precursors are present, but do not become BAT, and can function similarly to white adipocytes. Beige adipocytes induced by cold challenge contribute to nonshivering thermogenesis, and latent beige adipocytes remain for some period of time afterward. Beige adipocytes also appear to protect and possibly increase bone mass, both by limiting the cold-induced increase in sympathetic tone and via an unidentified secreted factor.

These findings allow us to create a testable model for BAT activity in humans (Fig. 4). In industrialized humans living at thermoneutrality, robust BAT function is induced by cold challenge at 16–19°C (60.8–66.2°F). However, as discussed above, humans can sense cold, and BAT thermogenesis can be activated, as soon as temperatures fall below thermoneutrality (27–31°C, 80.6–87.8°F). Sympathetic tone and particularly thyroid hormone are known to increase BAT thermogenesis in humans, while obesity suppresses BAT activity. Thus, in lean individuals in seasonally cold climates, we expect BAT thermogenesis and corresponding metabolic cost to increase in winter and fall in summer. These annual excursions would be greater in children, who have higher surface area:volume ratios compared to adults, and in women, who are shown to have higher BAT activity than men despite higher %body fat. BAT activity would be blunted or absent in warmer climates and in individuals who are rarely exposed to cold, including the obese.

A crucial area for future research is the ontogenetic plasticity of beige fat: can these cells be induced at any age, or are there critical windows for formation, e.g., during childhood or adolescence? A related question is how labile beige fat cells are once formed. How long do beige fat cells persist after cold challenge is removed, and does positive energy balance impair thermogenesis by causing these cells to behave more like white adipocytes? The answers to these questions will help to delineate the "normal" amount and activity of BAT in humans across the lifespan, and in warmer vs. cooler climates. For example, based on the model above, the seasonal changes in metabolic rate seen in circumpolar populations could mediate metabolically significant fluctuations in beige fat quantity and activity, while the magnitude of seasonal changes in BAT activity might be much smaller in the temperate zone.

To elucidate the function of BAT in extant humans and its role in combating the obesity epidemic, more data are needed to determine whether the body's caloric intake adapts to higher energy expenditure in BAT, as it does for other types of energy expenditure, and whether BAT activation has side effects including cardiovascular risk. More work on how exercise affects BAT function in trained athletes should also help to resolve the question of the relationship between BAT, exercise and shivering. The prevailing hypothesis, that BAT stimulation by muscle contractions is an adaptation that evolved to limit shivering, is consistent with experimental data. However, exercise upregulation of irisin and BAT function remains puzzling, given that it would seem to worsen hyperthermia and waste energy. Perhaps there is a counterregulatory mechanism to suppress BAT thermogenesis during exercise that remains to be identified.

Finally, in terms of human evolutionary history, the capacity to form beige fat may have been a key adaptation for hominin expansion to new environments, including out of Africa. Given that BAT is present in extant humans and a variety of other primates, it is likely that hominins have had active brown and beige fat throughout evolutionary history, and particularly in cold climates (Steegmann et al., 2002; Steegmann, 2007). Although this hypothesis is difficult to test directly, genomic comparison among Neanderthals, Denisovans, modern humans from different climates, and the other great apes might reveal signatures of selection on brown and beige fat function. Further, it remains to be determine why active BAT is associated with higher bone mass. More work in humans and primates from varied climates and controlled experiments in animal models will be needed to fully delineate how interactions between brown and beige fat, metabolism, and bone have shaped human evolutionary history.

ACKNOWLEDGMENTS

Thanks to Abigail Bigham, Lisa Nevell, John Speth, and three anonymous reviewers for thoughtful discussion and comments that greatly improved this manuscript.

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