

# Adipose tissue browning for the treatment of obesity and metabolic diseases

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**Keywords:** Beige adipocyte, White adipocyte, Brown adipocyte, Obesity, Diabetes mellitus, Differentiation.

## ABSTRACT

**Adipose tissue is the most abundant endocrine organ in humans with an important influence on many events throughout life. Many studies that highlight the different phenotypic characteristics of fat cells in adults are becoming more frequent. Perhaps, one of the most important properties of fat cells is their flexibility to adapt to different environmental and nutritional conditions. White adipocytes can receive hormonal stimuli from other tissues and differentiate into cells with a greater thermogenic potential. In this process, lipid inclusion changes and the number of mitochondria increases, leading to functional characteristics similar to those of brown adipocytes. Recently, beige fat cells have been studied in the attempt to elucidate their role in the regulation of obesity and insulin resistance. Therefore, understanding the beige adipocyte embryonic origin and the ability of these cells to transdifferentiate is a major research challenge, with the aim of elucidating the role of these cells as a possible therapeutic strategy for obesity and metabolic diseases. In this manuscript, we will focus on the origins of the different fat cells and the possible therapeutic properties of beige fat cells.**

## INTRODUCTION

Obesity is a disease that induces a series of cardiovascular, metabolic and osteoarticular complications that reduce life expectancy. Obesity is prevalent on a global scale, with multiple factors contributing to its

development. Medical therapy for obesity is limited by the paucity of anti-obesity drugs and their side effects that are often responsible for drug withdrawal<sup>1,2</sup>. The availability of anti-obesity medications is variable between different countries, in part because obesity is not considered as a disease in many health systems around the world<sup>3,4</sup>. Recently, it has been observed that adipose tissue has a much greater functional flexibility than previously considered<sup>5</sup>. White adipose tissue (WAT) is the largest adipose tissue in adults; its main function is to store energy in the form of triglycerides during periods of low caloric intake. A more energetically active adipose tissue is the brown adipose tissue (BAT), which has the ability to dissipate energy through increased mitochondrial activity and heat production<sup>6</sup>. In human adults, it is believed that WAT predominates because most of BAT is observed only in the first few months of life<sup>7</sup>. However, an adipose tissue with high energy capacity that originates from WAT has been observed in adults under special conditions, such as cold exposure or sympathetic nervous system activation<sup>8</sup>. Even though the characteristics of this type of tissue are the same as brown adipose tissue, these likely correspond to an adipose tissue variant, which has been termed beige adipose tissue<sup>9,10</sup>. Embryologically, fat cells derive from mesenchymal cells. However, a notable difference was observed in the differentiation process: brown fat cells appear earlier than white fat cells and have more features in common with muscle cells than with white fat cells<sup>11,12</sup>. Furthermore, certain factors can induce precursors of white adipose cells to give rise to beige fat cells, described as being more energetically active<sup>13,14</sup>. Multiple factors may modulate the differentiation process of beige adipocytes from adipocyte precursors.



sor cells. Factors that can induce white adipocyte precursors to give rise to beige adipocytes are signals originated in the sympathetic nervous system, cytokines released by immune cells and multiple epigenetic signals that influence the activity of specific transcription factors and genes<sup>15,16</sup>.

In the present manuscript, the distinct types of fat cells are described, and emphasis is placed on the therapeutic potential exhibited by beige fat cells.

#### FEATURES OF DIFFERENT ADIPOCYTES

Adipose tissue allows mammals to adapt to changes related to energy requirement, environmental conditions, and nutrient availability. In recent years, knowledge of adipose tissue has been ostensibly expanded due, in part, to the increase in metabolic diseases and cardiovascular risk<sup>17-19</sup>. Although fat cells are the main constituent of adipose tissue, almost 40% of the adipose tissue is made up of vascular components, macrophages, fibroblasts, endothelial cells and adipocyte precursor cells<sup>20-22</sup>. Adipocyte size can vary considerably from 20 to 200 micrometers in diameter, which means that they have great plasticity and a high capacity to modify their volume<sup>23,24</sup>. Adipose tissue can be dysfunctional, as a consequence of lipid accumulation and adipocyte cell hypertrophy. WAT secretes endocrine and pro-inflammatory factors under hypertrophic conditions, inducing a prothrombotic state and leading to chronic low-grade inflammation<sup>25,26</sup>. This condition causes many of the cardiovascular complications exhibited by patients with metabolic diseases<sup>27,28</sup>. Most of the adipose tissue in adults is represented by WAT, which has the peculiar property of storing energy for periods of famine. Phenotypically, white adipocytes have a single cytoplasmic lipid droplet, which is responsible for storing triglycerides as a result of lipogenesis. According to recent observations, WAT is characterized by a marked phenotypic plasticity<sup>5,29</sup>. These adipose cells, under the regulation of the sympathetic nervous system, can release fatty acids and secrete substances with endocrine properties<sup>30</sup>. Since the discovery of leptin in the 1990s, several adipokines secreted by WAT with distinct biological activities have been described<sup>31,32</sup>. Leptin regulates energy homeostasis and interferes with various neuroendocrine and immune functions. Likewise, leptin regulates food intake and increases energy expenditure through hypothalamic signals<sup>33</sup>. Adiponectin is another ad-

ipokine secreted predominantly by WAT, although it can also be secreted by skeletal muscle and cardiomyocytes. Adiponectin levels increase upon the use of insulin sensitizing drugs and are inversely correlated with insulin resistance. Adiponectin also has anti-inflammatory properties with an anti-atherogenic effect and promotes angiogenesis<sup>34,35</sup>. Resistin is secreted by WAT and macrophages, and has an important role in inflammatory processes triggering insulin resistance; some studies have shown that elevated plasma levels of resistin represent a predictive biomarker of future type 2 diabetes development<sup>36,37</sup>. Visfatin - also known as nicotinamide phosphoribosyltransferase (Nampt) - is an adipocytokine secreted by adipocytes, macrophages, and inflamed endothelial tissues. High levels of visfatin are observed in patients with obesity, type 2 diabetes, chronic inflammatory conditions and cancer. An association between serum visfatin levels and cardiovascular disease has recently been observed in patients with type 2 diabetes<sup>38-40</sup>.

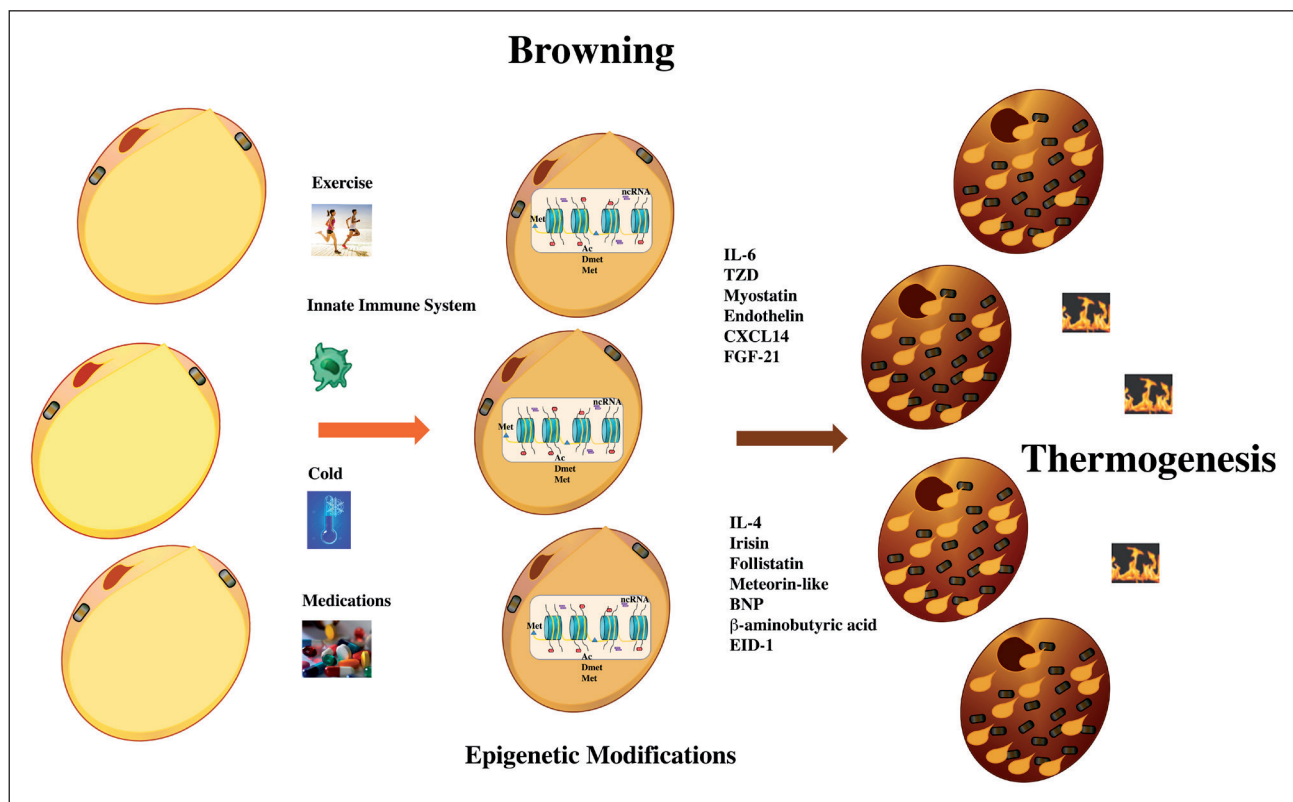
Moreover, BAT has multiple cytoplasmic lipid inclusions and numerous mitochondria. Compared to WAT, BAT is highly vascularized and rapidly metabolizes fatty acids, favoring optimal oxygen consumption and heat production<sup>41</sup>. Many environmental or molecular stimuli can promote the appearance of BAT<sup>42</sup>. Brown adipocytes are mainly observed in the small mammals and in the newborns. The embryological formation of BAT precedes that of WAT, due to its thermogenic function in the newborns. BAT originates from a subpopulation of the dermomyotome that has molecular markers such as Paired Box 7 (Pax7), Engreiled-1 (En1), and Myogenic factor 5 (Myf5)<sup>5,43-45</sup>. BAT can secrete cytokines that have an effect on different tissues and prevent diet-induced obesity. Follistatin secreted by BAT is a soluble glycoprotein that can block the activities of some members of the transforming growth factor (TGF) family. Follistatin can induce insulin sensitivity and prevents diet-induced obesity<sup>46,47</sup>. The c-terminal fragment of slit guidance ligand 2 (SLIT-C) belongs to the Slit family of secreted proteins that play an important role in various physiologic and pathologic processes, including inflammatory cell chemotaxis. SLIT-C is secreted by BAT and induces WAT browning and metabolic processes associated with substrate supply to fuel thermogenesis<sup>44,48</sup>. Growth differentiation factor 8 (GDF8/Myostatin) and growth differentiation factor 15 (GDF15) are

members of the transforming growth factor family, which are involved in the control of hunger-related neural circuits. GDF15 overexpression was shown to prevent obesity and insulin resistance by increasing the expression of thermogenic genes<sup>49</sup>. Fibroblast growth factor 21 (FGF21) is a regulator of energy homeostasis and is secreted mainly by the liver. FGF21 secreted by BAT prevents hyperglycemia and hyperlipidemia in mice<sup>50</sup>. FGF21 analogues tested in overweight/obese patients with type 2 diabetes have been shown to reduce dyslipidemia and hepatic steatosis, although they do not lead to improvement in glucose control and body weight<sup>51</sup>. Even though FGF21 has been shown to exhibit anti-inflammatory effects on white adipocytes, it remains to be determined if FGF21 has a similar action in BAT<sup>52,53</sup>.

Beige adipose tissue is the most recently discovered adipose tissue; it has morphological characteristics in common with WAT and BAT. The nature

of beige adipocytes is controversial, although their origin is thought to be secondary to the differentiation of WAT. Differentiation from cell precursor itself has also been observed<sup>54,55</sup>. Beige adipocytes have a simple lipid inclusion similar to WAT, but when exposed to stimuli such as cold exposure their physiological behavior becomes similar to that of BAT. The thermogenic capacity of beige adipocytes and its possible role in the regulation of body weight, obesity and insulin resistance are currently being studied<sup>56,57</sup>.

Beige adipocyte biogenesis, also called beige adipogenesis (or browning/beiging), is induced by chronic exposure to external stimuli such as cold, adrenergic stimulation, long-term treatment with peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) agonists, among others<sup>58</sup> (Figure 1). Browning is a temporary adaptive response which persists even after the dissipation of external environmental signals<sup>57,59</sup>. The origin of beige ad-



**Figure 1.** Factors influencing the differentiation of beige adipocytes. White adipocytes are modulated by external factors (cold, physical exercise, cells of the innate immune system type 2, medications). Environmental factors can affect gene expression (DNA Methylation, covalent modifications of histones; Ac: Acetylation; Dmet: Demethylation; Met: Methylation, ncRNA: non-coding RNA) and increase the expression of genes that modify the phenotype towards beige fat cells with thermogenic properties. Abbreviations: BNP: Brain natriuretic peptide; CXCL14: C-X-C Motif Chemokine Ligand 14; EID-1: EP300 inhibitor of differentiation-1; FGF-21: Fibroblast growth factor 21; IL-4: Interleukin 4; IL-6: Interleukin 6; TZD: Thiazolidinediones.

ipocytes is complex; some beige adipocytes arise in epididymal white fat from precursors that express platelet-derived growth factor receptor alpha + (PDGFR $\alpha$ ), CD34, and spinocerebellar ataxia type 1+ (SCA1+) proteins<sup>60-62</sup>. Beige adipocytes may also arise from Myf5-negative precursors of inguinal WAT<sup>54</sup>. A number of studies suggest that beige adipocyte precursors, like white adipocyte precursors, reside in the adipose tissue vasculature<sup>58, 63, 64</sup>. Some beige adipocytes have been shown to have specific smooth muscle cell markers, such as myosin heavy chain (Myh11)<sup>58</sup>. These observations suggest that beige adipocytes have a different embryonic origin than brown adipocytes<sup>23, 65-67</sup>. Beige adipocytes are cells with a high functional flexibility. The embryonic origin of beige adipocytes can be influenced by external factors. Mature white fat cells can differentiate into beige fat cells when exposed to specific factors that induce epigenetic changes. This phenomenon may correspond to transdifferentiation or to a direct differentiation of white adipocytes into beige adipocytes; they also could correspond to beige adipocytes that already resided in the WAT<sup>68</sup>. One of the aspects which are currently under investigation in human adults is whether adipocytes displaying thermogenic properties are brown or beige adipocytes<sup>66, 67</sup>. In adults, most of the energetically active adipocytes have molecular markers corresponding to beige adipocytes<sup>68, 69</sup>. However, brown adipocytes are found in adults, especially in some regions of the posterior neck area. The volume of adipose tissue is established during the adolescence, in which a proportionality is established with other organs, such as liver or heart<sup>70</sup>. Ultrastructure and dynamics studies of adipose tissue established that adipocyte progenitor cells (APCs) continuously support the replacement of adipose cells through the human lifespan. The number of fat cells remains constant during adulthood; even in obesity, the underlying process consists of fat cell hypertrophy rather than hyperplasia<sup>26, 64, 71</sup>. In humans, APCs are currently considered to be located in the vascular stromal fraction (SVF), which is similar to that found in rodents; however, in these animals, APCs are positive for PPAR $\gamma$ <sup>71, 72</sup>. Using genetic-tracing methodologies, it was found that PPAR $\gamma$ -expressing APCs are critical for adipogenesis *in vitro* and *in vivo*<sup>73</sup>. *In vivo* tracking of PPAR $\gamma$ + cells indicated that these cells reside in the blood vessel wall.

In line with a vascular residency, these APCs resemble mural cells (pericytes and vascular smooth muscle cells) because they express several mural cell markers, such as platelet derived growth factor receptor-beta (PDGFR $\beta$ ) and alpha-smooth muscle actin ( $\alpha$ -SMA)<sup>74, 75</sup>.

Mapping studies of the genetic fate of smooth muscle cells demonstrated that cells labeled with Myh11, PDGFR $\beta$  and SMA have the ability to give rise to beige-colored adipocytes in response to cold exposure. In particular, perivascular cells positive for SMA were able to give rise to 50-70% of the new beige adipocytes after 1 week of cold exposure. Interestingly, reduction of adipogenesis within SMA + cells or ablation of SMA + cells reduced the ability of beige adipocytes to differentiate upon cold exposure<sup>76, 77</sup>.

#### BEIGE THERMOGENESIS

In mammals, reduced substrates provide the cells with the energy needed for heat production. The main source of energy depends on the oxidative phosphorylation carried out in the mitochondria<sup>78, 79</sup>. Free energy is transformed by the equilibrium reaction [ATP = ADP + Pi]. However, only a fraction of the thermal energy flow coming from substrate oxidation is stored as free energy, whereas most of it produces heat<sup>77</sup>. Therefore, oxidative phosphorylation begins with the entry of electrons into the series of electron carriers called the “respiratory chain”. In cells where the mitochondrial respiratory chain dominates oxidative metabolism, the rate of mitochondrial respiration is the major determinant of heat production<sup>77, 80</sup>. Since brown and beige adipose tissue metabolism is predominantly oxidative, thermogenesis in these cells is effectively controlled by the manipulation of rate limiting steps in respiration<sup>81</sup>. Substrate oxidation by the mitochondrial respiratory chain drives an electrochemical proton gradient across the mitochondrial inner membrane<sup>82</sup>. The inner membrane is impermeable to most small molecules and ions, including protons (H<sup>+</sup>); the only species that cross this membrane do so through specific transporters. The proton-motive force (Dp) attempts to maintain the equilibrium by transporting the protons to the mitochondrial matrix through the ATP synthase, thus providing energy for the phosphorylation of the ADP molecule (ADP+Pi/ATP)<sup>83</sup>. Brown and beige adipocytes thermogenesis mechanism is

based on uncoupling respiration from ATP synthesis. The identification of uncoupling protein 1 (UCP1) reveals a way that provides a path for protons to return to the mitochondrial matrix without passing through the inner membrane<sup>84</sup>. As a result of this short-circuiting of protons, the energy of oxidation is not conserved by ATP formation but it is dissipated as heat, which contributes to maintaining body temperature<sup>85</sup>. Mitochondria of brown and beige adipocytes show a lack of respiratory control (relationship between ATP/ADP), which can be relayed by the balance between the purine nucleotides and the free fatty acid sequestration<sup>86, 87</sup>. Recent experiments in brown fat cells have shown that an increase in the concentration of free fatty acids leads to a higher conductance of protons by UCP1<sup>88</sup>. Moreover, it has been observed that purine nucleotides produce an inhibition of UCP1 activity. In this process, purine nucleotides bind to the cytosolic portion of UCP1 and appear to interfere with the pathway of proton translocation<sup>89</sup>. Under basal conditions, the inhibition made by purine nucleotides predominates and UCP1-mediated proton leak is reduced<sup>90</sup>. Basal proton leak accounts for 20–30% of the resting metabolic rate of hepatocytes and for up to 50% of the respiration of skeletal muscle of rats. Considering the high metabolic activity of the liver and the large proportion of skeletal muscle in relation to body mass, basal proton leak contributes significantly to basal metabolic rate of a resting mammal at thermoneutrality in the postabsorptive state<sup>91</sup>. However, cell cultures and *in vivo* experiments reveal that the thermogenic capacity of brown and beige adipocytes can be modified. In mammals and humans, environmental cold is a powerful trigger of brown/beige fat respiration<sup>6, 92</sup>. These stimuli are coupled to the thermosensitive receptors that transmit information through an afferent signal to the hypothalamus and the brain stem. In this way, sympathetic nerve endings in the adipose tissue release noradrenaline<sup>24, 93, 94</sup>. Noradrenaline acts on adrenoreceptors on the adipocyte plasma membrane, which ultimately results in the release of free fatty acids from stored triglycerides. Upon adrenergic stimuli (which results in the activation of brown (and white) adipocyte lipolytic cascade), leak respiration increases in a UCP1-dependent manner<sup>95</sup>. However, thermogenesis has proven to be independent of lipolysis; in fact, it has been demonstrated that stimulating lipolysis of cytosolic lipids droplets in brown adipocytes

is not required for cold-induced non-shivering thermogenesis<sup>96</sup>. Amongst other factors triggering thermogenesis, there are reactive oxygen species (ROS). The induction of ROS in fat cells by genetic modifications, some drugs or as result of cell oxidation, is sufficient to increase thermogenesis in adipocytes<sup>90</sup>. Moreover, activation of thermogenesis in murine BAT by applying either thermal stress (4 °C) or  $\beta$ -adrenergic stimuli results in augmented levels of mitochondrial superoxide, mitochondrial hydrogen peroxide, and lipid hydroperoxides. A possible mechanism by which mitochondrial ROS can induce thermogenesis is related to modifications of cysteine thiol redox status<sup>97, 98</sup>. A recent study identified a mechanism by which substantial accumulation of the mitochondrial metabolite succinate can act as a potent molecular source of thermogenic ROS in BAT and beige fat<sup>99</sup>. In addition to the strong evidence for UCP1 contribution to thermogenesis in beige cells, an increasing number of studies are evaluating if the presence of UCP1 protein alone is necessary and sufficient to induce thermogenesis<sup>84, 100</sup>. Indeed, some studies conducted in UCP1-KO mice have shown that induction of thermogenesis may have distinct routes. In a study using UCP-1 KO mice, it was observed that cold sensitivity can be fully regained by crossing this strain with mice expressing the transgenic PR domain containing 16 (PRDM16), which had the promoter of the fatty acid binding protein 4 (Fabp4 / aP2)<sup>101</sup>. Remarkably, UCP1-KO mice are resistant to diet-induced obesity at sub-thermoneutral temperatures, presumably via activation of poorly defined alternative pathways of energy loss in the absence of UCP1. However, the possibility that thermogenesis is independent of UCP-1 is controversial. In this regard, it cannot be ruled out that in UCP-1 KO mice thermogenesis is induced by muscle activity, which leads to shivering thermogenesis<sup>102</sup>. Recently, it has been observed that depleting creatine levels leads to reduced thermogenesis and obesity. Creatine is required to support full thermogenesis activation after adrenergic stimulation<sup>97, 103, 104</sup>. The thermogenic action of creatine seems to occur only when ADP is limited, which is the expected parameter of the physiological cellular state. However, the mechanism by which creatine influences mitochondrial metabolism is yet to be established. A study analyzing the expression of regulators of creatine in purified human subcutaneous adipocytes found an inverse correlation

between creatine mRNA and body mass index (BMI)<sup>97</sup>. A recent analysis of 18F-FDG PET/CT scans in human subjects demonstrated that renal creatinine clearance was a significant predictor of total activated human BAT<sup>105</sup>. Since creatinine is a direct product of phosphocreatine metabolism, these results are consistent with the activation of creatine-dependent thermogenesis in human BAT and suggest that creatinine may be used as a biomarker of human BAT activity.

### TRANSCRIPTIONAL CONTROL OF BROWNING

The phenotypic change of WAT into more energetically active cells makes evident a control of gene expression. Internal or external regulators of WAT physiology must modify the chromatin structure or the DNA promoter methylation pattern of the target genes<sup>106-108</sup>. Recently, other modifications of non-coding RNAs have shown an additional degree of controlling gene expression<sup>109, 110</sup>. It should be noted that regulators act by modification of four transcriptional or coregulator factors: PPAR $\gamma$ , CCAAT Enhancer Binding Protein Beta (C/EBP $\beta$ ), PPAR $\gamma$  co-activator-1 $\alpha$  (PGC1 $\alpha$ ) and PRDM16. PPAR $\gamma$  and C/EBP $\beta$  act as transcription factors and bind to DNA directly<sup>111, 112</sup>, while PRDM16 and PGC1 $\alpha$  act as transcriptional coregulators. In fact, PRDM16 forms a transcriptional complex with canonical DNA binding transcription factors PPAR $\gamma$  and C/EBP $\beta$  through its zinc finger domains, in order to activate the selective gene program for browning<sup>113, 114</sup>. More recently, analysis of PRDM16 by chromatin immunoprecipitation along with sequencing (ChIP-seq) showed that a large proportion of PRDM16 target genes are localized along with PPAR $\gamma$  and C/EBP binding sites, further supporting their co-regulation<sup>115</sup>. Although still not completely defined, beige adipocytes are detected after the birth of a pre-adipocyte population that is positive for platelet derived growth factor receptor- $\alpha$  (PDGFR $\alpha$ ) and stem cell antigen 1 (SCA1), or precursors of MYH11. Their appearance occurs as a response to a variety of internal or external stimuli, including chronic exposure to cold, PPAR $\gamma$  agonists, cancer cachexia, exercise and various endocrine hormones<sup>68, 69</sup>. Some factors that control the differentiation of BAT adipocytes also regulate the differentiation of beige adipocytes. Early B-Cell transcription factor 2 (EBF2) is a pivotal factor for the differentiation of BAT and has an important role in inducing the expression of

beige adipocytes<sup>64, 116</sup>. EBF2 is highly expressed in PDGFR $\alpha$  positive cells and the overexpression of EBF2 in primary white adipocytes or WAT induces the expression of thermogenic genes, increases oxygen consumption and suppresses high-fat diet induced weight gain<sup>117</sup>. There are several proteins that can control beige adipocyte differentiation through functional control of PRDM16. Differentiation of beige adipocytes can be promoted by the activating or repressive action of PRDM16. The formation of a repressor complex of PRDM16 with CtBP1 and CtBP2 reduces WAT adipogenesis<sup>43</sup>. On the other hand, the family of retinoblastoma proteins (pRb) antagonizes the activity of PPAR $\gamma$  and PRDM16<sup>118</sup>. We observed that inhibition of pRb by EP300 Interacting inhibitor of differentiation-1 (EID-1) can induce the differentiation of beige adipocytes in humans<sup>31</sup>.

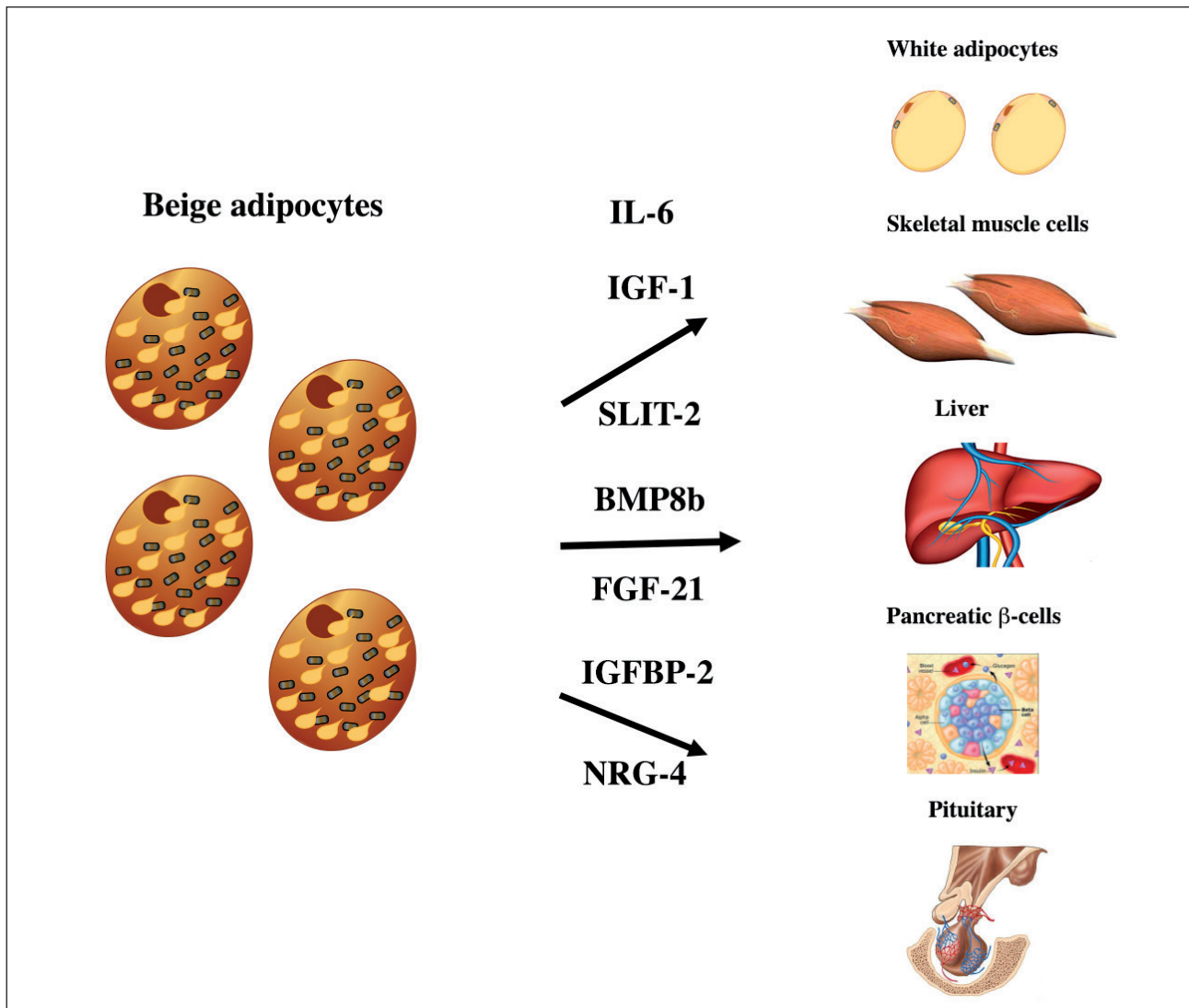
PGC1 $\alpha$  was first discovered as an interacting partner of PPAR $\gamma$  in brown adipocytes<sup>119</sup>. Pgc1 $\alpha$  gene expression is highly induced by cold exposure and is further activated following phosphorylation by the cAMP-PKA-p38/MAPK signaling pathway. Upon interaction with its binding partners, PGC1 $\alpha$  recruits histone acetyltransferases such as CBP/p300 and GCN5 to augment transcription<sup>120</sup>. PGC1 $\alpha$  binds to Nuclear Respiratory Factors 1 and 2 (NRF-1 and NRF-2) to promote the activation of several mitochondrial genes. PGC1 $\alpha$  also co-activates a number of nuclear hormone receptors, including PPAR $\gamma$ , PPAR $\alpha$ , and estrogen related receptors (ERR $\alpha/\beta/\gamma$ ), all of which participate in the transcription of brown fat genes<sup>121</sup>. Overexpression of PGC1 $\alpha$  in adipocytes, myotubes or cardiomyocytes promotes mitochondrial biogenesis and increases oxygen consumption<sup>122, 123</sup>. BAT pgc1 $\alpha$ -deficient mice display slightly increased lipid droplet accumulation but show normal levels of Ucp1 and other brown fat-selective genes<sup>124</sup>. Pgc1 $\alpha$ -deficient BAT in culture fails to efficiently activate the thermogenic machinery in response to adrenergic stimulation<sup>125</sup>. These results demonstrate that PGC1 $\alpha$  is required for the acute transcriptional activation of thermogenesis. Interestingly, deletion of Pgc1 $\alpha$  in adipocytes severely impairs the development of beige adipocytes in WAT<sup>126</sup>.

### THERAPY WITH INDUCTORS OF BEIGE ADIPOCYTES

Some factors have been used as potential anti-obesity drugs by regulating adipogenesis of beige adipocytes<sup>127, 128</sup>. Considerable attention has been paid to the secretory capacity of BAT and beige fat

cells. In this way, the molecules secreted by these cells have been called “batokines”, which can have paracrine or endocrine effects. Batokines play an important role in contributing to metabolic health through improvement of glucose and lipid homeostasis. Various BAT transplant studies have shown improvement in conditions associated with obesity, such as body weight and insulin sensitivity<sup>129, 130</sup> (Figure 2). Activation of  $\beta_3$ -adrenergic receptors is perhaps the most widely used pharmacological method for the induction of browning. Recently, a study investigated the use of Mirabegron (a drug approved by FDA for the treatment of overactive

bladder and exerting  $\beta_3$ -adrenergic receptor agonist properties) in patients with prediabetes. Mirabegron use for 3 months reduced insulin resistance without leading to reductions in body weight or any cardiovascular effects<sup>131</sup>. However, developing adrenergic agonists for obesity and metabolic diseases could lead to unwanted autonomic, bone, and cardiovascular effects over time<sup>132-134</sup>. Similarly, bone morphogenetic proteins 4, 7, and 8b (BMP4, BMP7, BMP8b), atrial and brain-type natriuretic peptides (ANP and BNP), FGF21, Vascular Endothelial Growth Factor- $\alpha$  (VEGF- $\alpha$ ), and prostaglandins, have all been shown to promote browning *in*



**Figure 2.** Adipokines secreted by beige adipocytes, which are also known as “batokines”. Beige adipocytes secrete molecules which have endocrine effects on some of the most important tissues involved in the regulation of body weight and lipid and carbohydrate metabolism. BMP8b: bone morphogenetic protein 8b; IGF-1: insulin-like growth factor 1; IGFBP-2: insulin-like growth factor binding protein 2; IL-6: Interleukin 6; NRG-4: Neuregulin 4; SLIT-2: Slit homolog protein 2.

*vivo*<sup>20, 135-137</sup>. However, these factors may also potentially exert unwanted pleiotropic effects when translated into drugs. FGF21 is an activator of thermogenesis that has gained the interest of various pharmaceutical companies. However, studies conducted in humans using FGF21 analogs have not shown a significant effect of these compounds on body weight or glycemic control<sup>138, 139</sup>, although a beneficial effect on hepatic steatosis has been observed<sup>140</sup>. New factors, such as SLIT2, are being assessed as possible therapeutic targets. SLIT2 is secreted from beige adipocytes and is transcriptionally regulated by PRDM16, which promotes a thermogenic, PKA-dependent pathway in adipocytes and improves overall metabolic parameters in response to high-fat diet challenge<sup>44</sup>. The c-terminal fibrinogen like domain of angiopoietin-like 4 (FLD of Angptl4), induces cAMP-PKA-dependent lipolysis in white adipocytes and reduces diet-induced obesity<sup>141</sup>. Angptl4 increases the thermogenic program and promotes the subsequent protection against weight gain and improvement of glucose tolerance in high-fat diet-fed mice<sup>142</sup>. Recently, it was observed that kynurenic acid increases energy expenditure by activating G-protein-coupled receptor 35 (Gpr35), which in turn stimulates a thermogenic program in adipose tissue and increases the levels of regulator of G protein signaling 14 (Rgs14) in adipocytes, resulting in enhanced  $\beta$ -adrenergic receptor signaling<sup>143</sup>. There have been several clinical studies in human adults that suggest the beneficial effect of activating browning from WAT. In an interesting study, Yoneshiro *et al.*<sup>144</sup> showed that daily 2-h cold exposure at 17 °C for 6 weeks resulted in an increase in BAT activity and cold-induced increments of energy expenditure, along with a concomitant decrease in body fat mass. Chondronikola *et al.*<sup>145</sup> showed that prolonged cold exposure for 5 to 8 h was able to increase resting energy expenditure (REE) by 15%; plasma glucose (30%) and FFA (70%) contributed to the observed increase in REE. Glucose disposal was increased in brown/beige adipocytes and whole-body glucose disposal was significantly increased. In subjects with type 2 diabetes, 10 days of cold acclimation increased peripheral insulin sensitivity by 43%<sup>146, 147</sup>. Basal skeletal muscle glucose transporter type 4 (GLUT4) translocation was markedly increased and glucose uptake in skeletal muscle was increased after cold acclimation<sup>148</sup>. These observations lead us to argue that cold exposure facilitated energy expenditure and could have beneficial effects on glucose metab-

olism. Also, these observations support the role of temperature reduction in the treatment of obesity and related metabolic disorders in humans. Most likely, the effect of the cold will arise from the activation of beige adipocytes.

Mesenchymal stem cells can be modulated in the attempt to give rise to beige adipocyte. This effect can be accomplished in different ways by modulating specific transcription factors. The large number of investigations in this area suggests a high possibility that numerous potential drugs will be tested in the upcoming years<sup>149-155</sup>. Despite the fact that the mechanisms by which these effects are produced have not been sufficiently elucidated, the use of modulation of beige adipocytes in adipose cell progenitors in adults is clearly a therapeutic approach, especially for obesity and type 2 diabetes<sup>156</sup>. Bearing in mind that in type 2 diabetes one of the most important pathophysiological components is peripheral resistance to the action of insulin, a change in insulin sensitivity mediated by an increase in the number of beige adipocytes may be a highly valuable therapeutic strategy<sup>157, 158</sup>. Furthermore, a decrease in body weight can lead to a lower load on the activity of the endocrine pancreas, protecting insulin-producing beta cells within the pancreatic islets. Some experiments have shown that a reduction in glucose levels can be obtained together with an increase in insulin sensitivity with the induction of beige/brown fat in humans<sup>145</sup>. Certain therapeutic effects must be evaluated before the possible clinical use of beige adipocyte induction. First, it should be determined whether the metabolic effects of beige adipocytes are subject to an increase in UCP1 or there are alternative metabolic pathways that could improve the condition of the adipose cells<sup>103</sup>. It is possible that stimulated beige adipocytes, like the brown adipocytes, are not only heat generators but also contribute to the improved metabolism of glucose and lipids through the secretion of specific factors<sup>20</sup>. Another important aspect is to address whether there is a more specific technique to detect beige adipocytes in humans; although 18F-FDG-PET has improved considerably, it is desirable to develop new tools or instruments that can quantify the amount of beige adipocytes in the body. The biology of beige adipocytes is so novel that it is necessary to gain a better understanding of their physiological actions, the number of days that these cells can survive, the factors that may be required to maintain the functionality of these cells, and so on.



Finally, it will be important to determine which are the specific factors that account for plasticity of beige adipocytes and make possible their differentiation from mature white adipocytes. The browning process from both adipocyte precursor cells, and white adipocytes may be a desirable therapeutic tool for weight loss and treatment of metabolic diseases.

#### ACKNOWLEDGMENTS:

The authors thank the members of the laboratory for the helpful suggestions and Katherin Calvo Martinez for the great help in editing the manuscript. This work was supported by the School of Medicine and DIN (Research Department) from Universidad de La Sabana.

#### FUNDING:

The work of the authors was supported in part by MED-255-2019 from DIN (Dirección de Investigación) Universidad de La Sabana. DM is supported by Young Researcher Program from MinCiencias 2019.

#### CONFLICT OF INTEREST:

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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