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RESEARCH ARTICLE

BROWN/BEIGE ADIPOSE TISSUE: NOVEL PLAYERS IN THE FIGHT AGAINST OBESITY

Gökhan BAĞCI
Department of Medical Biochemistry, Faculty of Medicine, Altınbaş University, Istanbul, Türkiye,
gokhan.bagci@altinbas.edu.tr, ORCID: 0000-0003-4554-2391

Sara ÇIBIK
Department of Medical Biochemistry, Faculty of Medicine, Altınbaş University, Istanbul, Türkiye,
ORCID: 0000-0003-0100-3409

Hatice ÖKTEN
Department of Medical Biochemistry, Faculty of Medicine, Beykent University, Istanbul, Türkiye,
ORCID: 0000-0003-3084-8064

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Abstract

Obesity is a metabolic disease which its prevalence is increasing worldwide. Multidisciplinary strategies are required to combat obesity. Many methods, from diet to surgery, are tried in obesity treatment. However, these methods have not been successful enough in the treatment of obesity. In recent years, a new adipose tissue type has been mentioned, with very important developments in adipose tissue biology. This type of adipose tissue is named as beige adipose tissue, different from white adipose tissue and classical brown adipose tissue. It has been observed that the beige adipocytes have a Brown-like characteristic and have thermogenesis abilities. It has been shown that beige adipocytes can develop in the white adipose tissue by a mechanism called browning, with the effect of various stimuli such as cold, hormones, exercise and dietary compounds. Brown/beige adipocytes are a candidate to be a new generation weight loss strategy and it is likely to have benefits against both obesity and its related metabolic diseases such as insulin resistance, diabetes, etc. To date, an increasing number of studies have been carried out to combat obesity by inducing browning of WAT by trying many compounds or methods, including cold exposure, various drugs, hormones, and plant-based agents. With the use of new generation nanotechnology-based therapies in the near future, specific molecules that can directly bind to brown/beige fat cells and activate the thermogenic program will be able to treat obesity. However, the therapeutic use of browning agents in people with obesity in the coming years will depend on the outcome of further randomised controlled trials.

Keywords: Obesity, Brown adipose tissue, Beige Adipose Tissue, Browning of white adipose tissue

1. INTRODUCTION

Obesity is a metabolic disease that threatens public health caused by both genetic factors and environmental, psychological and social factors, and its prevalence is increasing worldwide. Currently, it is reported that 2.2 billion people worldwide are overweight and 712 million people are obese (Cheng et al., 2021). A healthy adult person's body mass index (BMI) should be below 25 kg/m2. However, in diseases such as obesity, BMI in humans rises above 30 kg/m^2. Obesity occurs when energy intake exceeds energy expenditure (Pan et al., 2020).

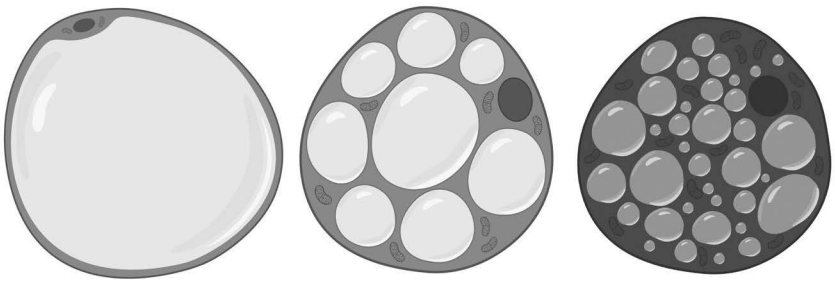
Multidisciplinary strategies are needed to combat obesity. Many methods, from diet and weight loss programs to endoscopic or bariatric surgery, are tried in the treatment of obesity. (Yoneshiro et al., 2013). However, these methods are unsuccessful in some obese patients. Therefore, it is of great importance to develop alternative methods for the fight against obesity. In recent years it has been recognized that brown/beige adipocytes have enormous metabolic benefits: Only 63 g of brown/beige adipocytes can burn about 4 kg of WAT per year (Cypess et al., 2013; Virtanen et al., 2009). Many studies have shown that activation of brown/beige adipogenesis can contribute to energy expenditure, suppressing obesity. To date, an increasing number of studies have been carried out to combat obesity by inducing WAT browning by trying many compounds or methods, including cold exposure, various drugs, hormones, and plant-based agents (Wankhade et al 2016; Herz and Kiefer, 2019). In this review, we will summarize subjects including adipose tissue types and characteristics of especially beige/brown adipose tissue. In addition, we will discuss in detail WAT browning, which is a current approach in the treatment of obesity, and the agents used for this purpose. Finally, we will consider nanotechnology-based drug delivery approaches in the treatment of obesity.

2. ADIPOSE TISSUE

In recent years, with increasing research, it has been revealed that adipose tissue is not only a tissue where energy is stored, but also a tissue with many functional roles for metabolism, which plays a fundamental role in food intake, energy homeostasis, insulin sensitivity, blood pressure and angiogenesis with various hormones and adipokines secreted from adipocytes. It has been shown to be a very important tissue and consequently an endocrine organ (Halberg et al., 2008). There is a large amount of adipose tissue around the subcutaneous, abdominal cavity, skeletal muscle, mammary glands and vessels of a normal adult (Berry et al., 2013). Adipose tissue acts as a fuel tank for metabolism and contributes to most of the vital needs of the organism. It also has crucial roles in thermogenesis, hormone synthesis and release, lactation and immune response (Cinti, 2012; Harms and Seale, 2013).

Traditionally, two types of adipose tissue are mentioned. First, white adipose tissue (WAT) is responsible for storing excess energy as triacylglycerol (TAG) and releasing free fatty acids (FFA) for energy when needed, while the second type is brown adipose tissue (BAT), which mainly performs thermogenesis

function (Berry et al., 2013). However, a third new class of adipose tissue has been mentioned in recent years. These adipocytes, on the other hand, were named beige adipocytes as a new and separate cell type different from white and brown adipocytes (Wu et al., 2012; Harms and Seale, 2013). The main characteristics of white, brown and beige adipocytes are shown in Figure 1.



	White Adipocyte	Beige Adipocyte	Brown Adipocyte
Function	Storage of triglycerides, endocrine (secretion of adipokines and vasoactive factors)	Thermogenesis, anti-inflammatory properties, cardioprotective	Thermogenesis, anti-inflammatory properties, cardioprotective
Depots	Visceral and subsutaneous and most PVAT depots	Cervical*, supraclavicular*, axillary*, renal*, thoracic PVAT*, paraspinal* subcutaneous (only rodents)	Interscapular (human only infants) thoracic PVAT*
Lipid Droplet	Uni-locular	Multi-locular	Multi-locular
UCP-1 Expression	-	+	+++
Mitochondrial Density	Low	Medium	High
Progenitor	PDGFRα^{+}, CD24^{+}, CD34^{+}	Vascular smooth muscle origin	Myogenic origin En-1, Myf5^{+}, Pax7^{+}
Changes during obesity	Hyperplasia, hypertrophy, secretion of vasoconstrictors, immune cell infiltration	Whitening, loss of UCP-1 expression	Potentially resistant to obesity-induced inflammation

Figure 1. The characteristics of white, brown and beige adipocytes

2.1. White Adipose Tissue

WAT is generally found all over the body and is classified as visceral (vWAT) and subcutaneous (sWAT) depots according to its location. Following excessive food intake or low energy expenditure, WAT can take both FFA and glucose from blood plasma and convert them to triglycerides. However, in humans, re-esterification of fatty acids is more preferable than de novo lipogenesis from glucose (Virtue et al., 2012). WAT is dominated by a single large vacuole that stores triglycerides, but still shows vascularity. WAT is also highly innervated with both afferent and efferent sympathetic nerves (Bartness and Song, 2007; Bartness et al., 2014; Merlin et al., 2016). WAT can regulate nutritional status by secreting inflammatory mediators, including adipokines such as leptin, adiponectin, resistin, and tumor necrosis factor-α (TNF-α) into the vascular and lymphatic systems. Considered in the context of obesity, these mediators play a central role both locally by affecting adipocyte proliferation and differentiation, and by controlling satiety and energy catabolism (Galic et al., 2010; Ouchi et al., 2011). On the other hand, in the case of obesity, hyperplasia, hypertrophy, secretion of vasoconstrictors, and immune cell infiltration are observed in the white adipose tissue.

2.2. Brown Adipose Tissue

BAT functions by consuming energy rather than energy storage and generating heat when activated. This process is known as non-shivering thermogenesis (Cypess et al., 2009). The most well-known role of BAT for a long time is that it plays an important role in protecting animals from hypothermia, mostly in hibernating animals. Furthermore, BAT is a rapidly activating tissue to maintain body temperature at birth in both humans and rodents and therefore functions in neonates. However, it is known that BAT in humans decreases with age. (Harms and Seale, 2013). But recent studies have shown that BAT exists in adults as well. (Nedergaard et al., 2007). In recent years, significant amounts of brown/beige adipocytes have been found in adult humans, especially in supraclavicular, cervical, paravertebral, perirenal, and mediastinal regions, as a result of the contributions of an in-depth examination of adipose tissue with 18F-fluorodeoxyglucose Positron Emission Tomography combined with Computed Tomography (18F-FDG-PET/CT). In humans, BAT depots are found to be much less than WAT. Moreover, it has been reported that BAT depots decrease with age (Bartelt and Heeren, 2014).

BAT is also called multilocular adipose tissue. BAT has many blood capillaries and contains a lot of mitochondria. It shows dense nervous networks. Macroscopically, the tissue appears brown due to the presence of heme cofactors in the mitochondrial enzyme cytochrome oxidase. BAT has a more limited distribution than WAT, which is found throughout the body. Compared to WAT cells, BAT cells are small and polygonal. It contains large amounts of lipid droplets of various sizes in its cytoplasm. It also has a centrally located nucleus and abundant long cristae mitochondria (Cedikova et al., 2016).

Low ATP synthase activity is shown in mitochondria found in brown adipocytes, Therefore, mitochondria cannot use the proton gradient to produce ATP. Instead, they use uncoupling protein-1 (UCP-1), which

uncouples cellular respiration and ATP synthesis, thereby releasing heat instead of ATP (Kajimura and Saito. 2014). Knock-out UCP-1 mice have been reported to be obese. (Feldmann et al., 2009).

2.3. Beige Adipose Tissue

Recent studies have shown that fat cells expressing UCP-1 can develop in WAT in response to various stimuli (cold, thyroid hormone, some hormones, various drugs, nutrients, etc.). These adipocytes, on the other hand, were named "beige" (Brite, brown-white, brown-like, induced brown) adipocytes as a distinct group from WAT and BAT (Harms and Seale, 2013; Wu et al., 2012). The reason why it is named this way is because beige adipocytes; Unlike myocytes and "classic" brown adipocytes, it do not originate from myogenous factor 5 (MYF5)-positive adipomyoblasts, but from MYF5-negative mesodermal stem cells such as white adipocytes (Merlin et al., 2016).

Principally, the idea that targeting brown/beige adipose tissue can be a therapeutic strategy to combat obesity is prominent. Brown and beige adipocytes in humans were found more than a decade ago with 18F-FDG-PET/CT imaging. (Nedergaard et al., 2007; Cypess et al., 2009; Virtanen et al., 2009). The presence of beige adipocytes in humans has been demonstrated by anatomical and transcriptomic methods in addition to 18F-FDG-PET/CT imaging. As a result of all these findings, it was revealed that beige adipocytes are mainly located in the supraclavicular regions (Jespersen et al., 2013), while the cervical region consists of classical brown adipocytes (Cypess et al., 2013). In recent years, WAT browning has been imaged using Mitochondrial Complex-I Tracer (^{18}F-BCPP-EF) (Goggi et al., 2022). With increasing evidence, it has been demonstrated that active human adipose tissues are heterogeneous. Although the deeper cervical adipose tissue in humans is similar in many respects to classical BAT in rodents, it has been shown that adipose tissue in the supraclavicular region of humans has a mixture of brown and beige adipocytes (Cypess et al., 2013; Shinoda et al., 2015; Jespersen et al., 2013).

Beige adipocytes were infiltrated in WAT. They contain more dense mitochondria than white adipocytes but less dense than brown adipocytes. Like BAT, beige adipocytes consist of multilocular lipid droplets and show high vascularization and sympathetic nervous system (SNS) innervation and expression of thermogenic genes such as UCP-1, peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), PPARα, and cell death-inducing DFFA Like Effector A (CIDEA) (Ahmad et al., 2021; Harms and Seale, 2013; Wang and Seale, 2016; Wankhade et al., 2016). Beige adipocytes are similar in structure to white adipocytes under normal conditions. But they have the ability to increase heat production and energy expenditure under certain stimuli (β-adrenergic stimulation, diet or exposure to cold). This makes it similar in function to brown adipocytes. However, after the stimulus is withdrawn, beige adipocytes begin to change their expression profile and morphological structure and display white adipocyte features again (Altshuler-Keylin et al., 2016). Aging, obesity, and overall poor metabolic responses are associated with loss of BAT ("whitening") and reduced capacity to induce browning (Harms and Seale, 2013). Findings from cell lineage studies in mice show that beige adipocytes develop

directly from white adipocytes and smooth muscle cells after exposure to cold. (Lee et al., 2014; Long et al., 2014). In humans, beige adipocytes, which are the intermediate form of adipose tissue, are found in both subcutaneous and visceral regions (Saito, 2014).
Studies on mice have shown that beige adipocytes play an important role in the clearance of lipids from plasma and the treatment of hyperlipidemia (Bartelt et al., 2011). Beige adipose tissue also has effects on glucose tolerance (Guerra et al., 2001). It is predicted that the conversion of WAT to beige adipocytes may be a promising target in the treatment of obesity and related complications such as insulin resistance, type 2 diabetes, hypertension, and cardiovascular diseases (Tan et al., 2011).

2.4. Lineage of White, Brown and Beige Adipocytes

Figure 2 demonstrates the lineage of white and brown beige adipocytes. Adipogenic, myogenic, or osteogenic cells arise from common mesenchymal stem cells (MSCs). White adipocytes are formed from MSCs from the adipogenic lineage (Myf 5^-) (Ahmad et al., 2021). It appears that white adipocytes are derived from mural progenitor cells expressing CD24, CD34 and PDGFRα (platelet-derived growth factor receptor alpha), and subcutaneous and visceral white adipocytes originate from different progenitor populations (Rodeheffer et al., 2008; Berry and Rodeheffer, 2013; Gesta et al., 2006). There are many factors in determining the adipogenic lineage of stem cells such as bone morphogenetic proteins (BMPs), transforming growth factor β (TGF-β), fibroblast growth factor 1 and 2 (FGF 1 and 2), insulin-like growth factor 1 (IGF1), activin, interleukin 17 (IL-17), etc. Generally, white adipocytes are believed to arise from $Myf5^-$ progenitor cells. However, by showing that white adipocytes can also develop from $Myf5^+$ progenitor cells, it has been argued that the developmental origin of white adipocytes has a very complex character. Despite all this complexity, the notion that white adipocytes develop mainly from $Myf5^-$ progenitor cells is more dominant. (Sanchez-Gurmaches et al., 2012).

Because BAT protects the newborn against cold, its development and differentiation occur before birth (Park et al., 2014). In mice, classical brown adipocytes begin to express Pax7, Engrailed-1 (En-1), and Myf5 around days 9.5 to 11.5 of embryonic development. BAT originates from $Myf5^+$ progenitor cells (Koenen et al., 2021). These progenitor cells are cells that have the potential to differentiate into either myocytes or brown preadipocytes which then develop into mature brown adipocytes (Becerril et al., 2013; Ahmad et al., 2021). This process is influenced by BMP-7 (Tseng et al., 2008), PR domain containing 16 (PRDM16) (Harms et al., 2014), and early beta-cell factor 2 (EBF2) (Wang et al., 2014).

With the induction of these markers, an increase is observed in the expression of BAT-specific markers including Peroxisome proliferator-activated receptor gamma (PPARγ), PPARγ Coactivator-1α (PGC-1α) PR domain containing 16 (PRDM16), CCAAT enhancer-binding proteins (C/EBPs) etc. As a result of this increase in the expression of BAT-specific markers, the development of progenitor cells into brown preadipocytes is induced (Ahmad et al., 2021).

Unlike BAT, beige adipocytes develop in a different developmental way. Studies have revealed that beige adipocytes are formed from Myf5- progenitor cells or by trans differentiation of white adipocytes (Rui, 2017; Ikeda et al., 2018). They also have the characteristics of white adipocytes (Herz and Kiefer, 2019). Although beige and white adipocytes come from the same common origin, beige adipocytes appear to have a different transcriptional profile and metabolic role than those white adipocytes (Wang et al., 2015). Both brown and beige adipocytes also highly express PGC-1α, a key regulator of energy metabolism. Homeobox c9 (Hoxc9) is expressed in WAT and mouse beige adipocytes of subcutaneous adipose tissue origin. Lower expression is observed in human supraclavicular samples (Merlin et al., 2016). Previously, cell surface markers T-box transcription factor 1 (TBX1), transmembrane protein 26 (TMEM26) and CD137 were thought to be specific for beige adipocytes (Wu et al., 2012). However, as a result of recent studies, it has been questioned whether CD137 and TBX1 are suitable markers for beige adipocytes (Srivastava et al., 2020; Markan et al., 2020).

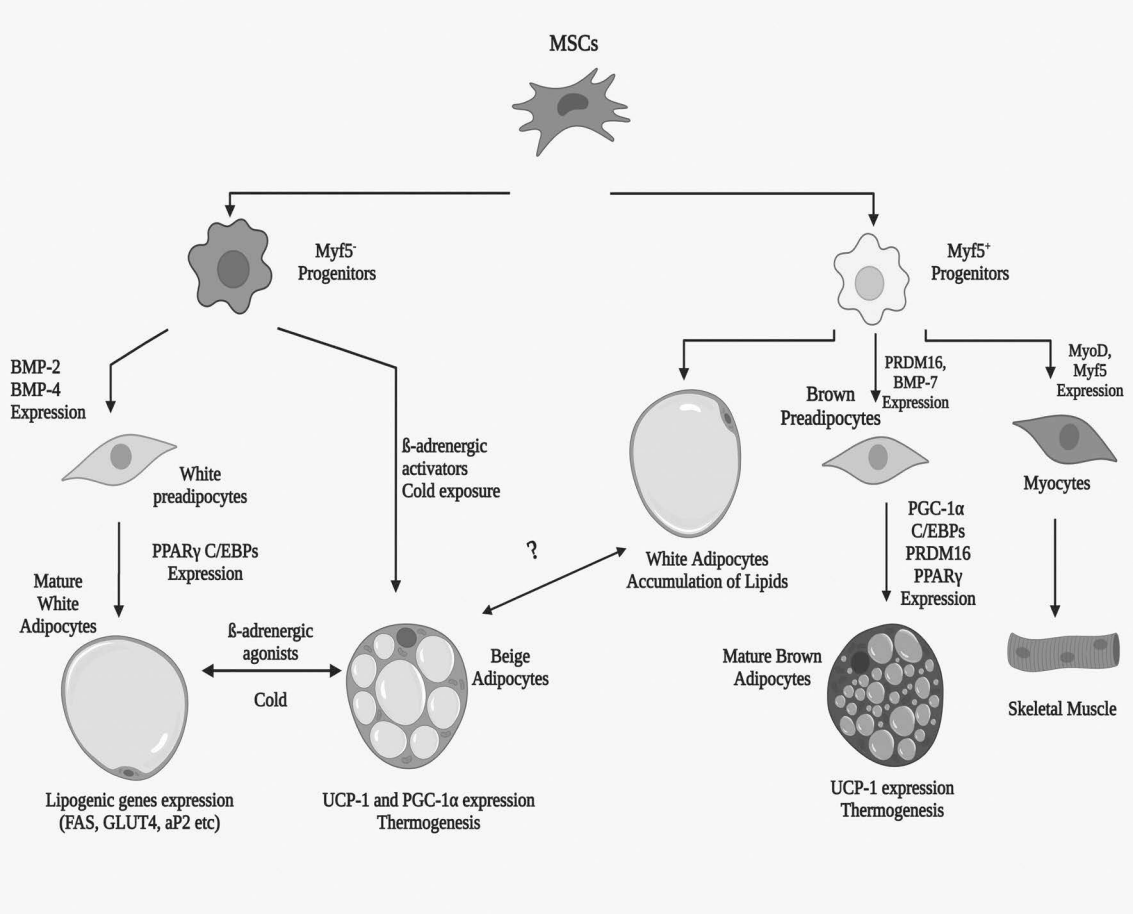


Figure 2. The lineage of white, brown and beige adipocytes

Interestingly, Chen et al. (2019) discovered a different form of thermogenic cell, which they called the glycolytic beige adipocytes. They reported that these cells showed thermogenesis and energy homeostasis even without β-AR signaling in cold conditions. The developmental origins, regulation and enhanced glucose oxidation of glycolytic beige adipocytes have been demonstrated showed to be different compared to conventional beige adipocytes.

2.5. Functions of Brown/Beige Adipose Cells

While the main function of WAT in our body is to store the excess energy as TAG, brown and beige adipocytes are a type of adipose tissue that is metabolically highly active to generate heat through their thermogenesis abilities and contribute to energy expenditure by using chemical energy. Brown/beige adipocytes are known to play a critical role mainly in thermogenesis, energy homeostasis, body temperature and body mass control. In addition to all these functions mentioned above, recent findings have revealed that BAT and beige adipocytes are undeniably involved in their metabolic function. Therefore, the possibilities of using them as possible therapeutic target cells against metabolic diseases are gaining importance (Wankhade et al., 2016).

The process of converting chemical energy into heat is called thermogenesis. Thermogenesis, which utilizes rapid muscle tremors to produce heat, is called shivering thermogenesis, while the type of thermogenesis created by BAT, a special tissue type that produces heat, is called non-shivering or adaptive thermogenesis (Azhar et al., 2016). The mitochondrial respiratory chain establishes a proton gradient for ATP production across the inner mitochondrial membrane. However, the UCP-1 protein, which is specific for brown/beige adipocytes, causes proton leakage from the inner membrane, preventing ATP generation through the phosphorylation of ADP after the mitochondrial respiratory chain and instead provides heat production (Chouchani et al., 2019). As a result, UCP-1 expression in BAT disrupts the electrochemical gradient that drives ATP synthesis in mitochondria and heat energy is released instead of ATP synthesis (Cinti, 2012). Although this process, called thermogenesis, is controlled by sympathetic stimulation of the β3-adrenergic receptor, fatty acids and thyroid hormones also play an important role in the necessary regulation (Harms and Seale, 2013).

The main receptor involved in the thermogenesis program is the Beta-3 adrenergic receptor (β-3 AR). The release of catecholamines such as norepinephrine as a result of sympathetic stimulation by cold exposure leads to mitochondria activation in brown/beige adipocytes. This provides more heat generation. Mechanistically, following cold exposure, the sympathetic nervous system releases norepinephrine. Norepinephrine binds to β3-AR, resulting in an increase in cAMP levels via activation of adenylyl cyclase and subsequently increased activation of protein kinase A (PKA) (Cypess et al., 2015). By activating this signal, p38 mitogen-activated protein kinase (p38 MAPK) stimulates transcription factor 2 (ATF-2), thereby activating the transcription of PGC1-α (Robidoux et al., 2005). PGC1- has significant downstream effects inducing mitochondrial biogenesis and PPAR activation (Hondares et al., 2011). One of the most important

effects of PGC1-α is that it activates nuclear respiration factor 1 (NRF1), whose nucleus communicates with mitochondria and triggers mitochondrial replication with the activation of mitochondrial transcription factor A (TFAM) (Piantadosi and Suliman, 2006). Consequently, the binding of norepinephrine to β3-AR triggers the signal that causes the release of FFA from brown/beige adipocytes, which is the main energy source for UCP-1-induced thermogenesis (Saito, 2014). In fact, reducing body mass through thermogenesis is not a novel concept and it should be noted that it still needs careful consideration for safety. For example, 2, 4-dinitrophenol (DNP), a mitochondrial uncoupling agent, has been shown to be effective for weight loss, but hyperthermia and other serious problems have been seen as side effects in patients treated with 2, 4-dinitrophenol (Grundlingh et al., 2011).

As a result of the balance between energy intake and energy expenditure, body mass is kept at a relatively constant level (Rui, 2013). It is further understood by experimental evidence that contributing to energy expenditure by activating thermogenesis through BAT activation or browning of WAT will be protective against obesity. In line with this concept, it has been shown that various nutrients, metabolites and some metabolic hormones can induce brown/beige adipogenesis. (Seale et al., 2009; Wankhade et al., 2016; Herz and Kiefer, 2019). BAT and beige adipocytes mediate diet-induced thermogenesis, at least in part. Thus, it prevents weight gain (Feldmann et al., 2009). Conversely, ablation of brown/beige fat has been observed to result in severe obesity in mice (Lowell et al., 1993). More importantly, chronic cold exposure has been reported to activate brown/beige adipocytes, thereby reducing body mass and fat mass in adult humans (Yoneshiro et al., 2013).

It is not surprising that brown/beige adipocytes have regulatory functions on metabolic homeostasis, given their critical role in the control of energy expenditure and regulation of body mass. Brown/beige adipocytes primarily use fatty acids for thermogenesis. In addition, BAT and beige fat may regulate glucose and lipid metabolism by a mechanism independent of body mass. Human brown and beige adipose cells are likely to similarly regulate glucose/lipid metabolism (Rui, 2017). It has been shown that keeping overweight/obese men in the cold for 5-8 hours (19.9 ± 0.8°C) activates BAT and increases gene expression related to lipid metabolism. (Chondronikola et al., 2016). Furthermore, it was observed that glucose uptake, glucose oxidation and insulin sensitivity increased in BAT of individuals exposed to cold for 10 days. (Chondronikola et al., 2014). BAT transplantation had positive effects on hyperglycemia and glucose intolerance in treptozotocin-induced or type 1 diabetes mice. (Gunawardana and Piston, 2012; Gunawardana and Piston, 2015). Insulin resistance was also reduced with BAT transplantation in high-fat diet (HFD)-induced obese recipient mice (Srivastava et al., 2012). In humans, body mass index, resting plasma glucose and lipid levels appear to be inversely related to BAT/beige fat mass (Saito et al., 2009). However, the mechanistic relationship between BAT/beige adipose tissue and insulin sensitivity and glucose metabolism has not been fully elucidated. In rodents, brown/beige adipocytes express and secrete abundant lipoprotein lipase (LPL) with cold exposure. Brown/beige adipocytes appear to play a critical role in maintaining blood TAG homeostasis in rodents (Bartelt et al., 2011).

It has been understood that WAT secretes many hormones and mediator molecules (adipokine) such as leptin and adiponectin, thereby helping to manage energy and nutrient metabolism. Similarly, it has been reported that adipokines such as leptin and adiponectin are also secreted from brown/beige adipocytes. However, because of their very small mass relative to WAT, brown/beige adipocytes are likely to contribute little to human blood levels of leptin and adiponectin. In recent years, it has been understood that brown adipose tissue is not only involved in thermogenesis but also secretes endocrine factors called "batokines", which have important contributions to the metabolic health of our body. It has been shown that these batokines have autocrine, paracrine or endocrine effects. Some of the important batokines are as follows: Neuregulin 4, insulin-like growth factor-1 (IGF-1), fibroblast growth factor 21 (FGF21), and interleukin-6 (IL-6), fibronectin type III domain-containing protein 5 (irisin/FNDC5), ependymin-related protein 1 (EPDR1), C-X-C motif chemokine ligand-14 (CXCL14), (Fisher et al., 2012; Wang and Wahl, 2014; de Jong et al., 2015; Gunawardana and Piston, 2015; Cereijo et al., 2018; Deshmukh et al., 2019; Gaspar et al., 2021). One of these batokines, neuregulin 4, suppresses hepatic lipogenesis (Wang and Wahl, 2014). FGF21 and IL-6 stimulate brown/beige adipocyte thermogenesis by paracrine or autocrine pathway (Hondares et al., 2010; Fisher, 2012; Knudsen et al., 2014). It is believed that the level of IGF-1 increases in mice with type 1 diabetes who underwent BAT transplantation, thereby plays a role in reducing hyperglycemia (Gunawardana and Piston, 2015). EPDR1, which is defined as a new batokine, has been reported to have a great contribution to energy homeostasis in mice, in addition to having a role in adipocyte thermogenic differentiation. In addition, since EPDR1 is detected in human plasma, it also suggests that it has important contributions to human metabolism (Deshmukh et al., 2019). CXCL14 has been demonstrated to mediate brown fat-macrophage communication in thermogenic adaptation. CXCL14 has been shown to improve glucose homeostasis and induce browning in WAT in obese mice. (Cereijo et al., 2018).

3. BROWNING AGENTS

Beige adipocytes are found in WAT and have a WAT-like phenotype, but when induced they acquire a BAT-like phenotype with an increased capacity for thermogenesis. This phenomenon is called browning (Bargut et al., 2017). Browning of the WAT means that transcription factors such as PRDM16 and PPAR-α, and especially UCP-1, which is the hallmark of thermogenesis, should be expressed in white adipose tissue (Wu et al., 2013). Until now, in addition to stimuli such as exposure to cold, exercise, thyroid hormones, various hormones such as leptin, melatonin; pharmacological agents such as (PPAR) agonists; Plant-based substances such as capsaicin, resveratrol, curcumin have been shown to induce WAT browning or protect against HFD-induced obesity. It has also been observed that blocking some genes or overexpressing some genes have similar effects. Many agents have been tried for this purpose, especially in recent years, and many of them have been found to have significant effects on browning and against HFD-induced obesity (Wankhade et al., 2016; Kaisanlahti and Glumoff, 2019).

The number of browning agents is increasing very rapidly. In this part of our review, we will mention some of the important browning agents reported in the literature so far. Table 1 shows the list of browning agents. Cold exposure is the stimulus that we will describe as the most well-known and most

studied and even the oldest agent that causes the browning of WAT (Cousin et al., 1992). Similarly, β-3 AR agonists are another important group of browning agents. β-3 AR agonist CL 316243 has been shown to induce the development of brown adipocytes in conventional WAT depots, such as mesenteric, epididymal, inguinal, and retroperitoneal fat depots. (Ghorbani and Himms-Hagen, 1997). Increased expression of UCP1 in periovarian WAT depots has been observed in rodents with treatment with BRL 26830A (Chapman et al., 1988)

The potential impact of exercise on the induction of browning remains controversial, as previous studies in humans and rodents have reported negative reports of exercise for BAT activation and WAT browning (Segawa et al., 1998; Shibata and Nagasaka, 1987). On the other hand, studies in recent years have shown that exercise increases the expression of PGC-1α. In addition, irisin, an exercise-associated adipomyokine, has been shown to induce browning in humans and rodents (Bostrom et al., 2012).

It is well known that many different hormones, primarily thyroid hormones, can induce WAT browning (Weiner et al., 2017). Parathyroid hormone (PTHr) (Thomas and Mitch, 2017), glucagon like peptide 1 (GLP1) (Lopez et al., 2015), leptin (Dodd et al., 2015), melatonin (Jiménez-Aranda et al., 2013), and natriuretic peptides (NPs) also showed effects on WAT browning and BAT metabolism by several different mechanisms (Liu et al., 2018; Bordicchia et al., 2012) Moreover, in a recent report, maternal secretin promoted white adipose tissue browning in offspring (Xue et al., 2022). Batokines such as FGF21 (Fisher et al., 2012) IL-6 (Kristóf et al., 2019) and apelin (Than et al., 2015) have also demonstrated browning of WAT effects. In addition, several metabolites including, lactate, β-hydroxybutyrate (Carriere et al., 2014) and retinoic acid (Wang et al., 2017) have also shown browning effects on WAT.

To date, studies in mice or in vitro have shown that many plant-based compounds, most of which are in our diet, have WAT browning effects. These compounds are found in the foods we eat, and the use of these compounds in the treatment of obesity is a very smart strategy since they do not produce side effects compared to pharmacological drugs. Some important ones of these compounds are mentioned below. In studies with mice, capsaicin and related capsinoids have been reported to induce WAT browning through many different mechanisms (Baskaran et al., 2016). Similar browning effects have also been observed in the plant-based compound including resveratrol (Azhar et al., 2016), berberine (Zhang et al., 2014), decaffeinated green tea extract (Chen et al., 2017), cinnamon (Kwan et al., 2017), curcumin (Lone et al., 2016), quercetin (Lee et al., 2017). Furthermore, fish oil intake leads to upregulation of UCP-1 and the β3-AR in inguinal WAT of mice (Kim et al., 2015). In addition to the above compounds, natural bioactive constituents from herbs and nutraceuticals, which have effects on white adipose tissue browning, were reviewed in a recent review by Ma et. al (2022).

MiRNAs such as miRNA-32 (Ng et al., 2017) and miRNA-455 (Zhang et al., 2015) have also been reported to regulate both subcutaneous WAT browning and BAT activation. On the other hand, several miRNA types were found to be negatively regulated by BAT activity and WAT browning (Shamsi et al., 2017).

Various drugs have also been found to have effects on WAT browning. It was reported that a well-known agonist thiazolidinedione (TZD) compound, rosiglitazone induces browning of WAT in mice (Ohno et al., 2012). Similarly, Prostaglandin E2 (Garcia-Alonso and Claria, 2014) and Gleevec (Choi et al., 2016) showed similar effects on WAT Browning.

Table 1. Several agents used for the browning of white adipose tissue

Browning Agent	**Reference**	**Browning Agent**	**Reference**
Cold exposure	Cousin et al., 1992	***Hormones***	
β-3 adrenergic receptor agonists		Thyroid hormones	Weiner et al., 2017
CL 316243	Ghorbani and Himms-Hagen, 1997	Parathyroid hormone (PTH)	Thomas and Mitch, 2017
BRL 26830A	Chapman et al.,1988	Glucagon-like peptide 1 (GLP1)	Lopez et al., 2015
Dietary factors		Leptin	Dodd et al., 2015
Capsaicin	Baskaran et al., 2016	Melatonin	Jimenez-Aranda et al., 2013
Resveratrol	Azhar et al., 2016	Natriuretic peptides (NPs)	(Bordicchia et al., 2012; Liu et al. 2018
Berberine	Zhang et al., 2014	Maternal secretin	Xue et al., 2022
Green tea	Chen et al., 2017	Irisin	Bostrom et al., 2012
Fish oil	Kim et al., 2015	***Batokines***	
Quercetin	Lee et al., 2017	FGF21	Fisher et al., 2012
Curcumin	Lone et al., 2016	IL-6	Kristóf et al., 2019
Cinnamon	Kwan et al., 2017	Apelin	Than et al., 2015
MicroRNAs		***Metabolites***	
miRNA-32	Ng et al., 2017	Lactate	Carriere et al., 2014
miRNA-455	Zhang et al., 2015	β-hydroxybutyrate	Carriere et al., 2014
Drug agents		Retinoic acid	Wang et al., 2017
Thiazolidinediones (TZDs)	Petrovic et al., 2010	***Other factors***	
Prostaglandin E2	Garcia-Alonso and Claria, 2014	Gut microbiota	Moreno-Navarrete and Fernandez-Real, 2019
Gleevec	Choi et al., 2016		

The relationship between the gut microbiota and browning of WAT and also the activity of BAT has been reviewed by Moreno-Navarrete and Fernandez-Real (2019). Both the amount and composition of the gut microbiota have important effects on energy expenditure. Exposure to cold, calorie restriction and intermittent fasting cause changes in GUT microbiota composition, resulting in browning of WAT and increased BAT activity. Moreover, depletion of the microbiota has also been shown to activate the browning process in subcutaneous fat. (Suárez-Zamorano et al., 2015).

Finally, body conditions such as cachexia (Tsoli et al., 2012) and burns (Patsouris et al., 2015) can induce browning as well. In conclusion, the discovery of WAT browning has highlighted the importance of brown/beige adipose tissue targeted strategies to fight diseases such as obesity. With the discovery of new browning agents that have increased in recent years, promising results are expected in the treatment of obesity using dietary or pharmacological approaches (Bargut et al., 2017).

3.1. Nanomedicine-Based Strategies for Browning of WAT

In recent years, nanoparticles and transdermal patches have been used for the spesific delivery of browning agents to adipose tissue. In a recent review, nanomedicine based strategies for browning agent delivery have been summarized. We will focus on these delivery strategies. The many side effects as a result of the oral or injection administration of various browning agents have led researchers to find different delivery strategies. In this context, the tissue-specific drug delivery option seems to solve this problem. Various polymer nanoparticles such as poly(lactide-co-glycolide) (PLGA), poly(ethylene glycol) (PEG) and Polyethylenimine (PEI), and lipid nanoparticles (LNPs) and hepatitis B core (HBc) protein virus-like particles (VLPs) are used for these purposes (Zhang et al., 2021). In a recent study using resveratrol-loaded nanoparticles, trans-resveratrol was observed to significantly induce differentiation of adipose stromal cells (ASCs) into beige adipocytes after 5 weeks of intravenous administration to obese C57BL/6J mice. Similarly, a 40% reduction in fat mass was observed. In addition, it was accompanied by improved glucose homeostasis and reduced inflammation (Zu et al., 2021). In another study using peptide-functional nanoparticles containing rosiglitazone or a prostaglandin E_2 analog (16,16-dimethyl PGE_2), when injected into the adipose tissue vasculature of mice, both were found to stimulate the browning of WAT and angiogenesis (Xue et al., 2016).

Transdermal drug delivery is another option for specific delivery of browning agents to adipose tissue. Transdermal delivery of an agent allows a local, convenient, and painless alternative to oral and injectable administration. Various transdermal systems have been developed, such as microneedles and hydrogel patches, since the intrinsic physiological barrier of the skin is difficult to penetrate and consequently delivery efficiency is reduced. Similar strategies have been used for adipocyte browning Zhang et al., 2021; Zhang et al., 2018).

4. CONCLUSION

In conclusion, with the discovery of the browning of adipose tissue in humans, we have gained a new generation method to be used in the fight against obesity. Since the biology of beige adipocytes is a novel subject, the biochemical, genetic and physiological factors in these processes need to be analyzed and investigated comprehensively. Brown/beige adipocytes are a candidate to be a new-generation weight loss strategy and it is likely to have benefits against both obesity

and obesity-related metabolic diseases such as insulin resistance, diabetes, etc. Currently, there are numerous dietary compounds, various drugs and hormones, etc., which can be used as browning agents. However, in the future, with the development of a new generation of nanomedicine-based therapies, obesity can be treated by designing specific molecules that can bind directly to brown/beige adipocytes and activate thermogenesis. The therapeutic use of browning agents in people with obesity in the coming years will depend on the outcome of further randomised controlled trials.

Conflict of Interests "Authors declare no conflict of interests

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