Published in final edited form as:

Endocrinol Metab Clin North Am. 2016 September ; 45(3): 605–621. doi:10.1016/j.ec1.2016.04.010.

Brown and Beige Adipose Tissue:

Therapy for Obesity and Its Comorbidities?

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Keywords

Brown adipose tissue; Beige adipose tissue; Energy expenditure; Thermogenesis; Obesity; Type 2 diabetes; Lifestyle intervention

INTRODUCTION

Overweight and obesity are now among the greatest health challenges in both developed and developing countries. In 2013, worldwide estimates for the percentage of overweight/obese adults was 36.9% for men and 38% for women, and the prevalence in children and adolescents—23.8% for boys and 22.6% for girls is equally alarming.¹ The situation in the United States is even more dire, where 65% of US adults are overweight/obese, and 32.2% and 25.3% of children (aged 2–19 years) are overweight and obese, respectively. Obesity is associated with a number of comorbidities, including the metabolic syndrome, insulin resistance, cardiovascular diseases, type 2 diabetes, and cancer, all of which impact the social and economic burden on the population, and is contributing to the substantial increase in direct and indirect cost of health care. Thus, there is an urgent need to identify new therapeutic strategies to combat obesity and its comorbidities.

Obesity is a chronic condition caused by excess energy intake relative to energy expenditure, creating positive energy balance and weight gain. Much of this extra energy is stored in the form of triacylglycerols in white adipose tissue (WAT). One of the strategies to reverse obesity is to generate “negative energy balance” with a focus on decreasing energy intake and increasing energy expenditure.² Understanding the physiologic, cellular, and molecular mechanisms of energy intake, storage, and expenditure to achieve whole body energy homeostasis may allow us to identify effective and durable (treatment) strategies for treating and managing obesity. One of the exciting possibilities of brown adipose tissue (BAT) is that it can oxidize fatty acids and glucose, and thus could in theory, dissipate energy in the form

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of heat via uncoupling mechanisms. This approach is supported by the strong negative association between BAT and body weight, body fat, and visceral fat.^{3–5} Thus, further understanding of the genetic, molecular, and physiologic attributes of BAT may generate empirical data to support the idea that upregulation of BAT activity could lead to the discovery of a novel, effective, and durable therapy for treating and managing obesity. However, the significance and viability of this approach is the subject of some skepticism. In this review, we focus on how brown/beige fat cell activation may impact obesity therapy and its potential to mitigate obesity-related comorbidities.

FEATURE CHARACTERISTICS, AND PHYSIOLOGIC AND METABOLIC PHENOTYPE OF THERMOGENIC ADIPOCYTES

In mammals, thermogenic adipocytes are classified into BAT and the recently discovered “brownlike”/“beige”/“brite” adipocytes.⁶ BAT is highly vascularized, is characterized by a light pink color, and is densely innervated by the sympathetic nervous system. Brown adipocytes are packed with multilocular small vacuoles that are rich in mitochondria that contain dense cristae.⁷ In rodents and small mammals, the most classical brown fat presentation is around the upper back (interscapular), the kidney (perirenal), and heart (periaortic) regions. In humans, active BAT was traditionally thought to be restricted to newborns and the early childhood period, and was viewed as a natural defense mechanism against hypothermia.^{8–10} More recently, several groups of investigators have reported functional active BAT in healthy adult humans using a combination of radiolabeled glucose tracers, 2-deoxy-2-[¹⁸F]-fluoro-D-glucose ([¹⁸F]-FDG), and PET and computed tomographic (CT) scanning technology.^{3, 4, 11, 12} These data show that BAT is present in the upper trunk, including the cervical, supraclavicular, paravertebral, and pericardial regions, and to a lesser extent in the mediastinal and mesenteric areas.^{3, 4, 11} Glucose uptake and perfusion of the tissue are both increased in human BAT in response to cold, indicating active thermogenesis.¹³ The amount of active BAT in humans is heterogeneous, but the exact mass has never been measured precisely. Estimates suggest that healthy humans have about 50 g of active BAT (approximately 0.1% of body mass).^{14, 15}

Beige adipocytes have similar morphologic features to classical brown adipocytes, with central nuclei, multilocular lipid droplets, and are rich in mitochondria.¹⁶ Beige adipocytes arise upon external cues, such as stimulation of sympathetic activity during chronic cold exposure or administration of β_3 -adrenergic receptor agonists, or exercise. Unlike brown adipocytes, which seem to have a discrete anatomic location, beige adipocytes reside within the WAT depot, mainly in inguinal WAT in rodents. In humans, there remains uncertainty regarding the presence of both the precursor of white adipocytes committed to “browning” or functional beige adipocytes, and whether trans-differentiation of white into beige adipocytes is indeed a real physiologic phenomenon. Several reports suggest that human BAT, particularly from the neck region, represent both the classical brown as well as beige adipocytes found in mice.^{6, 17–19} Recent findings, using a combination of both RNA sequencing and genome-wide expression analysis of a preadipocyte lineage derived from supraclavicular BAT of nonobese individuals, found a smaller population (10.8%) of uncoupling protein (UCP)⁺ human adipocytes that were representative of beige

adipocytes.²⁰ To support this observation, they also reported the presence of potassium channel K3 and mitochondrial tumor suppressor 1, which are molecular markers of beige adipocyte differentiation and thermogenic function. Nonetheless, the study of beige adipocytes in humans remains challenging because (1) [¹⁸F]-FDG-PET/CT scans do not distinguish brown versus beige adipocytes, and (2) investigations on the biological stimulation and thermogenic features of inducible brown adipocytes in humans is difficult owing to the requirement of more than minimally invasive intervention to assess the mass and activity.

Classical brown and beige adipocytes originate from distinct cellular lineages. Classical brown adipocytes share the same cellular lineage as skeletal myocytes. During embryonic development, classical brown adipocytes arise from Engrailed-1 (En1)-expressing cells in the central dermomyotome,²¹ and a myf5⁺ cell lineage, which is a similar progenitor as that of skeletal muscle myocytes. Transcriptional profile data from recent microarray experiments support the view that brown preadipocytes and myocytes share a common lineage.²² In addition, high-resolution quantitative mass spectrometry has been used to show that the mitochondrial proteomic profile of brown fat is more similar to skeletal muscle than to white fat cells.²³ The transcriptional coregulator PRD1-BF-1-RIZ1 homologous domain-containing protein-16 (PRDM16) acts as a switch that controls the bidirectional cell fate between myoblasts and brown preadipocytes.²⁴ Loss of PRDM16 from brown preadipocytes favors a muscle cell differentiation program, whereas ectopic expression of PRDM16 in myoblasts induces programming that leads to brown fat cell differentiation.²⁴ The cellular origin of beige adipocytes is also complex. It is thought that, depending on the metabolic milieu, beige adipocytes emerge from a myf5⁻ lineage of precursor cells similar to white adipocytes.²⁵ However, it has been shown that a subset of beige adipocytes can also originate from myf5⁺ cells.²⁶ Recently, Long and colleagues²⁷ used a ribosomal profiling approach to demonstrate that a subset of beige adipocytes (about 10%–15%) originated from a smooth muscle-like precursor driven by Myh11. Clonal analysis of adipogenic cell lines from the inguinal WAT in rodents led to the realization that beige and white adipocytes are distinct cell types with distinctive molecular profiles.⁶ Distinct cellular origin and molecular signatures at the precursor stage between brown/beige and white adipocytes have also been found in adult humans.^{20, 28} These data suggest that (1) brown fat cells originate differently from beige or white fat cells, (2) brown adipocytes share a similar developmental program with myocytes, and (3) beige adipocytes may be derived from a heterogeneous population of cell lineage.³

Another unique biochemical and molecular feature of brown and beige adipocytes is the expression of thermogenic proteins. A key thermogenic protein is UCP1 (also called thermogenin), a 33-kDa protein located in the inner mitochondrial membrane that functions as a proton and anion transporter.²⁹ The role of UCP1 in BAT has been elegantly demonstrated by Nicholls and colleagues.³⁰ Activation of UCP1 is mediated by free fatty acids (FFAs), particularly long-chain fatty acids.^{31, 32} Beige adipocytes resemble white adipocytes in that they have an extremely low basal expression of UCP1, but after full differentiation, beige adipocytes turn into UCP1⁺ cells, which is similar to classical brown adipocytes. Consequently, beige adipocytes are classified as nonclassical/inducible brown

adipocytes.⁶ Of note, the process of generating beige adipocytes is called “beiging” or “browning.”

White (WAT) and brown (BAT) adipose tissue have distinct physiologic functions and this is particularly evident when we consider whole body energy homeostasis. Under conditions of increased energy intake, WAT maintains energy homeostasis by storing extra energy in the form of triacylglycerols and by breaking down this stored energy into FFA and glycerol to generate energy in the form of adenosine triphosphate when energy demands increase. In contrast, BAT predominantly promotes energy expenditure. It can dissipate chemical energy by oxidizing glucose and lipids for heat generation via uncoupled mitochondrial respiration mediated by UCP1 in a process called adaptive thermogenesis. The number of visceral white fat cells increases when there is a positive energy balance, whereas new brown fat cells are generated in response to cold temperature. Beige adipocytes are thought to be associated with the maintenance of energy balance, and this may be similar for classical brown adipocytes.

THE MECHANISM OF BROWN ADIPOSE TISSUE ACTIVATION AND BROWNING OF WHITE ADIPOSE TISSUE

The thermogenic activity of BAT is stimulated by cold exposure and activation of the sympathetic nervous system via β 3-adrenergic agonism.³² Upon cold exposure norepinephrine binds to β 3-adrenergic receptors that are coupled with G-proteins on the cell surface of brown adipocytes. This leads to activation of adenylate cyclase, catalyzing the formation of cyclic adenosine monophosphate (cAMP), which in turn activates the cAMP–protein kinase A signaling pathway. β 3-Adrenergic stimulation generates both acute and chronic effects on BAT. Acutely, stimulation of β 3-receptors results in activation of UCP1. In brief, stimulation of the cAMP–protein kinase A pathway induces hormone-sensitive lipase, adipose triglyceride lipase, and monoacylglycerol lipase, enzymes that regulate the hydrolysis of triacylglycerols, diacylglycerols, and monoacylglycerides, respectively, leading to the release of FFAs.³² These FFAs are imported into the mitochondria where they bind and activate UCP1^{33, 34} and are oxidized through cellular respiration, amplifying the free flow of protons across the inner mitochondrial membrane,³⁵ and the rapid release of chemical energy as heat.^{30, 32} Besides activation of lipolysis, norepinephrine also increases glucose transporter and lipase expression to increase glucose and FFA uptake, and thus provide the substrate that is needed for thermogenesis to proceed. The long-term effect of β 3-adrenergic stimulation results in upregulation of the thermogenic gene program (UCP1), mitochondrial biogenesis, and both hyperplasia and hypertrophy of BAT,³⁶ resulting in increased total BAT mass. In humans, long-term effects can occur through cold acclimation and this results in a 37% increase in BAT volume, comparable changes are observed in rodents.³⁷ The mechanism requires upregulation of the cAMP-protein kinase A pathway, followed by activation of the transcription factor CREB, which is bound to the CRE promoter within the thermogenic program (UCP1),³² or upregulation of the mitogen-activated protein kinase and extracellular signal-regulated kinases 1/2 pathways that control mitochondrial biogenesis and oxidative phosphorylation genes, such as PGC-1 α .³⁸

The mechanism of browning of WAT was recently reviewed by Warner and Mittag.³⁹ They concluded that browning of WAT can be achieved by different mechanisms. First by the activation of the central nervous system through modulation of sympathetic output to WAT. This mechanism is primarily mediated by neurons in the hypothalamus, including proopiomelanocortin⁴⁰ and agouti-related peptide⁴¹ producing neurons. Second, browning of WAT can be achieved by the recruitment and activation of immune cells (such as eosinophils and the interleukin [IL]-14/13 signaling pathway,⁴² or ILC2 immune cells^{43, 44}) to WAT; or lastly by direct action of stimuli on beige adipocyte precursor cells. Current nonpharmacologic and pharmacologic strategies for WAT browning was reviewed by Lee and Greenfield,⁴⁵ and can be categorized into sympathomimetics (β 3-adrenergic receptor agonists), nonadrenergic cold mimetics (eg, fibroblast growth factor [FGF]21 and irisin), peroxisome proliferator activated receptor- γ agonists, or capsaicin/capsinoidlike compounds.

MODULATION OF BEIGE ADIPOCYTE FORMATION IN HUMAN INTERVENTION TRIALS

Exercise Intervention

Exercise is associated with numerous health benefits, but from a metabolic perspective one of the most important is whole-body glucose homeostasis via adaptation in insulin-sensitive tissues, including fat, liver, and skeletal muscle. Although adaptation in skeletal muscle is perhaps the most widely studied, changes in the structure and function of adipose tissue is also important. In WAT, exercise decreases adipocyte size^{46, 47} and lipid content, which reduces adiposity. At the cellular level, exercise increases mitochondrial protein abundance and activity, and these responses are mediated by endothelial nitric oxide synthase and PGC-1 α .^{48–51} Recent data suggest that exercise may also induce “beiging” of subcutaneous WAT. When previously sedentary mice increase physical activity, there is an increased expression in the “beige” adipocyte marker genes including UCP1, Prdm16, Cidea, Elovl3, PGC-1 α , PPAR γ , Tbx1, and other related markers of BAT or beige adipocytes.^{49, 50, 52, 53} This increased gene expression is also associated with increased UCP1 immunofluorescence.

The mechanism responsible for exercise-induced “beiging” of subcutaneous WAT may be related to irisin (gene: *FNDC5*), an exercise-induced myokine that works through the PGC-1 α pathway. Boström and colleagues⁵² reported that irisin stimulates the expression of UCP-1 in white adipocytes, stimulates beige adipocyte formation and improves metabolic outcomes. However, the role of irisin in “beige” adipocyte development in humans remains controversial. Although some research groups have reported that circulating irisin is increased acutely after exercise,^{54, 55} several groups have not been able to detect or confirm a role for irisin in humans, and there has been a failure to demonstrate a positive correlation between plasma irisin levels following exercise training and the browning of fat in humans.^{56–60} Moreover, genomic sequencing of human *FNDC5* reveals that humans have an alternative start codon for *FNDC5* (ATA), whereas rodents have a highly conserved ATG start codon.⁶¹ Thus, humans may have a lower translational efficiency for the gene, and this is verified by in vitro data showing that only 1% of the full-length *FNDC5* protein is

translated with an ATA start codon. This difference may explain some of the contradictory observations on circulating irisin levels in humans and rodents.⁶¹ In addition to the confusion surrounding the detection of circulating irisin levels and the response to exercise training, recent findings also challenge the view that irisin may play a beneficial role in obesity therapy. It seems that irisin is not only a myokine, but it is also an adipokine,⁶² and plasma irisin levels are unexpectedly higher in obese humans.^{63, 64} Furthermore, Viitasalo and colleagues⁶⁵ found a positive and unfavourable correlation between plasma FFAs and irisin in overweight children. More reliable and sensitive detection methods and greater in depth study of the mechanistic regulation of irisin are required to establish whether irisin is indeed a mediator of pathways that propagate obesity, or whether irisin has potential as a therapeutic target for treating or managing obesity.

In addition to irisin, there are several other proteins that may mediate exercise-induced beige adipocyte biogenesis. IL-6 is a myokine that is released from contracting skeletal muscle cells,⁶⁶ and it may impact metabolism in adipose tissue.⁶⁷ Knudsen and colleagues⁶⁸ found that exercise training increased the expression of IL-6 in inguinal WAT, and upregulated UCP1 messenger RNA expression in wild-type control mice, but not in IL-6 knockout mice. However, cold exposure failed to stimulate the expression of genes responsible for brown fat activation in this model. In 2014, Rao and colleagues⁶⁹ reported that muscle PGC-1 α 4 overexpression in mice induces the expression of a protein that they named meteorin-like hormone. This protein was linked to the promotion of “beige” adipocyte formation via eosinophil-dependent M2 macrophage activation. Other metabolites secreted from skeletal muscle after exercise, such as lactate⁷⁰ and β -aminoisobutyric acid⁷¹ have also been associated with the “beiging” of subcutaneous adipose tissue.

Dietary Intervention

Food ingestion leads to an increase in body temperature. This physiologic response is called diet-induced thermogenesis (DIT) and is associated with an increase in energy metabolism and activation of taste and smell senses by the intraoral sensory nervous system, as well as an increase in energy for food digestion and absorption.⁷² Using an overfeeding rat model (cafeteria diet), Rothwell and Stock¹⁵ performed seminal studies that established the role of DIT in energy balance. They found that overfeeding stimulates an adaptive response mechanism that serves to eliminate excess calories by activating the thermogenesis pathway. The responses include increased body temperature, energy expenditure, and resting oxygen consumption, and these responses are directed through BAT thermogenesis.

All macronutrients (lipid, carbohydrates, and protein) stimulate thermogenesis. However, the effect of high-fat feeding on BAT activation in rodents remains controversial. Some data suggest that increased fat consumption reprograms BAT,^{15, 73} whereas other data conclude that there is no, or only a marginal increase in BAT mass after a high-fat diet.⁷⁴ Despite the controversy regarding BAT mass, there is general agreement that high-fat feeding upregulates UCP1 expression in BAT in rodents,⁷⁴ whereas in humans, high-fat feeding seems to increase BAT activity.⁷⁵ The source of dietary fat is an important determinant of the effects of diet on UCP1 upregulation; polyunsaturated fatty acids have the most potent effect.^{74, 76} There are several reports that DIT is a significant mechanism for weight loss

induced by a high-protein diet. This is supported by the observation that a high protein diet has a higher thermic response and higher energy expenditure than a high carbohydrate diet.⁷⁷ Moreover, protein type also impacts DIT, with whey protein having a greater thermic effect compared with casein or soy.⁷⁷ Although a protein rich diet stimulates DIT, the effect of a high protein diet on BAT activation is less clear. When a high protein diet was compared against a high-fat diet, the protein diet did not affect UCP1 messenger RNA expression in BAT.⁷⁸ In addition to the major nutrients, several food ingredients have been shown to activate the thermogenic response in BAT through stimulation of transient receptor potential (TRP) channels (such as TRPV1, TRPM8, and TRPA1) that release norepinephrine.⁷⁹ Capsaicin, and its pungent analogs (capsinoids; capsiate, dihydrocapsiate, and nordihydrocapsiate) found in red peppers, has been shown to increase energy expenditure and promote weight reduction in individuals who have greater amounts of BAT than in BAT⁻ subjects.⁸⁰ Other food ingredients that have been reported to affect BAT activation include green tea extract,⁸¹ curcumin,^{82, 83} and ginger extract.⁸⁴

Bariatric Surgery

Physical reconstruction of the gastrointestinal tract through bariatric surgery is the most effective therapy for weight loss and the remission of type 2 diabetes. The mechanisms that facilitate these improvements are unclear. However, there are data to suggest that gastric bypass surgery improves energy expenditure in rodent models as well as in humans.^{85–89} Whether the improvement in energy expenditure is due to increased BAT activation after surgery is unknown. Vijgen and colleagues⁹⁰ used [¹⁸F]-FDG-PET/CT imaging to assess BAT activation and found greater activation 1 year after laparoscopic adjustable gastric binding surgery; however, the number of patients was small and definitive conclusions require larger studies. The mechanisms that determine how bariatric surgery may promote higher BAT activation remain elusive. One possible mechanism may act through bile acids, nutrient-responsive hormones that modulate energy balance. Obese subjects have a lower postprandial excursion of conjugated bile acids than lean individuals. Roux-en-Y gastric bypass surgery essentially normalizes the meal-induced increase in conjugated bile acids in morbidly obese subjects. The effect is evident within 2 weeks after surgery⁹¹ and is sustained for at least 40 weeks.⁹² Another possible mechanism is activation of the sympathetic nervous system signaling pathway by natriuretic peptides. Roux-en-Y gastric bypass surgery increases B-type natriuretic peptide expression in humans,⁹³ and Neinast and colleagues⁹⁴ have shown that Roux-en-Y gastric bypass increased natriuretic peptide receptors 1 and 2, B-type natriuretic peptide, and β -adrenergic receptor messenger RNA expression in gonadal adipose tissue. This adaptation was associated with increased UCP1 expression, which is consistent with increased BAT and a greater capacity for energy dissipation through thermogenesis.

FAT MASS AND OBESITY GENES AND BROWNING OF ADIPOSE TISSUE

Genome-wide association studies have helped to identify fat mass and obesity (FTO)-associated polygene variants in chromosome 16 as the most relevant to date for human obesity.^{95–97} Several investigators report a strong correlation between genetic variance within the FTO gene and body weight regulation and fat mass, primarily in single nucleotide

polymorphism variants located at the first intron of FTO.^{95, 98} Frayling and colleagues⁹⁵ found that 16% of adults who were homozygous for the risk allele A, weigh an average of 3 kg more, and have a 1.67-fold increased risk of obesity compared with those not inheriting a risk allele. Furthermore, Dina and colleagues⁹⁸ demonstrated that the T allele of single nucleotide polymorphism rs1121980 located in the first intron of the FTO gene was strongly associated with severe adult obesity. The FTO gene is widely expressed throughout multiple tissues and organs, so how FTO plays a role in the development of obesity is unclear. The first mechanistic study using loss of function of the FTO gene in mice showed resistance to diet-induced obesity owing to an increase in daily energy expenditure without changes in BAT mass and brown fat UCP1 expression.⁹⁹ In contrast, mice overexpressing the FTO gene have increased body and fat mass, but with no difference in energy expenditure.¹⁰⁰ Additional studies in FTO deficient mice have shown upregulation of UCP1 expression in gonadal and inguinal WAT, and this is associated with increased WAT browning and uncoupled respiration in white adipocytes, suggesting that FTO is significant for the induction of a brown adipocyte phenotype.¹⁰¹ Recent data by Claussnitzer and colleagues¹⁰² show that the single nucleotide variants rs1421085 of the FTO allele is associated with a reduction in mitochondrial thermogenesis. T-to-C single nucleotide variants within the ARID5B repressor were identified, which led to an increase IRX3 and IRX5 expression, causing a developmental shift from energy-dissipating beige adipocytes to energy-storing white adipocytes, with a reduction in mitochondrial thermogenesis by a factor of 5 and an increase in lipid storage capacity. Manipulation of the ARID5B-rs1421085-IRX3/5 regulatory axis by knocking down IRX3/IRX5 in primary adipocytes from participants with the risk allele reversed the obesity signature as observed by an increase in thermogenesis.¹⁰² All of these findings suggest that the role of FTO in the control of energy homeostasis and body composition may be mediated by its role in regulating white adipocyte browning.

CONTRIBUTION OF BROWN ADIPOSE TISSUE THERMOGENESIS TO ENERGY EXPENDITURE

Energy balance is composed of 2 main variables: energy intake, which is derived from ingested food, and energy expenditure in the form of heat and physical work. An imbalance between either of these 2 variables results in storage of excess energy in the form of fat (positive balance; ie, weight gain), or burning of fat storage (negative balance; ie, weight loss). From the total of energy ingested in human, about 4% to 8% of energy is lost during digestion and 3% to 5% leaves the body in the form of urine and through the skin.¹⁰³ Thus, approximately 90% of the ingested energy is metabolized^{14, 104} and can be used as an expendable energy source. van Marken Lichtenbelt and Schrauwen¹⁴ categorized this metabolizable energy into obligatory and facultative. The obligatory energy expenditure is vital, and covers the energy spent preserving normal cellular, tissue, and organ function (maintaining whole body temperature, respiration, protein turnover and other basic functions of tissues), DIT (energy for digestion and metabolism of ingested nutrients), and physical activity. Facultative energy expenditure covers adaptive thermogenesis, including cold-induced shivering and nonshivering thermogenesis, as well as exercise and nonexercise activity thermogenesis. The amount of expendable energy varies from person to person and is affected by age, gender, weight, height, body composition, environmental temperature,

hormonal status, genes, and stress level.¹⁰⁵ Energy spent maintaining whole body metabolism accounts for the greatest amount of the total daily resting metabolic rate, about 55% to 65%.¹⁴ Physical activity is the most variable and easily altered component, and depends on an individual's body mass and the intensity, duration, and frequency of exercise, as well as how efficient the individual is in performing the activity. Physical activity usually accounts for about 25% to 35% of the total daily metabolic rate, with a sedentary person expending less and a physically active person expending more energy.

Adaptive thermogenesis is defined as the process of heat production in response to environmental cues, such as changes in temperature or diet to protect the organism from cold exposure, or to regulate energy balance in response to change in calorie intake.³⁶ Maintenance of body temperature in small mammals or hibernating animals is achieved primarily by the ability of BAT and skeletal muscle to generate heat.¹⁴ BAT thermogenic activity is minimal in a thermoneutral environment. With cold exposure the BAT thermogenic program is activated and this physiologic response is evident both in animals as well as humans.³⁷ In mice, the maximal heat producing capacity of BAT is approximately 300 W/kg, and this is the highest metabolic rate among mouse tissues.³² In contrast, exposure to more comfortable warmer temperatures leads to a suppression of thermogenesis, owing primarily to a reduction in sympathetic drive to BAT.³⁷

The potential contribution of BAT thermogenesis to whole body energy expenditure in humans was elegantly reviewed by van Marken Lichtenbelt and Schrauwen.¹⁴ It is estimated that there is 40 to 60 g of BAT (approximately 0.1% of body mass) in a healthy human.^{3, 11} Rothwell and Stock¹⁵ estimated that BAT activity may contribute up to 20% of daily energy expenditure in a lean healthy human. However, this estimate is based on assumptions that BAT can be maximally activated, and allometric relations were not taken into account when extrapolating energy expenditure in tissues/bodies of animals that differ in size.¹⁴ When allometric corrections are included, Wang and colleagues¹⁰⁶ have estimated that BAT energy expenditure in humans is 80 W/kg, and with a 20% contribution from daily energy expenditure, a lean healthy human would need about 200 to 360 g of BAT (approximately 0.3 to 0.5% of body mass in a 70-kg human),¹⁴ which is considerably higher than the first estimate. Based on [¹⁸F]-FDG-PET/CT or ¹⁵O-PET imaging observations,^{3, 11, 14, 107} a BAT mass of 40 to 60 g is likely for a healthy human. Thus, when fully activated BAT could account for approximately 2% to 5% of basal metabolic rate in a human.¹⁴ Recently, Muzik and colleagues¹⁰⁷ examined the role of BAT on whole body energy expenditure in BAT⁺ and BAT⁻ human subjects. During thermoneutrality, whole body energy expenditure was similar for the 2 groups. Exposure to cold increased total energy expenditure in the BAT⁺ group. It was estimated that human BAT contributed up to 25 Kcal/d, which represents approximately 1% of total energy expenditure in an average person. By extrapolation, if 1% of daily energy expenditure represents an energy consumption of 100 Kcal/d, this translates into a loss of approximately 0.25 kg of fat per year.¹¹ Such a negligible amount is unlikely to impact the overweight and obesity problem that is facing the US population.

When these estimates are combined with physiologic and biochemical observations in humans and mice, the case for BAT and beige adipose tissue in the management or treatment of obesity in humans becomes challenging. Based on the observations reported, arguments

against BAT and beige adipose tissue as current therapeutic targets for humans include (1) the presence of beige adipose tissue and the conversion of white fat cells into beige fat cells in humans is inconclusive at this point, (2) the capacity of BAT to contribute significantly to total daily energy expenditure is minimal and assumptions in favor of the alternative are based in part on a condition of continuous maximal activation, (3) obese people have modest BAT mass compared with lean individuals, and (4) pharmacologic stimulation of BAT in a thermoneutral environment would increase core body temperature, which may create an uncomfortable state including increases in respiration and/or perspiration.

THE ROLE OF BROWN ADIPOSE TISSUE BEYOND ENERGY BALANCE

Apart from its role in promoting energy expenditure for body weight regulation, activation of BAT may contribute to whole body physiologic and metabolic processes unrelated to weight modulation, but with a positive modulating impact on obesity-related comorbidities. Recent findings comparing BAT⁺ and BAT⁻ adults reveal that the BAT⁺ group has lower hemoglobin A_{1C}, total cholesterol, low-density lipoprotein cholesterol, and liver enzymes (alanine aminotransferase and aspartate aminotransferase).^{5, 108} These data suggest that activation of BAT may contribute to regulation of whole body glucose and lipid homeostasis, preventing states of insulin resistance, liver steatosis, cardiovascular disease, and immune system-related diseases. Possible mechanisms for these effects may be linked to the ability of BAT to channel blood glucose and lipid toward oxidation, or to secrete novel adipokines that target important metabolic tissues including the liver, skeletal muscle and pancreas.

There is considerable interest in the potential role of BAT as a mediator of glucose homeostasis and insulin resistance, and this is an area of active research. This work is primarily motivated by the need to find new therapies for treatment and prevention of type 2 diabetes. Because a reduction in fat mass typically improves insulin sensitivity, there is the potential that targeting BAT may uncover a new treatment approach. Recent reports from Chondronikola and colleagues¹⁰⁹ and Lee and colleagues¹¹⁰ are supportive of this line of work and the data suggest that glucose metabolism in BAT in adult humans is associated with improved peripheral glucose homeostasis and insulin sensitivity. Peirce and Vidal-Puig¹¹¹ reviewed the potential mechanism of action whereby BAT activation regulates glucose homeostasis. One likely scenario is that BAT activation facilitates an increase in glucose disposal. One human study in which the hyperinsulinemic–euglycemic clamp was used to assess insulin-mediated glucose disposal found an acute bout of mild cold exposure for 5 hours increased glucose disposal in BAT⁺ subjects, but this did not occur in subjects with undetectable BAT activation.¹⁰⁹ Based on empirical data, it was predicted that continuously activated BAT (approximately 70 mL) could clear approximately 20 to 30 g of glucose from the circulation over a 24-hour period.^{109, 112} In mice, the transcriptome profile of BAT was assessed using digital gene expression profiling and it was determined that cold exposure induced genes related to glucose metabolism,¹¹³ suggesting that the use of glucose was upregulated in these animals and this may contribute to increased whole body glucose disposal. This improvement in whole body glucose metabolism would attenuate glucotoxicity and may also reduce β -cell dysfunction.¹¹¹ In addition, the effect of BAT activation on glucose homeostasis may be mediated by proteins secreted from brown fat cells. One such protein is FGF21, which is highly upregulated in BAT during prolonged cold

exposure.¹¹⁴ FGF21 is recognized as a metabolic regulator owing to its glucose lowering effects¹¹⁵ and its role in promoting enhanced insulin sensitivity.¹¹⁶

The presence of BAT is also associated with a lower risk of nonalcoholic fatty liver disease (NAFLD) in human adults. Yilmaz and colleagues¹¹⁷ performed whole body PET-CT scans on 1832 patients and found that the odds ratio for having NAFLD was significantly higher in BAT[−] subjects, and that the maximal standardized uptake values of [¹⁸F]-FDG into BAT was significantly correlated with the ratio of mean liver attenuation to spleen attenuation. It is speculated that the role of BAT on attenuation of liver steatosis is mediated through brown fat adipokines, namely neuregulin (Nrg4). Nrg4 is a member of the epidermal growth factor family of extracellular ligands that are highly enriched in brown fat and expression and secretion is markedly increased during cold exposure and during brown adipocyte differentiation.^{118, 119} Analysis of serum Nrg4 level in humans has shown that NAFLD patients have significantly lower Nrg4 than non-NAFLD patients.¹²⁰ Gain-of-function and loss-of-function studies in mice have shown that Nrg4 protects against diet-induced insulin resistance and hepatosteatosis.¹¹⁹ The protective role of Nrg4 may be mediated by binding with the extracellular domain of receptor tyrosine kinase ErbB3 (Erb-B2 receptor tyrosine kinase 3) and ErbB4 (Erb-B2 receptor tyrosine kinase 4) in hepatocytes. This binding down-regulates hepatic de novo lipogenesis, which is mediated by the transcription factors LXR and SREBP1c through *Acc1*, *Scd1*, and *Fasn*.¹¹⁹

SUMMARY

Our understanding of the role of brown/beige adipose tissues on body weight in obese humans is evolving. Several unanswered questions remain, including the abundance of beige adipocytes, the mechanism of “WAT browning,” the contribution of BAT thermogenesis to whole body homeostasis, and the extent to which thermogenesis can be upregulated to have sufficient impact on weight loss in humans. Resolution of these issues will determine the future for BAT as a therapeutic target in obesity and obesity-related comorbidities.

Acknowledgments

Disclosure: The authors have received funding for NIH, R01 DK089547.

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KEY POINTS

- Our understanding of the role of brown (BAT)/beige adipose tissues on body weight in obese humans is evolving.
- Unanswered questions include the abundance of beige adipocytes, “browning” of white adipose tissue, contribution of BAT thermogenesis to homeostasis, and the extent to which thermogenesis can be upregulated to have sufficient impact on weight loss.
- Resolution of these issues will determine the future for BAT as a therapeutic target in obesity and obesity-related comorbidities.