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Brown Fat in Humans: The Significance of Thermogenic Active Tissue

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Abstract

Brown adipose tissue is a metabolically active form of fat in the body that performs a crucial function in non-shivering thermogenesis. It can be compared to the prevalent white adipose tissue which is generally understood to be energy storage in the body, with brown tissue performing an opposing role. The tissue itself contains unique gene and protein markers such as uncoupling protein 1 (UCP1) which allows for the thermogenic process inside the cell, burning lipids to do so. These gene and protein markers have proven to be crucial in the detection of brown adipose tissue, which had previously been thought to be lost in humans after early childhood. Activation and proliferation of brown adipose tissue has been linked with acute and chronic cold exposure, diet, obesity, age, and more. Ways to increase or monitor this are of considerable interest to the field of obesity studies. Insight into brown adipose tissue corresponds to insights into further energy expenditure processes in the body in areas such as muscles, potentially offering a wide variety of therapeutic options for obesity treatment.

Keywords

Brown Fat, Brown Adipose Tissue, Adipose Tissue, Human Brown Adipose Tissue, Thermogenesis

Disciplines

Analytical, Diagnostic and Therapeutic Techniques and Equipment | Nutritional and Metabolic Diseases | Physiology

Brown Fat in Humans: The Significance of Thermogenic Active Tissue

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Introduction

Brown adipose tissue is a metabolically active form of fat in the body that performs a crucial function in non-shivering thermogenesis. It can be compared to the prevalent white adipose tissue which is generally understood to be energy storage in the body, with brown tissue performing an opposing role. The tissue itself contains unique gene and protein markers such as uncoupling protein 1 (UCP1) which allows for the thermogenic process inside the cell, burning lipids to do so (Jung et al., 2018). These gene and protein markers have proven to be crucial in the detection of brown adipose tissue, which had previously been thought to be lost in humans after early childhood. The standard for detection of this tissue is in Positron Emission Topography (PET) and X-ray Computed Tomography (CT) with the measurement of ^{18}F-2-deoxyglucose uptake (Saito et al., 2009). However, issues such as the exposure of radiation during such measurements, the field of detection of this tissue is ever expanding (Okla et al., 2017).

Activation and proliferation of brown adipose tissue has been linked with acute and chronic cold exposure, diet, obesity, age, and more (Fenzl & Kiefer, 2014). Ways to increase or monitor this are of considerable interest to the field of obesity studies. Insight into brown adipose tissue corresponds to insights into further energy expenditure processes in the body in areas such as muscles, potentially offering a wide variety of therapeutic options for obesity treatment (Betz & Enerbäck, 2017). This paper will aggregate research into brown adipose tissue, with a focus on its presence in adult humans, and discuss characteristics of brown adipose tissue as well as what its unique thermogenic function means for current and future therapeutic techniques. It will progressively explain the anatomy of the tissue, creation and stimulation (including effect of factors such as diet), and its presence in adult humans. Subsequently, the full functioning of

thermogenesis will be detailed. Finally, future research perspectives and therapeutic techniques will be considered.

Anatomy and Morphology

Cell Overview

Brown adipose tissue (BAT) is a metabolically active form of adipose tissue, so it is functionally different than white adipose tissue deposits. The color distinction between the two reflects their functional difference, with the brown color of BAT stemming from the deposits of mitochondria, which contain high amounts of iron (Jung et al., 2018). Furthermore, in white adipose tissue, the lipid deposit is one large, singular droplet (unilocular) making up most of the cellular space itself. There is also a low mitochondrial density. In brown adipose tissue, the mitochondrial density is much higher, and there is a large amount of lipid droplets, smaller in size than in white adipose tissue, but much greater in number (multilocular). These droplets therefore have a much larger surface area to volume ratio, which likely assists in metabolite exchange with the mitochondria. The specialization and separation of these mitochondria allow the BAT to oxidize uncoupled fatty acids as well as generate adenosine triphosphate (ATP) for fatty caid activation (Benedor). BAT also has abundant vascularization compared to white adipose tissue. Another differentiating marker is the high amount of uncoupling protein-1 (UCP1) expression in BAT compared to white tissue, thanks to the protein's role in BAT thermogenesis (Fenzl & Kiefer, 2014).

Location in Body

At birth, human BAT deposits are primarily present in deposits between the scapula and around the kidneys. These are thought to provide core body temperature maintenance while the body is developing, but could have other neonatal functions as well (Jung et al., 2018). Recent developments concerning brown fat in humans have shown that BAT is not only present in

newborns, but adult humans as well. The deposits of BAT in adults are located in supraclavicular, axillary, and paravertebral regions around bones, as well as around major cardiac regions (around the aorta, carotid artery, and near the heart wall), lung bronchi, and some other solid organs (liver, spleen, pancreas, liver, and adrenal gland) (Jung et al., 2018).

Genetic Factors

Genetic indicators for different types of adipose tissue have been discovered, primarily through rodent studies. Certain marker genes in adipocytes correspond to the specific type of fat tissue (Fenzl & Kiefer, 2014). For white adipose tissue, TCF21 and Leptin genes have been identified, and BAT contains the marker genes LHX8 and XIC1 (Waldén et al., 2012) (Cypess et al., 2013). However, determining genetic markers for BAT in adult humans has proven to be significantly more challenging. These challenges have so far included a high degree of variability based on the anatomical location of the fat deposit, and conflicting results between BAT with similar markers to rodent BAT and BAT which contain markers for beige adipose tissue as well (Fenzl & Kiefer, 2014). UCP1-expressing adipose tissue does exist in distinct human deposits, but their presence seems to depend on a wide variety of factors such as sex, age, location of deposit, and metabolic state (Fenzl & Kiefer, 2014). Notably, brown fat seems to correspond to leanness in humans (Wang & Seale, 2016).

Beige Adipose Tissue

Adipocytes that contain many mitochondria and express UCP1 also develop within white fat tissue deposits with cold or other environmental stimuli (Harms & Seale, 2013). As these adipocytes lie outside brown fat deposits but perform a similar function of thermogenesis, they are commonly referred to as beige fat deposits (Wang & Seale, 2016). Their morphology as reflected by lipid droplet size and vasculature is intermediate between white tissue and brown tissue (Jung et al., 2018). When stimulated by stressors, these adipocytes differentiate into their

thermogenic active form from a low basal level of mitochondria and UCP1 expression similar to standard white adipose tissue (Fenzl & Kiefer, 2014). However, the exact method of this differentiation is as yet unclear. Beige adipose tissue could differentiate when stimulated from a precursor cell pool, interconversion from dormant to active states during stimulus, or a combination of both of these mechanisms (Jung et al., 2018).

One of the transcriptional co-activators mediating UCP1 expression, PGC1-α, also controls oxidative metabolism and mitochondrial biogenesis in other cell types. It is perhaps most prominent in muscle cells, and is linked with beneficial effects of exercise such as mitochondrial proliferation. PGC1-α has been shown to stimulate the gene FNDC5 to create the hormone irisin, which in turn stimulates surrounding adipose tissue to increase UCP1 expression and take on beige characteristics. Thus, exercise leads to the increase of metabolically active tissue types, which help maintain the benefits of exercise by further burning dietary substrates (Boström et al., 2012).

Brown Adipocyte Origins

Differentiation

While the pathways of brown adipocyte lineage are not fully determined, it is understood that they originate from the mesoderm during embryonic development, thus linking their origins closely with skeletal muscle, bone, connective tissue, and white adipocytes. During this stage, mesenchymal stem cells for adipose tissue can commit to Myf5-negative cells, which differentiate to white adipocytes, or otherwise to Myf5-positive cells, which differentiate to brown adipocytes and skeletal myoblasts (Fenzl & Kiefer, 2014) (Timmons et al., 2007), (Seale et al., 2008).

Genetic Commonalities

The commonality between cells of this lineage type has been mapped to the *Engrailed-1* (En1) gene (Atit et al., 2006). Those cells expressing this gene at different stages of development differentiate into skeletal muscles located in the back, dorsal dermis cells, or, and most relevant, brown adipocytes of the scapular and cervical regions. Evidence has so far determined a timeline of differentiation for cells expressing the En1 gene (Wang & Seale, 2016). Early expression of the gene is primarily linked with brown adipocytes in anterior deposits. Later expression is more open, leading to brown fat, muscle, and dermis cells (Atit et al., 2006). This body of evidence suggests that the En1 gene marks cells for brown fat differentiation, and cells that do not become brown fat must necessarily lose this marker before proceeding.

The evidence supports the idea that brown adipose tissue and skeletal muscle are closely linked in developmental stages. In the mitochondria, rather than similarity to white adipose tissue, BAT mitochondria proteomes are more similar to skeletal muscle (Forner et al., 2009). MicroRNAs, which help regulate the expression of protein-coding genes, demonstrate many forms, some of which are myogenic, found in muscle precursor cells. These types of MicroRNAs have too been found in brown adipocytes, a feature that can help define the precursor lineage between them and myocytes, as well as further clarify cell traits between energy-storing and energy-dissipating cells (Walden et al., 2009). These factors are just some of many in a long list of cell-fate determinators for BAT and muscle cells.

Dietary Factors in BAT Formation and Function

Beyond cold temperature activation and proliferation of BAT, another important subsection of BAT development is in dietary research. As the future of BAT research points toward ways to treat obesity, the connection to one's diet is of paramount importance. Breakthroughs in this area would allow for a twofold goal of dieting to treat obesity: limiting

excess dietary molecules that cannot be burned to limit their accumulation in the short term, and promoting BAT growth and activation to continue to burn accumulated molecules in the long term. It has been demonstrated that a high-fat diet leads to some adaptation of white adipose tissue ("browning", or creation of beige adipose tissue) to counter increased adiposity (García-Ruiz et al., 2015). Reviews such as Okla et al. discuss the interactions of many different dietary molecules with both the development and thermogenesis of BAT. As the discovery of BAT's significance in adult humans is still recent, the authors recommend more human trials, as well as analysis into combinations of dietary molecules that have a compound effect on BAT more than the sum of their individual effects (Okla et al., 2017).

Discovery of BAT in Adult Humans

BAT function in humans was long thought to be important for cold-temperature protection in infants, with thermogenesis of BAT thought to be halted in adulthood. From 2009 onward, the capability to stimulate BAT in adults has been experimentally demonstrated and research has proceeded into what this means for energy expenditure and metabolism (Fenzl & Kiefer, 2014). The most popular discussion around BAT in adults is its correlation with both temperature and obesity, and even some of the first studies detecting BAT in adults discuss its potential in treating obesity and other metabolic disorders (Cypess et al., 2009) (Virtanen et al., 2009). From these initial studies it has been revealed that cold activation of BAT increases energy expenditure, possibly providing insight into obesity treatments.

One of these initial 2009 studies by Virtanen *et al.* demonstrated the cold-induced glucose uptake by supraclavicular adipose-tissue deposits is increased by a factor of 15. Based on data from a section of tissue found in the experiment, cold-induced activation for 24 hours would have had 11 mmol of glucose taken up by the tissue. Furthermore, maximal activation of

the site would have increased this further, bringing the total estimated energy burn over the course of one year to be over four kilograms of adipose tissue. The study concludes with the determination that the findings identify functional brown adipose deposits. The conclusion is supported by the presence of BAT mRNA markers in the targeted tissue -- UCP1, DIO2, PGC1α, PRDM16, and ADRB3 -- and the protein UCP1, evidenced by western blot analysis and mitochondrial localization. These early findings for adult human BAT are significant because this evidence marks the beginning of a standard paradigm of evidence for human BAT -- UCP1 expression, high levels of mitochondria, and so on (Virtanen et al., 2009).

Another pioneering 2009 study by Saito *et al.* analyzes the uptake of 2-[^{18}F]fluoro-2-deoxyglucose (FDG) into adipose tissue using Positron Emission Topography (PET) and X-ray Computed Tomography (CT). The authors note that PET is a powerful tool for detecting malignant tumors via FDG tracking, yet sometimes detects symmetrical FDG uptake by tissues with no tumor present, in the shoulder and thoracic spine regions. Prior studies interested in these findings were designed to diagnose cancer more accurately, therefore Saito *et al.* was among the first to detect and evaluate for BAT itself. The study's results show a marked increase in FDG uptake in the supraclavicular and paraspinal regions with cold exposure, increased uptake in winter versus summer, increased uptake in younger subjects versus elderly, and increased uptake with lower obesity and BMI versus higher. These findings detail patterns of BAT function and activation that have informed the growing field of research since. Saito *et al.* concludes with the observation that trends in BAT activation in humans are similar to those in rodents, prompting a reevaluation of the relevance of prior studies (Saito et al., 2009).

Thermogenic Function of BAT

Non-shivering thermogenesis

The central principal of energy expenditure of BAT is its activation during cold temperature exposure. It's unique place in the body's biological processes allows it to metabolize from dietary substrates in the mitochondria. Historically, body temperature maintenance during cold exposure has been analyzed from a primary position of shivering thermogenesis, as shivering is the body's primary way method of accomplishing this goal. Chronic cold exposure eventually leads to a near-complete cessation of shivering, yet a persistent increased metabolic rate. Thus is the historical principal of non-shivering thermogenesis -- heat maintenance that replaces shivering (Cannon & Nedergaard, 2004). As one of BAT's characteristics is its increased vasculature over other types of adipose tissue, one of the first methods for determining its role in non-shivering thermogenesis was through measuring blood flow and oxygen consumption of the tissue. Foster and Frydman's 1979 experiment demonstrated that BAT indeed takes up oxygen in order to perform it's thermogenic role. In doing so, the author's concluded that BAT is the primary anatomical site of increased heat production in the cold, and considerably assists shivering in maintaining body temperature (Foster & Frydman, 1979). Subsequent research has concluded that, under the definition of "post-shivering thermogenesis", non-shivering thermogenesis is only accomplished by BAT (Cannon & Nedergaard, 2004).

In the cold, the sympathetic nervous system is stimulated to release norepinephrine. This catecholamine binds to β-adrenergic receptors on BAT, which prompts the transcription of UCP1 in the mitochondria. This sympathetic stimulation of the tissue is possibly the only route for the stimulation and recruitment of this tissue. β_3-adrenergic receptors interact with norepinephrine, beginning a cascade of signalling mediated by Adenylyl cyclase, cAMP, and protein kinase A (PKA) (Zhao et al., 1994). PKA marks a split pathway, mediating both lipolytic processes

directly creating heat, as well as the enhanced protein differentiation leading to UCP1 expression. Thus this split ensures both the process of thermogenesis and tools to do so (Cannon & Nedergaard, 2004) (Chaudhry & Granneman, 1999). The purpose of UCP1 is to uncouple respiration from ATP synthesis, generating heat (thermogenesis) (Fenzl & Kiefer, 2014). UCP1 is unique among similar proteins (UCP2, UCP3) because of its increased expression in the cold, performing a reactive thermogenic role that cannot be replicated by these other proteins (Nedergaard et al., 2001).

Involved Substrates

To fuel thermogenesis, the tissue takes in glucose. BAT has a very high uptake of glucose per gram of tissue, making it a major glucose-clearing organ despite its limited actual amount in the body. Inside the cell, triglyceride lipolysis triggered by catecholamines results in loose glycerol and free fatty acids. These free fatty acids are quickly captured by binding proteins, resulting in a low amount of free fatty acids in cytosol despite the high rate of lipolysis. These fatty acids are brought to the mitochondria and used as the primary substrate in thermogenesis by UCP1 (Cannon & Nedergaard, 2004).

It is known that fatty acids are involved in activation of UCP1, and there exists multiple theories for how this interaction occurs within the UCP1 complex. However, a review of the process by Nicholls and Locke advises an overall approach to the process rather than individual mechanical steps, citing Hess' constant heat summation law. As the purpose of the process is to create heat, which can be measured by initial reactants and final products, a general overview of UCP1's mechanisms should remain general (Nicholls & Locke, 1984). Thus, this general mechanism of fatty acids in to heat out has been proposed as three distinct mechanisms: an H^+ or OH^- uniporter activated by allosteric binding of fatty acids, a "stepping stone" model where the

fatty acid imbeds within UCP1's proton conducting channel to facilitate proton transfer, and a "flip flop" mechanism where fatty acids alternate across the mitochondrial wall and eventually carry in H^+ protons via the fatty acids' pronated form (Cannon & Nedergaard, 2004) (Fedorenko et al., 2012). Whatever the mechanism, fatty acids are integral to the process of UCP1 carrying protons into the mitochondria (Fedorenko et al., 2012).

This electrochemical gradient in the mitochondria is dispersed as heat. UCP1 uncouples oxidative phosphorylation from ATP regeneration. More specifically, the mitochondrial process without UCP1 activation proceeds along the electron transport chain, accomplishing this by using the proton gradient between the mitochondrial membrane and intermembrane space. UCP1 activation "short-circuits" the normal chain, which disrupts the gradient and uses it as energy (Betz & Enerbäck, 2017). This heat dissipation is rapid and significant; in a study by Liu et al., mice without UCP1 gradually exposed to cold demonstrated reduced metabolic efficiency. This suggests that UCP1-mediated non-shivering thermogenesis is the most calorie-efficient pathway of heating the body, and without it more costly methods and inefficient methods are resorted to (Liu et al., 2003).

Conclusion

Ongoing Clinical Significance

Obesity is a global epidemic with varied lifestyle and genetic factors affecting it, making the challenge of treating the disease on a systemic level one of the most daunting the medical field faces today (Mahmoud, 2022). Furthermore, the ongoing coronavirus 2019 (COVID-19) pandemic has overlapped itself on the obesity crisis, compounding many risk factors and creating much greater risk to the patient (Gammone & D'Orazio, 2021). While lifestyle treatment of the obesity crisis is its own field, BAT research has increasing become the focus of metabolism

research (Fenzl & Kiefer, 2014). Epigenetic drugs designed for the treatment of obesity have been created, targeting aspects such as insulin production and gluconeogenesis (Mahmoud, 2022). Appropriate utilization of genetic markers of BAT such as the En1 gene may lead to effective drug treatment of obesity via stimulation of BAT or white adipose tissue to take on a thermogenic role (beige tissue) (Atit et al., 2006) (Wang & Seale, 2016). Such is one theoretical example of the value of BAT to the study of obesity treatment.

Beyond simply BAT, thermogenic or otherwise metabolically active tissues are significant clinically and subject to ongoing analysis. Irisin, the novel hormone created from PGC1-α cascade in muscles following exercise, has the potential to be prepared as an injectable peptide. Further research into the effect of exercise on creating long-term metabolically active sites has the potential to extend the benefits of exercise far past the actual active period (Boström et al., 2012).

Rather than injectable peptides, a recent study by Wang et al. displays remarkable results concerning the artificial creation of entire metabolically active cells. These cells, dubbed human brown-like (HUMBLE) cells, were engineered using CRISPR/Cas9-SAM-gRNA to express UCP1 on human white adipocytes. These HUMBLE cells were injected into obese mice, and subsequently these mice displayed lasting glucose tolerance, increased energy expenditure, and insulin sensitivity. Such an implant was demonstrated to avoid side effects that typically arise from sympathomimetic drug injection, like increased blood pressure or heart rate. Thus, such a treatment adapted for human use potentially avoids uncomfortable or dangerous intervention in treating obesity (Wang et al., 2020).

Future Perspective

For humans, especially faced with a widespread obesity crisis, the significance of BAT and similar tissue types extends far beyond simply keeping the body warm in the cold. Not long ago was BAT thought to be an insignificant presence in adult humans, yet its role in adults is rapidly being realized. As an inverse to metabolically inactive storage tissues like white adipose tissue, BAT is a lasting point of metabolic activation, theoretically akin to a small, permanent increase in metabolic rate. Therefore, the discovery of this tissue in adult humans marks the beginning of an important paradigm in thermogenic activity, obesity treatment, exercise physiology, and more. Not least of all, this finding reveals the lasting possibility of "forgotten" aspects of the human body, as what is created in the human body from birth may be more present than previously thought, to serve possibly any biological process.

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