A Literature Review on Thermogenesis as a Prospective Obesity Treatment

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Abstract

The disease of obesity has quickly escalated from an epidemic to a worldwide pandemic in the last few decades, and viable treatment methods are being investigated with urgency. One such treatment method is increased induction of the thermogenesis pathway that is active in brown adipose tissue. This pathway is typically activated in mammals when they are exposed to cold temperatures for extended periods of time but can also be induced exogenously. Thermogenesis is a metabolically inefficient process that occurs in the mitochondria of a cell, meaning it uses substrate energy without generating ATP. The inefficiency of this process allows excess energy storage to be used and therefore serves as possible method of treatment. This review investigates the thermogenesis pathway and the ways it can be used to prevent and treat obesity.

A Literature Review on Thermogenesis as a Prospective Obesity Treatment

Approximately 42% of American adult citizens are impacted by obesity and roughly 173 billion US dollars go towards paying medical costs for this populace annually (Stierman et al., 2021). Not only can obesity be physically debilitating, but several other health detriments coincide with obesity including heart disease, type two diabetes, and certain cancers. Excessive fat tissue, formally termed adipose tissue, is a major health detriment in obese persons. New treatment methods have sought to uncover viable techniques to minimize adipose tissue levels. Recent advancements in this search for treatment have investigated the thermogenesis pathway in mammals as thermogenesis metabolizes excess energy storage to generate heat in response to cold temperatures. The aim of this review will be to analyze the thermogenesis pathway on a tissue and cellular level and determine the role thermogenesis may have in treating obesity.

As obesity rates steadily rise in this country, treatment methods are being established which include strict dieting, bariatric surgeries, and weight-loss medicines. More recent advancements in the search for weight loss treatment has examined the benefits of increasing energy expenditure as opposed to decreasing caloric consumption. This search led to the thermogenesis pathway which was initially discovered in hibernating animals and infants (Fenzl and Kiefer, 2014). Thermogenesis is stimulated in mammals when they are exposed to cold temperatures, and the shivering reflex is stimulated. The shivering reflex causes skeletal muscle filaments to move rapidly to generate heat and maintain internal body temperature. These skeletal muscles stimulate the sympathetic nervous system to secrete signaling molecules and through a cascade of events, fat cells dissipate energy as heat to maintain a high metabolic rate (Lee et al., 2014). This high metabolic rate started by the shivering reflex is spurred on by thermogenesis at the cellular level, meaning that shivering ceases and thermogenesis begins

during prolonged cold temperature exposure. The substrates catabolized in this process include fatty acids and glucose. The burning of these substrates gives thermogenesis the potential to be a sustainable therapy for various metabolic diseases. Because obesity develops due to energy intake exceeding energy expenditure, the high metabolic state that thermogenesis puts the body in could efficiently burn excess energy stored in obese persons. The uniqueness of brown adipose tissue, the cellular pathway of thermogenesis, and the treatment potential of thermogenesis will be assessed in this review.

The Distinctiveness of Brown Adipose Tissue

Thermogenesis is localized to brown adipose tissue. Until recently, brown adipose tissue was only thought to be present in hibernating animals and infants, but it has been proven that it is not only present, but active in adults. In 2009, functional brown adipose tissue in humans was officially recognized in the scientific community after imaging scans revealed cervical fat tissue with high metabolic activity (Maliszewska and Kretowski, 2021). Brown adipose tissue (BAT) has significant metabolic potential as it has been estimated that less than 50g of BAT can burn approximately 20% of daily basal caloric levels when operating at maximum capacity (Townsend and Tseng, 2012). The distinctiveness of brown adipose tissue will be summarized below.

Brown Adipocytes Compared to White Adipocytes

Adipose tissue is present in both white and brown forms, yet thermogenesis only occurs in brown adipose tissue. White adipose tissue is what one typically thinks of when thinking of fat tissue as its primary purpose is energy storage. In contrast, brown adipose tissue is specialized in metabolic activity. Adipose stem cells can either differentiate into white adipose tissue or brown adipose tissue. Myogenic factor 5 (Myf5) negative cells lead to the development of white

adipocytes, while Myf5-positive cells lead to the development of brown adipocytes or skeletal myoblasts (Fenzl and Kiefer, 2014). Whether Myf5 is activated or not is due to the presence of the transcriptional regulator PR domain containing 16 (PRDM16). Additional transcription factors that lead to the development of BAT will be outlined later in the insulin/IGF-1 signaling pathway.

Histologically, brown adipocytes are characterized by a high concentration of mitochondria, many small lipid droplets, dense vascularity, and centrally located nuclei (Townsend and Tseng, 2012). In contrast, white adipocytes differ morphologically as they have a single cytoplasmic lipid droplet, few mitochondria, and a peripherally located nucleus (Fenzl and Kiefer, 2014). Beige/brite adipocytes within white adipose tissue have the potential to be converted to brown adipocytes in a process called browning (Maliszewska and Kretowski, 2021). This process of browning will be explained in the treatment section of this review. The contrast between the histology of these three tissues can be seen in Figure 1. Additionally, BAT is innervated by the sympathetic nervous system and is highly sensitive to catecholamines, like adrenaline, dopamine, and noradrenaline, while WAT primarily serves as storage with little sympathetic innervation.

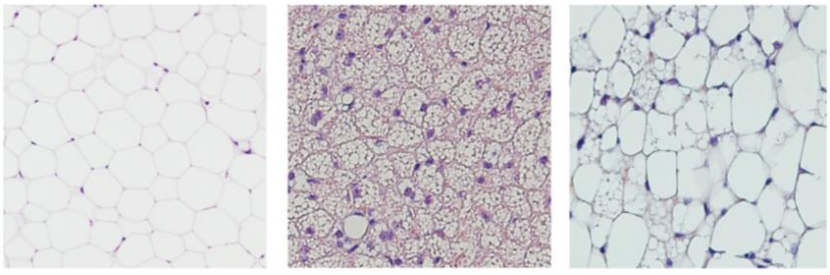

Figure 1. The histological differences between adipocytes in white adipose tissue (WAT), adipocytes in brown adipose tissue (BAT), and beige/brite adipocytes in WAT. Retrieved from "Brown adipose tissue and control of body weight: A new potential target for the treatment of obesity," by J.A. Villena, 2013, Obesity Epidemic, 1. Copyright [2013] by iConcept Press Ltd.

Brown Adipocyte Immune Response

In obese people, the immune system is constantly activated due to metabolic inflammation; however brown adipocytes differ from white adipocytes immunologically. Lipotoxicity, the harmful accretion of unoxidized long-chain fatty acids in non-adipose tissue, has a greater impact on WAT (Engin, 2017). Inflammation is often observed in WAT during lipotoxicity as cytokines and chemokines recruit immune cells to the adipocytes (Townsend and Tseng, 2012). The continuous growth of WAT causes constant hypertrophy, the growth of muscle cells, and constant hypoxia (Maliszewska and Kretowski, 2021). Hypertrophy and hypoxia cause macrophages to amass in WAT which increases the release of pro-inflammatory cytokines. Hypertrophied cells also secrete tumor necrosis factor alpha (TNFα), which is another type of cytokine that has a variety of phenotypic effects on the immune response. Adipose tissue itself also secretes adipose specific cytokines called adipokines, and one of these adipokines is monocyte chemoattractant protein-1 (MCP-1), which contributes to the accumulation of macrophages. Each of these features cause lipotoxicity to be more harmful and uncontrollable in WAT.

In contrast, BAT is not prone to a high degree of inflammation. Because BAT is highly metabolic and able to burn fatty acids, inflammation is hardly observed in this tissue because lipotoxic levels are never reached (Townsend and Tseng, 2012). Furthermore, the number of macrophages in BAT is low, and often undetectable (Maliszewska and Kretowski, 2021). Macrophages are organized in multinucleate ring-like structures in BAT and perform phagocytosis more often than cytokine secretion. In a 2017, a study observed that Macrophage concentration and inflammatory secretions are lower in BAT of obese mice. It also observed that macrophage marker genes, M1 and M2, were lower in mice living in cold temperatures as

opposed to higher levels in mice living in neutral temperatures (Dowal et al., 2017). Recently, it has been observed that brown and beige adipocytes release growth and differentiation factor 15 (GDF15), which mediates the downregulation of local inflammatory pathways as it inhibits macrophage cytokine activity (Campderros et al., 2019). Norepinephrine and cyclic adenosine monophosphate (cAMP) stimulate protein kinase A (PKA) to generate and release GDF15. Norepinephrine originates from sympathetic stimulation, while cAMP is generated within the thermogenesis pathway, as will be outlined below.

The Thermogenesis Pathway

When mammals are exposed to the cold, they first respond by shivering. During shivering, muscles break down adenosine triphosphate (ATP) to generate heat (Nedergaard, 2001). The breakdown of ATP describes the exergonic process where the phosphate bonds within the ATP molecule are broken to release energy, such as heat. Heat generated by ATP breakdown is the first line of defense against cold temperatures. However, physical shivering is only a temporary response to cold that is quickly replaced by non-shivering thermogenesis in brown adipose tissue. When subjected to cold temperatures, skin receptors signal to the greater neural network, which includes the hypothalamus located in the ventral region of the brain (Maliszewska and Kretowski, 2021). Once activated, the hypothalamus ensures homeostasis throughout the body by stimulating the autonomic nervous system or by secreting hormones.

In the case of thermogenesis, the hypothalamus maintains a steady internal body temperature by causing the peripheral nervous system to secrete signaling molecules, like norepinephrine. Norepinephrine is the signaling molecule that binds receptors on brown adipose tissue and activates an intracellular signaling cascade. Non-shivering thermogenesis (NST) maintains a high metabolic rate not by breaking down ATP as does the shivering response, but by

uncoupling an ATP gradient in the mitochondria of adipose cells and releasing free energy as heat. This process is compounded as more adipose tissue is recruited to maintain this high metabolic rate. The molecular background for the thermogenesis response is outlined below.

UCP1

Thermogenesis is dependent upon the presence of uncoupling protein 1 (UCP1), a transmembrane protein with six alpha-helical domains (Sentis et al., 2021). To understand the action of UCP1, it is first important to understand the process of oxidative phosphorylation. In the context of thermogenesis, oxidative phosphorylation begins with lipolysis. Lipolysis is the breakdown of fat during which fatty acids are released and shuttled into the mitochondria via the carnitine system (Fenzl and Kiefer, 2014). These fatty acids are oxidized and generate NADH, $FADH_2$, and acetyl coenzyme A (acetyl-CoA). Acetyl-CoA is used in the tricarboxylic acid cycle (TCA cycle) to produce more molecules of NADH and $FADH_2$. NADH and $FADH_2$ are electron carriers that travel to the inner mitochondrial membrane where they transfer their electrons to molecular oxygen.

The passing of energized electrons to oxygen happens sequentially through a series of complexes known as the electron transport chain (ETC). As electrons are passed between these complexes, free energy from the electrons is used to pump hydrogen ions against their gradient from the mitochondrial matrix into the intermembrane space of the mitochondria. This process is diagrammed in Figure 2. The energy stored in this concentration gradient of hydrogen ions is used to generate ATP as the ions pass back through a complex, ATP synthase, into the matrix of the mitochondria. ATP synthase joins a molecule of adenosine diphosphate (ADP) to a free phosphate to form a completed molecule of ATP. In summary, this process uses the energy released during the re-oxidation of oxygen to make ATP (Maliszewska and Kretowski, 2021).

The free energy harvested from the passing of electrons through each complex is coupled to the synthesis of ATP. This process is extremely efficient and a major source of cellular energy as each cycle produces 32 molecules of ATP (Cooper, 2000).

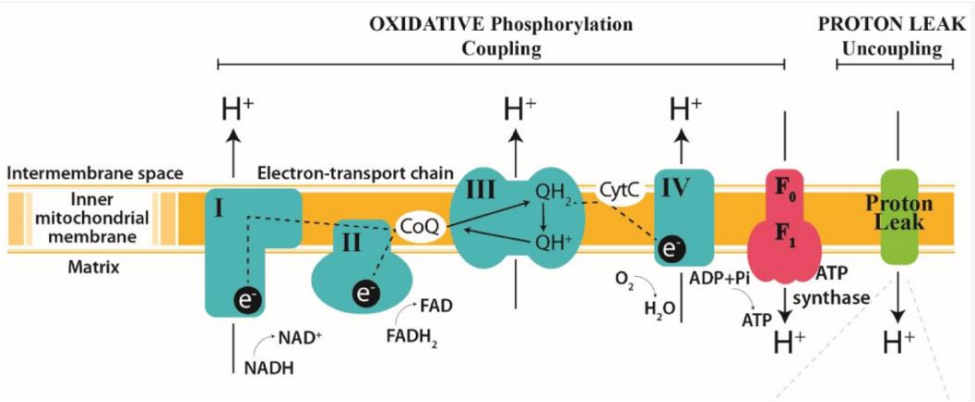

Figure 2. The process of oxidative phosphorylation in the inner mitochondrial membrane and the adverse work of an uncoupling protein. Retrieved from "Regulation of oxidative phosphorylation of liver mitochondria in sepsis," by P. Eyenga, 2022, Cells 11(10), 1598. Copyright [2022] by MDPI.

In oxidative phosphorylation, a portion of energy is lost as heat, but in brown adipocytes, energy is dissipated as heat without the production of ATP. In contrast to the work of ATP synthase, thermogenesis is metabolically inefficient as substrate energy is used without generating ATP (Nedergaard et al., 2001). The mechanistic action of UCP1 is inefficient because it works to equalizes the ATP gradient in the inner mitochondrial membrane generated by oxidative phosphorylation, as seen in Figure 2 as "PROTON LEAK". Brown adipose tissue mitochondria dissipate energy from the hydrogen ion gradient as heat instead of generating ATP. Although brown adipocytes have a high number of mitochondria, they have a very low capacity to generate ATP through ATP synthase as this complex has extremely low activity in brown adipocytes and UCP1 has such high activity (Kramarova et al., 2008). Low ATP synthase activity along with high UCP1 activity contributes to a diminished proton gradient and high metabolic inefficiency.

UCP1 does not work in isolation but requires help from a long chain fatty acid (LCFA) substrate. LCFAs permanently attach to and assist UCP1 as the protein carries hydrogen ions through the membrane. A recent study identified UCP1 as a long chain fatty acid (LCFA) anion/H^+ symporter that transports both a LCFA and hydrogen ion at the same time (Fedorenko et al., 2012). Figure 3 displays UCP1 transporting just one LCFA and one H^+ per cycle. A LCFA anion does not directly bind H+ but provides electroneutrality to the UCP1 protein. Unlike the hydrogen ion, the LCFA anion infrequently dissociates with UCP1 and instead works as a virtual cation carrier. When UCP1 uncouples the proton gradient and releases H+ into the mitochondrial matrix, a charge translocation, known as the UCP1 current, is created as the $LCFA^-$ head returns to the opposite side of the membrane. UCP1 can transport hydrogen ions on both sides of the inner mitochondrial membrane, although it only operates in one direction as an uncoupler.

UCP1s function is dependent upon LCFA binding and the pKa of that LCFA, therefore giving LCFAs the title of principle substrate and H+ the title of secondary substrate (Fedorenko et al., 2012). If the pKa of the LCFA is low, the LCFA is unable to dissociate from UCP1 due to strong hydrophobic interactions, and H^+ ions will not bind, as diagrammed in Figure 3. Temporary UCP1 currents result as the LCFA will move within UCP1 when the membrane potential changes. On the other hand, if the pKa of the fatty acid is high, as is the case in short chain fatty acids (SCFAs), only weak hydrophobic interactions result between UCP1, and detachment is common. In this case, real UCP1 currents are produced as opposed to transient currents (Fedorenko et al., 2012). Because fatty acid presence is required for the binding of H^+ ions, the high pKa of these fatty acids will prevent H^+ translocation. Because of these characteristics, UCP1 is pKa dependent and functions best with a middle ground pH around 7.0

that is close to the pKa binding site of H+ on UCP1, and the pKa of that binding site is dependent upon what type of fatty acid is bound to UCP1.

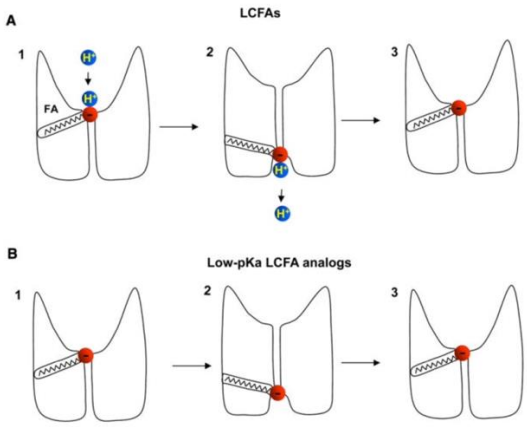

Figure 3. (A) The symport activity of UCP1 with long chain fatty acids (LCFAs) and hydrogen ions (H^+) within the inner mitochondrial membrane. A1-A3 display the LCFA working as a virtual cation carrier with UCP1 by providing electroneutrality to the UCP1 molecule as it carries the hydrogen ion through the mitochondrial membrane. (B) Optimal pKa of the LCFA is required for H^+ to bind UCP1. B1-B3 display UCP1s inability to transport a hydrogen ion through the membrane when paired with a LCFA with too low of a pKa. Retrieved from "Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria," by A. Fedorenko, 2012, Cell 151(2), 400-413. Copyright [2013] by HHSPA.

As previously stated, UCP1 is required for non-shivering thermogenesis to occur. High expressions of UCP2/UCP3 have no impact on brown tissue thermogenesis, though both are present in high concentrations in mitochondria of brown adipose tissue (Nedergaard et al., 2001). Genetically, the difference between UCP1 and UCP2/3 are two specific sequences: one starting at amino acid 144, SHLHGIKP, and one on the c-terminus region of the protein, RQTVDC(A/T)T. These sequences, conserved among all UCP1 proteins, and are undoubtedly essential for its specific function. Apart from these two sequence regions, UCP1/2/3 and identical genetically. Moreover, a significant increase in UCP1 expression in moderately cold temperatures has been shown, whereas there is no consistent change observed in UCP2/UCP3 expression. Ultimately, UCP1 expression is always simultaneous with metabolic inefficiency and required for activation of non-shivering thermogenesis. A recent study with UCP1 knockout mice

verified the necessity of UCP1. Amidst cold temperatures, a high metabolic rate was still maintained in the mice, but strictly through physical shivering as the mice were unable to transition to non-shivering thermogenesis (Nedergaard et al., 2001). Cells from these UCP1 knockout mice still performed lipolysis but did so ineffectively and inefficiently. Norepinephrine bound to a G-protein coupled receptor (GPCR) and caused a signal cascade, yet there was no metabolic effect as UCP1 was absent.

β3-adrenergic Signaling

The most understood pathway of non-shivering thermogenesis is signaling of the transmembrane ß3-adrenergic receptor ($ß_3$-AR). This receptor binds norepinephrine released by the sympathetic nervous system (Tabuchi and Sul, 2021). When $ß_3$-AR is bound, it activates a signal cascade where the transmembrane protein adenylyl cyclase (AC) creates cyclic AMP (cAMP) which then activates PKA. This signal cascade is shown in Figure 4. In turn, PKA activates both p38 and cAMP response-element binding protein (CREB), which can enter the nucleus and promote gene expression. When in the nucleus, p38, a serine/threonine protein kinase, phosphorylates activating transcription factor 2 (ATF2) and peroxisome proliferator activated receptor gamma coactivator alpha (PGC1α). This phosphorylation activates ATF2 and PGC1α which are transcription factors that then promote the transcription of UCP1. This activation of UCP1 is characteristic of the thermogenic pathway. To summarize, norepinephrine binds $ß_3$-AR, activating AC, which creates cAMP, which activates PKA, which in turn activates both p38 and CREB, promoting the expression of UCP1 in the nucleus.

In recent studies, p38 was also found to phosphorylate zinc finger CCCH-type containing 10 (ZC3H10) which binds upstream RNA and also promotes UCP1 production (Tabuchi and Sul, 2021). ZC3H10 can only be phosphorylated by p38 in cold temperatures during shivering

thermogenesis. How p38 distinguishes phosphorylation between non-shivering and shivering thermogenic transcription factors has not be discovered. PKA activates p38 as well as CREB which has a similar function to p38 in that it binds a proximal promoter of UCP1 to promote its transcription (Tabuchi and Sul, 2021). Ultimately, β3-adrenergic signaling plays a key role in thermogenesis, specifically in the transcription of UCP1. This entire pathway is illustrated in Figure 4 below.

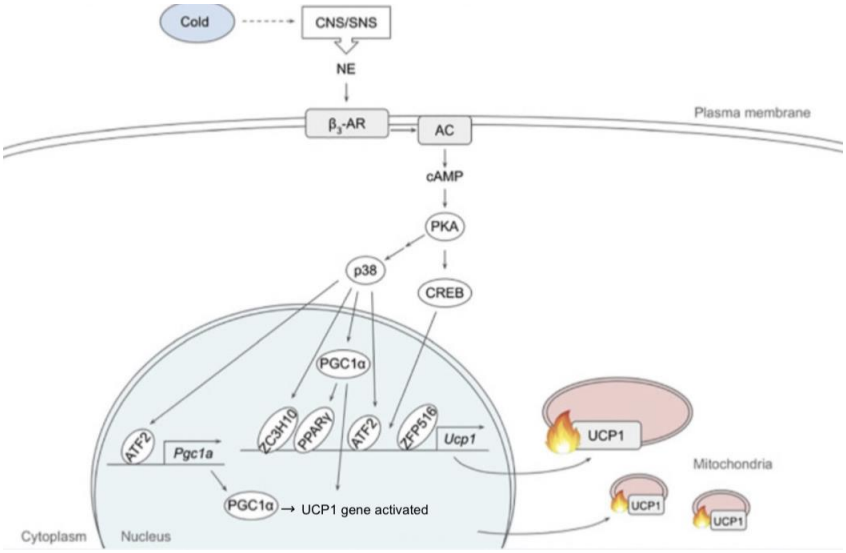

Figure 4. The intracellular signal cascade that occurs in the nucleus during β3-adrenergic signaling resulting in UCP1 expression in the mitochondria. Figure modified from "Signaling pathways regulating thermogenesis," by C. Tabuchi, 2021, Frontiers in Endocrinology 12, 595020. Copyright [2021] by Frontiers.

Thyroid Hormone Signaling

Yet another major pathway of thermogenesis is the thyroid hormone signaling pathway. The main function of the thyroid hormone is to regulate metabolism, weight, energy, and temperature (Sentis et al., 2021). Thermogenesis involves each of these aspects and is impacted by thyroid hormones. This idea aligns with the symptoms of hyperthyroid patients, who consistently display a higher body temperature and with the symptoms of hypothyroid patients, who have difficulty keeping a consistent body temperature. During the sympathetic response, the

thyroid hormone T_3 can provoke thermogenesis by inducing the expression of UCP1 and also by increasing how sensitive the body is to norepinephrine. Thyroid hormone release is then reinforced as activated BAT expresses deiodinase type 2 (DIO2) enzyme which promotes the creation and signaling of thyroid hormone in adipose tissue (Sentis et al., 2021).

One study revealed lower rates of UCP1 expression in mice with hypothyroidism (Bianco and Silva, 1987). Even more interesting was that UCP1 expression would not increase even when these mice were exposed to cold temperatures. On the other hand, thyroid hormone treatment was not able to cause an increase in the expression of UCP1 on its own. UCP1 over-expression only increased with both thyroid hormone and norepinephrine treatment. The findings of this study have encouraged research to focus on the synergistic relationship between thyroid hormone and norepinephrine in context of the thermogenesis pathway.

Receptors bind specific ligands, as is the case for the thyroid receptors on BAT cell membranes. Thyroid hormone must be locally converted from thyroxin 4 (T4) to 3,3',5-triiodothyronine (T3) in order to bind to receptors on the membrane of BAT. The DIO2 enzyme is necessary to cause T4 to transition into T3 through the removal of iodine on the phenolic ring of the molecule (Sentis et al., 2021). Furthermore, thyroid hormone receptors on BAT must be saturated by T3 to induce UCP1 expression. T3 saturation of receptors causes UCP1 gene expression by activating TH-response element (TRE) located on the UCP1 gene. Altogether, this leads to increased thermogenesis as more UCP1 protein is generated. To summarize, cold exposure causes norepinephrine release, which produces cAMP, which activates CREB, which increases DIO2 activity, which causes T4 to transition to T3, and T3 causes UCP1 expression.

Insulin/IGF1 Signaling

Studies have also shown that insulin/insulin growth factor 1 (IGF-1) is critical in the thermogenesis pathway since is vital in brown adipose tissue development. In mice with knockouts of both the insulin receptor (IR) and IGF-1 receptor (IGF-1R), there was an 85% decrease in BAT adiposity, therefore pointing to a correlation between insulin/IGF-1 and thermogenesis (Tabuchi and Sul, 2021). Brown adipocytes develop from stem cells that express engrailed 1 (EN1), paired box 7 (PAX7), myogenic factor 5 (MYF5), and early B cell factor 2 expression (EBF2). Transcription factor PR domain containing 16 (PRDM16) associates with peroxisome proliferator activated receptor gamma (PPARγ) to cause precursor cells to differentiate into brown adipocytes. Then, ZFP516 directly interacts with PRDM16 to cause UCP1 and other thermogenic proteins to be expressed. Zfp516 specifically binds the -70 to -45bp proximal region of the UCP1 promoter gene (Dempersmier et al., 2016). These are some of the major transcription factors and receptors that contribute to the development of brown adipocytes. In mice with insulin receptor knockout (IR KO), PPARγ expression significantly decreased, and BAT development was significantly impaired as this nuclear receptor is essential for the signaling cascade to induce UCP1 expression (Tabuchi and Sul, 2021). This emphasizes the fact that insulin promotes adipogenesis by promoting expression of transcription factors tand receptors.

Insulin/IGF1 also promotes adipocyte proliferation, which is the division and development of daughter cells from parent cells. This is done as insulin/IGF1 promotes insulin receptor substrate 1 (IRS1) to associate with growth factor receptor bound protein 2 (GRB2) to cause phosphorylation which can then activate the RAS-MAPK pathway (Tabuchi and Sul, 2021). The intricacies of this pathway will not be covered in this review, but it is important to know that the RAS-MAPK pathway is a major intracellular pathway that causes genes to be

activated leading to cell growth and division, which results in the proliferation of adipocytes. Furthermore, β3-adrenegric signaling has been shown to reduce the effects of insulin/IGF1 signaling. In mice who were pre-treated with β3-adrenegric signaling there was significantly less insulin receptor stimulated adipogenesis. Upon further examination, there appeared to be less IRS1-association with GRB2 leading to mitigation of the RAS-MAPK pathway. Glucose uptake was also hindered in these pre-treated mice, further pointing to the adverse effect β3-signaling has on insulin effectiveness. The implication of this relationship needs to be further studied as obese individuals typically already struggle with insulin effectiveness, and thermogenetic treatment could compound this struggle. In summary, insulin/IGF1 signaling plays a critical role in BAT development and should be further studied in future research.

Role in Treating Obesity

The hypercaloric state of obesity continues to present challenges for finding treatment options. Obesity has become a global pandemic with almost one-third of the global population fitting into this category (Fenzl and Kiefer, 2014). Thermogenesis provides hope in this search for treatment because brown adipocytes have such a huge metabolic capacity. In the past decade, investigation of thermogenesis has quickly gained momentum. Although little testing has been conducted on human subjects, other mammalian subjects offer hope for human treatment. The simplified issue of obesity is that energy intake exceeds energy use. The metabolic inefficiency of thermogenesis may allow for this imbalance to be reversed. Several pertinent studies will be reviewed below that have tested a variety of methods to increase thermogenesis. These techniques include increasing the availability of substrates used in oxidative phosphorylation, overexpressing transcription factors in the thermogenesis pathway, and increasing the amount of brown adipose tissue through browning.

Unfortunately, those with a high body mass index (BMI) often have lower levels of BAT in comparison to the levels of BAT in healthy individuals. The question remains as to whether obesity causes a decrease in thermogenesis or if a predisposed factor hinders thermogenesis and therefore contributes to obesity. Individuals with a genetic susceptibility for obesity display lower β3-adrenergic signaling aptitude, pointing to the latter contribution (Chouchani et al., 2018). Not only is BAT less prevalent, but the BAT that is present is desensitized to norepinephrine. As will be explained later, a high caloric diet can cause norepinephrine to be released from the nervous system to combat weight gain during diet induced thermogenesis (DIT) (Rothwell and Stock, 1997). The constant stimulation of norepinephrine in BAT because of constant food consumption leads to this desensitization of receptors on adipocyte membranes. The issue of obesity is truly complex as it compounds on itself. Treatments should focus on increasing the amount of BAT in those that are obese to amplify the metabolic impact of thermogenesis.

Increase in Substrate Availability

Since brown adipose tissue uses both fatty acids and glucose, an increase in supply of either of these substrates will increase adipose thermogenesis. Lipolysis is promoted when $ß_3$-AR is bound and PKA phosphorylates diacyl glycerol hydrolase (HSL) and perilipin proteins (PLINs) on lipid droplets. Perilipin proteins function to sequester lipids from lipase action, but when phosphorylated they are deactivated and leave lipid droplets unprotected and available for lipolysis (Sztalryd and Brasaemle, 2017). Lipolysis produces fatty acids which can be used in ß-oxidation to produce NADH and $FADH_2$, the main electron transporters used in oxidative phosphorylation. This contributes to more electron transport, increasing the proton gradient across the mitochondrial membrane, which UCP1 then uncouples during thermogenesis. Fatty

acids also contribute to thermogenesis as they can bind directly to UCP1 and promote greater uncoupling of the proton gradient, as diagrammed in Figure 3.

Overexpression of Transcription Factors

Brown fat has recently been found to be activated by retinoids, a class of vitamin A metabolites. Retinoids activate transcription factors in the thermogenesis pathway as retinoic acid can bind to the retinoic acid receptor (RAR) and retinoid X receptor (RXR) present in the nuclear membrane of adipocytes (Fenzl and Kiefer, 2014). When retinoic acid binds to either of these receptors, response elements bind to the enhancer region of the UCP1 promoter and cause transcription. In mice subjects, body weight reduction was observed amidst a high fat diet. Overexpression of other transcription factors, like ZFP516 and AIFM2, resulted in increased body temperature, oxygen use, and WAT browning, all of which contributed to a lower body weight (Tabuchi and Sul, 2021).

Members of the transforming growth factor beta (TGFβ) family have been recognized to upregulate transcription factors involved in thermogenesis (Tabuchi and Sul, 2021). One of these members is bone morphogenetic protein 7 (BMP7). BMP7 has shown to induce the expression of both adipogenic and BAT thermogenesis transcription factors including PPARγ, PRDM16, PGC1α, and UCP1. Inhibitors of thermogenesis were also downregulated. In mice with BMP7 knockout, the mass of BAT was significantly reduced and UCP1 was not expressed. In mice with upregulated BMP7, high energy expenditure, high body temperature, and decreased weight was observed. Administration of BMP7 in mice models was also found to induce BAT specific genes to be expressed in WAT and WAT size to decrease (Tabuchi and Sul, 2021). BMP7 was also found to cause adipose stem cells to differentiate into BAT at a higher ratio than normal. Other

members of the TGFβ family, like BMP8b, caused similar results in the induction of thermogenesis.

Insulin/Glucose Homeostasis

Insulin resistance and obesity are closely related, and the obese store excess fat in non-adipose tissues, like the liver and muscles, instead of it being confined to subcutaneous tissue. The WAT that accrues and the endocrine activity of this WAT have shown to contribute to insulin resistance (Maliszewska and Kretowski, 2021). Fortunately, there is a negative correlation between BAT activity and the amount of visceral adipose tissue, giving hope for treatment. The increased metabolic activity of BAT has been shown to combat insulin resistance in type 2 diabetes and to minimize amounts of visceral adipose tissue. To investigate the contribution BAT may have on insulin sensitivity, mice with detached sympathetic stimulation of BAT were studied. These mice developed hypertriglyceridemia, a symptom caused or worsened by diabetes mellitus (Dulloo and Miller, 1984). After this experiment in 1984, research began focusing on the correlation between sympathetic innervation of BAT and insulin receptivity.

Fatty acids are the main energy source oxidized by UCP1. When fatty acids are depleted, glucose is used as a secondary energy source in BAT. This finding was confirmed using 18F-FDG tracer imaging, a type of tomography scan that uses an analogue of glucose, 2-deoxy-2-[fluorine-18]fluoro-D-glucose, to reveal the metabolic function of curtain tissues by visualizing the radioactivity of the tracer as it is detected by a scanner (Almuhaideb et al., 2011). This imaging allowed researchers to view activity in two common glucose transporters, GLUT-1 and GLUT-4, in BAT of mice subjects (Maliszewska and Kretowski, 2021). In a warm temperature experiment, insulin caused BAT to uptake glucose molecules through GLUT-4, suggesting that BAT can use glucose independent of thermogenesis stimulation. Even more promising was an

experiment conducted in cold temperatures that demonstrated a significant increase of BAT glucose utilization (Maliszewska and Kretowski, 2021). Mice with induced high-blood sugar were placed in a cold environment, and GLUT-4 transporter activity was significantly higher than in the control mice. This same uptake of glucose by BAT was seen in a high insulin environment. These experiments point to the role that BAT plays in insulin-facilitated glucose disposal. Insulin has also been shown to increase UCP1 expression in mice by enhancing the sympathetic nervous system innervation of BAT (Maliszewska and Kretowski, 2021).

Progressive insulin resistance leads to the development of diabetes because insulin is constantly present in the bloodstream attempting to incite glucose uptake. Receptors that are constantly saturated with insulin eventually atrophy and lose their function, causing insulin resistance. Glucose in the bloodstream is unable to enter and be used by the cell, and high blood sugar is the result. Thermogenesis combats insulin resistance by utilizing glucose in the bloodstream. Ultimately, cold-activated BAT has improved glucose homeostasis in the bloodstream and therefore increases insulin sensitivity. This process is summarized in Figure 5 below. A recent study analyzing high endurance athletes recognized increased levels of fatty-acid-induced uncoupling and mitochondrial protein adenine nucleotide translocase 1 (ANT1), a protein needed in fatty acid uncoupling (Schrauwen and Lichtenbelt, 2016). Moreover, insulin sensitivity paralleled amounts of fatty-acid induced thermogenesis. In mice with reduced ANT1 levels, both fatty-acid uncoupling and insulin-stimulated glucose uptake reduced at a similar rate.

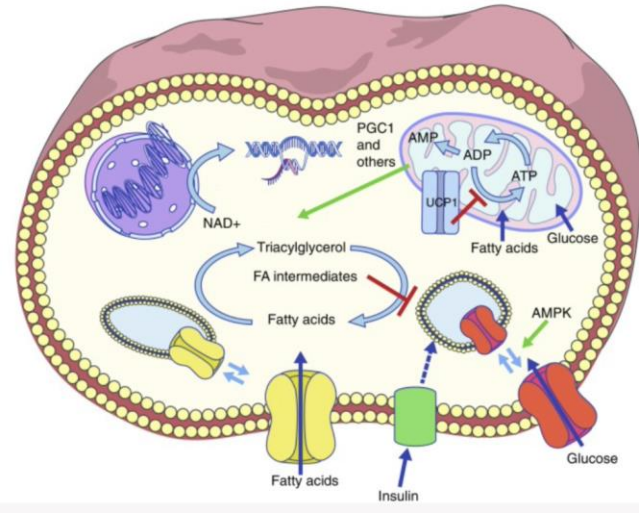

Figure 5. Insulin stimulated glucose uptake in cells contributing to energy storage used by thermogenesis. Figure modified from "Combatting type 2 diabetes by turning up the heat," by P. Schrauwen, 2016, Diabetologia 59(11), 2269-2279. Copywright [2016] by the Authors.

Browning of WAT

Because brown adipose tissue is the only type of tissue that can perform thermogenesis, techniques are being investigated to cause white adipocytes to transition to brown adipocytes in a process called browning. Browning of WAT has shown to be stimulated by cold exposure, β-adrenergic signaling, and regulation of transcription factors. Although the amount of BAT varies greatly among humans, there is an estimated 7% prevalence within adipose tissue in adults (Worku et al., 2020). Determining ways to increase this percentage should be one of the first steps in using thermogenesis as a treatment.

Beige/brite cells have seen to emerge naturally from white adipose tissue in the groin region during prolonged cold exposure. However, exposing someone to cold temperatures for a long period of time is not necessarily a practical treatment option. A more practical browning method currently being tested is causing an increase in expression of transcription factor Zfp516 (Dempersmier et al., 2016). The overexpression of Zfp516 has shown to cause inguinal WAT (iWAT) to brown and therefore increase body temperature and metabolic rates. Cold

temperatures are not required to brown the WAT as browning transpired at neutral temperatures. The overexpression of Zfp516 has not been found to increase the thermogenetic rate in BAT, but simply contribute to more cells performing thermogenesis as WAT is browned. Like Zfp516, the overexpression of PRDM16 has also been found to brown iWAT and leave BAT unaffected (Dempersmier et al., 2016). PRDM16 is involved in the differentiation of precursor cells into adipocytes, as outlined in the insulin/IGF-1 signaling pathway. Because Zfp516 and PRDM16 directly associate with one another, one may conclude that they both work together to brown iWAT. Inguinal white adipose tissue in mice is analogous to subcutaneous adipocytes in humans, supporting the idea that WAT browning could contribute to obesity treatments given at neutral temperatures.

PGC1α and PRDM16 are both transcriptional regulators than can be upregulated to produce BAT. PGC1α is a coactivator to the nuclear receptor PPARγ which causes adipocytes to differentiate when bound by certain hormones, as previously outlined in the insulin signaling pathway. As expected, mice that were induced to overexpress PGC1α in white adipocytes were able to express UCP1 and therefore parallel the function of BAT (Fenzl and Kiefer, 2014). In obese mice, PGC1α was necessary for metabolism of both glucose and lipids. This is supported by research showing patients with type 2 diabetes have minimal amounts of PGC1α expression, and poor glucose metabolism is characteristic of diabetes. Additionally, PRDM16 overexpression has also been shown to increase amounts of BAT (Fenzl and Kiefer, 2014). Adipose stem cells expressing Myf5 lead to the development of brown adipocytes or skeletal myoblasts. PRDM16 then causes brown adipocytes to develop as opposed to myoblasts. In a study conducted in 2014, PRDM16 was heavily expressed in myogenic precursor cells of mice models which resulted in a reprogramming of these cells into brown adipocytes. Similarly, PRDM16 expression was able to

brown WAT and increase energy expenditure. Browning of WAT by PRDM16 was only observed in subcutaneous fat, not visceral fat.

Diet Induced Thermogenesis

Thermogenesis can also be stimulated by food consumption or food stimulation in a process called diet induced thermogenesis (DIT). Typically, DIT utilizes roughly 10% of total daily energy expenditure (Maliszewska and Kretowski, 2021). Studies have shown that there is high metabolic efficiency during fasting as opposed to metabolic inefficiency during dietary overfeeding because of norepinephrine release (Nedergaard, 2001). Diet induced thermogenesis (DIT) has been a controversial topic since it can be stimulated by over-eating, and this is precisely the problem for those that are obese. Nevertheless, the benefits of DIT will be outlined below. The benefit of DIT is metabolically relevant because postprandial energy use can last for up to ten hours after a meal, as opposed to fasting which constricts metabolic activity (Rothwell and Stock, 1997). Ultimately energy input activates energy expenditure in DIT.

Diet induced thermogenesis (DIT) is not the same as non-shivering thermogenesis (NST), although both have the same effect on BAT. What separates these two methods of thermogenesis are their initial stimulation. DIT is stimulated postprandially while NST is typically stimulated by cold temperatures. In DIT, excessive food intake is sensed by the central nervous system and thermogenesis is then triggered to help fight against weight gain (Bachman et al., 2002). This was tested when mice with knockouts of three main β-adrenergic receptors (β-less) were given a normal chow diet and a high fat diet. Both groups of mice became obese, with the high fat mice becoming significantly obese. In contrast, control mice given a high fat diet did not develop obesity to this same extent. This proved the fact that β-adrenergic receptors are involved in the

DIT pathway. It has been further shown that DIT causes the nervous system to secrete norepinephrine which follows the classic pathway of UCP1 induction.

A recent study that was especially interesting pointed to the effect chewing has on inducing DIT. In this experiment, healthy subjects were split up into three groups: a control group, a taste group, and a chewing group. The control group was given food to be quickly swallowed, the taste group kept the food in their mouth for 30 seconds without chewing, and the chewing group chewed their food for 30 seconds before swallowing (Hamada and Hayashi, 2021). DIT was measured by gathering information on gas exchange, heart rate, and arterial blood flow. The results of this study showed that both the taste group and chewing group showed significant increase in DIT levels, with DIT activation being the highest in the chewing test group. This experiment provides hope for treatment using DIT as human subjects were used in this trial, as opposed to animal subjects used in most other experiments. Chewing is already a part of daily life, so refining this technique would be a simple, non-invasive treatment option for those that are obese. Future research should seek to replicate this experiment on obese subjects to determine if the same effect would be observed as that in healthy subjects and to see if a longer duration of chewing would cause even higher rates of DIT.

Conclusion

The assumption that fat tissue is only used for storage is overly simplistic. Fat tissue is quite complex with high metabolic capabilities. Weight loss regimens often focus on reducing energy intake, while thermogenesis treatment focuses on increasing energy expenditure. Not only does focusing on increasing energy expenditure promote weight loss, but it also improves insulin resistance, heart health, and reduces the risk for developing certain cancers in a way strict dieting

cannot. In this review, the uniqueness of brown adipose tissue, the thermogenesis pathway, and the treatment potential of thermogenesis were thoroughly analyzed.

Future research should continue to focus on unveiling the mechanism of thermogenesis. There remains a possibility that animals lacking UCP1 are able to perform non-shivering thermogenesis (Nedergaard, 2001). Whether this is through a different uncoupling protein, or another technique is currently unknown. Research should focus on genetic profiling of adult human BAT as it does not always parallel the genetic profiling gathered while researching mice. The location, sex, age, and metabolic state impact the genetic profiling of BAT in humans. Ultimately, there is much more genetic differentiation and environmental variety in humans than there is in mice which provides a challenge because much of the research promoting the therapeutic benefit of thermogenesis have been conducted on mice subjects. Verifying that the BAT genome is paralleled between human cells and mice model cells will be essential in applying current findings to future treatment options for humans. Hopefully, greater success in human subjects will be observed because the large surface area to volume ratio allows heat to be dissipated to a greater extent than mammalian subjects used in current studies (Sentis et al., 2021).

Since the discovery of the presence of BAT in humans in 2009, great strides have been made. Not only has the cellular metabolic mechanism been understood, but various treatment potentials have been discovered and understood more deeply. These possible treatment options include increasing the availability of substrates used in oxidative phosphorylation, overexpressing transcription factors in the thermogenesis pathway, stimulating DIT, promoting insulin sensitivity, and increasing the amount of BAT through browning WAT. The therapeutic potential of thermogenesis is constantly growing. Maybe someday thermogenesis stimulation

will be a renowned obesity treatment used in humans, but for now, research needs to continue analyzing the details of brown adipose tissue.

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