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REVIEW

Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus

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ABSTRACT

Mammalian adipose tissue can be divided into two major types, namely, white adipose tissue (WAT) and brown adipose tissue (BAT). According to classical view, the main function of WAT is to store excess energy in the form of triglycerides, while BAT is a thermogenic tissue that acts a pivotal part in maintaining the core body temperature. White adipocytes display high plasticity and can transdifferentiate into beige adipocytes which have many similar morphological and functional properties with brown adipocytes under the stimulations of exercise, cold exposure and other factors. This phenomenon is also known as 'browning of WAT'. In addition to transdifferentiation, beige adipocytes can also come from de novo differentiation from tissue-resident progenitors. Activating BAT and inducing browning of WAT can accelerate the intake of glycolipids and reduce the insulin secretion requirement, which may be a new strategy to improve glycolipids metabolism and insulin resistance of obese and type 2 diabetes mellitus (T2DM) patients. This review mainly discusses the significance of brown and beige adipose tissues in the treatment of obesity and T2DM, and focuses on the effect of the browning agent on obesity and T2DM, which provides a brand-new theoretical reference for the prevention and treatment of obesity and T2DM.

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Introduction

Obesity is a chronic metabolic disease caused by genetic, environmental, psychological and social factors. It is characterized by the imbalance of white adipose tissue (WAT) and brown adipose tissue (BAT). The global obesity rate continues to rise, which must be the result of a long-term imbalance between energy intake and energy expenditure. At present, about 2.2 billion people are overweight in the world, accounting for about one third of the global population, of which about 712 million people (ten percent of the global population) are obese people [1]. Obesity has a profound impact on tissue insulin sensitivity, and therefore has an impact on systemic glucose homoeostasis. Obesity is prone to a variety of diseases, which have been considered as potential causes or contributing factors of many obesity-related diseases, including insulin resistance and type 2 diabetes mellitus (T2DM) [2,3]. T2DM is a serious chronic metabolic disease that occurs when the blood glucose levels increase as a consequence of peripheral insulin resistance, which can be accompanied by the subsequent development of beta-cell failure and insulin deficiency. At present, there are 463 million diabetics. According to this growth trend, there will be 700 million diabetics in the world by 2045 [4]. Although there are many treatment methods for obesity and T2DM, including drugs that protect pancreatic cell function, increase insulin sensitivity and improve glucose and lipid metabolism, many drugs will bring adverse reactions. In addition, only a few drugs have been approved in many countries/regions, and the therapeutic effect of these drugs is not as expected. Therefore, it is necessary to find a novel therapy that can reduce calorie accumulation or increase energy expenditure to improve insulin sensitivity, reduce weight and maintain the activity of pancreatic beta cells.

It is known to all that there are two main types of mammalian adipose tissue: WAT and BAT. In the 1960s and early 1970s, it was thought that BAT was

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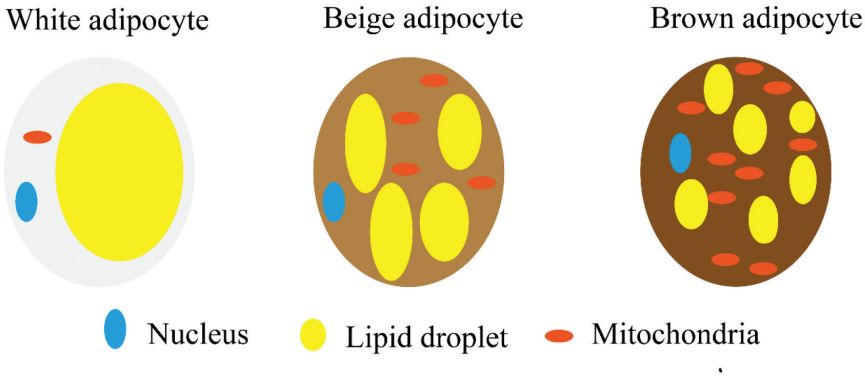
abundant in infancy, but regressed in adulthood, and there was almost no BAT or active BAT in adults [5,6]. However, with the in-depth researches of adipose tissue, a large quantity of bioactive brown/beige adipocytes were found in the neck and shoulder regions of adults by ^{18}F-FDG-PET/CT detection [7,8]. It is currently believed that white and brown adipocytes arise from distinct embryonic precursors. White adipocytes mainly originate from myogenic factor 5 negative cells (Myf5^{-}), while brown adipocytes originate from myogenic factor 5 positive cells (Myf5^{+}) [9,10]. Apart from different origins, their biological functions are totally different. White adipocytes (Figure 1) are specialized in lipid storage and mobilization. In times of caloric need, white adipocytes provide a long-term metabolic fuel via lipolysis and the release of fatty acids. Besides, white adipocytes are also involved in functions such as hormone secretion, immune function. On the other hand, brown adipocytes are specialized in energy expenditure and utilize chemical energy for thermogenesis, which acts a pivotal part in maintaining the core body temperature. Brown adipocytes (Figure 1) are characterized by multilocular lipid droplets and abundant mitochondria which contain a unique protein called uncoupling protein 1 (UCP1). Residing in the inner mitochondrial membrane, UCP1, a very significant regulatory factor in thermogenesis, uncouples mitochondrial respiration from ATP synthesis resulting in thermogenesis [11].

However, with further insight into adipose tissue, it has been observed that in the case of cold exposure or activation of β-adrenergic receptors (β-ARs), a distinct type of adipocyte named beige adipocyte (Figure 1) can be detected in WAT. This phenomenon is known as



	White adipocyte	Beige adipocyte	Brown adipocyte
Location in humans	Subcutaneous, supraclavicular	Supraclavicular, neck	Supraclavicular, neck, axillary
Location in mice	Gonadal, mesenteric, inguinal, retroperitoneal	Inguinal	Interscapular
Morphology	Unilocular/ large lipid droplets	Multilocular/ multiple small lipid droplets	Multilocular/ multiple small lipid droplets
UCP1 levels	Low or undetectable	Medium	High
Mitochondrial density	Low	Medium	High
Function	Energy storage	Thermogenesis and energy expenditure	Thermogenesis and energy expenditure
Enriched markers	TCF21, TLE3	CD137, TBX1, TMEM6	LHX8, ZIC1

Figure 1. Comparison of white adipocytes, beige adipocytes and brown adipocytes in location, morphology, UCP1 expression level, mitochondrial density, function and marker genes. In adults, the subcutaneous fat is characterized by classic WAT. The BAT on clavicle consists of white, beige and brown adipocytes, while the classic BAT can be found in the deep neck, armpit and adjacent to skeletal muscle tissue. The fat adjacent to gonads, groin, mesentery and peritoneum of adult mice has the characteristics of classical WAT. The beige adipocytes were found in the groin of mice, while the classical BAT was found in the scapular area. Transcription factor 21 (TCF21), Transducin-like enhancer of split 3 (TLE3), Tumour necrosis factor receptor superfamily, member 9(CD137), T-Box 1 (TBX1), Transmembrane protein 26 (TMEM26), LIM homeobox protein 8 (LHX8), zinc finger protein of the cerebellum 1 (ZIC1).

'browning of WAT' [12,13]. The origin of beige adipocytes is still controversial. It mainly includes the following points: (a) Beige adipocytes are derived from the transformation of mature white adipocytes; (b) Beige adipocytes exist a distinct beige adipocyte precursor; (c) Beige adipocytes come from de novo differentiation from tissue-resident progenitors [14,15]. Recently, it has been found that the number of UCP1 positive cells in beige adipose tissue and classical BAT is controlled by different genetic factors [16]. Beige adipocytes, at least those in the mouse subcutaneous depot, do not originate from myogenic factor 5 (myf5)-positive embryonic precursors that give rise to brown adipocytes [16]. Importantly, the metabolic characteristics of both brown adipocytes and beige adipocytes in the body have aroused great interest. Both brown adipocytes and beige adipocytes can uptake glucose and fatty acids to produce heat, which play a decisive role in regulating glucose and lipid metabolism in the whole body. It has been confirmed that the activation of brown and beige adipose tissues may represent a valid strategy to treat obesity and T2DM [17–19]. Therefore, elucidating the regulatory mechanisms of brown and beige adipose tissues may provide new targets for treating obesity and diabetes.

Role of brown and beige adipose tissue in obesity and T2DM

As previously stated, BAT acts a pivotal role in thermogenesis, contributing to energy consumption and preventing obesity. The thermogenic energy of BAT mainly comes from β-oxidation of fatty acids, while only a small part of the energy comes from glucose metabolism. However, BAT has a substantial impact on glucose homoeostasis in *vivo*, as BAT has a high rate of glucose uptake after activation compared with other metabolically active tissues [20]. BAT is one of the most insulin-sensitive tissues, and insulin enhances BAT glucose uptake, and glucose uptake by BAT can produce ATP through anaerobic hydrolysis in the cytoplasm. On the one hand, it can be used for the activation of free fatty acid (FFA) before entering the mitochondria for β-oxidation; on the other hand, it can prevent ATP deficiency caused by the uncoupling of oxidative phosphorylation in mitochondria [21]. In addition, glucose can also be used in the synthesis of FFA and triglycerides [22]. It has been found that activated BAT can take up and utilize glucose and lipid, and thereby improving beta-cell function and reducing the demand of insulin secretion from pancreatic beta cells [17]. FDG-PET/CT detection confirmed that the fasting glucose concentration of individuals with BAT was lower than that of individuals without BAT [23]. Under the conditions of cold exposure and insulin stimulation, both glucose uptake rate and insulin sensitivity of obese patients were lower than those lean subjects, suggesting that the effects of cold and insulin on BAT activity are markedly blunted in obesity [22]. Similarly, cold exposure or BAT transplantation can also improve glucose tolerance and insulin sensitivity in mice [24–26]. However, when the insulin receptor of brown adipocytes was knocked out, mice showed age-dependent interscapular brown fat loss, but the expression of UCP1 and UCP2 increased [27]. Moreover, these mice developed defective insulin secretion, leading to progressive glucose intolerance without insulin resistance [27]. The above results suggest a role of insulin receptors in brown fat development, as well as a role for BAT in regulating insulin secretion and glucose homoeostasis.

In addition to classic BAT activation to treat obesity and T2DM, the recruitment of beige adipocytes has recently attracted much attention as a novel therapeutic target for obesity and T2DM. Beige adipocytes also play a paramount role in weight control, energy balance regulation and amelioration of glucose and lipid metabolism. Under external stimuli (such as cold exposure, β_3-adrenergic agonists, etc.), beige adipocytes recruited by WAT accelerate the absorption of circulating glucose and lipids, and increase energy consumption and thermogenesis [28,29]. The consumption of glucose and lipids indirectly improves glucose tolerance, insulin sensitivity and beta-cell function. In fact, brown and beige adipose tissues are potential therapeutic targets to treat obesity and T2DM due to their inherent thermogenic capacity and their ability to improve glucose metabolism. The protective effects of brown and beige adipose tissues against obesity and T2DM rely on the recruitment of beige adipocytes and the enhancement of BAT amount and/or BAT activity, resulting in increased UCP1 expression, energy expenditure and thermogenesis (Figure 2). Activation of beige and brown adipocytes increases glucose uptake, fatty acid oxidation and lipolysis, which results in the increase of insulin sensitivity and reduction in blood glucose, blood lipids and fat mass, thus preventing obesity and T2DM [30]. Overall, brown fat and beige adipose tissue display a markedly high metabolic activity, which may be used as an underlying therapeutic target for the treatment of obesity and T2DM.

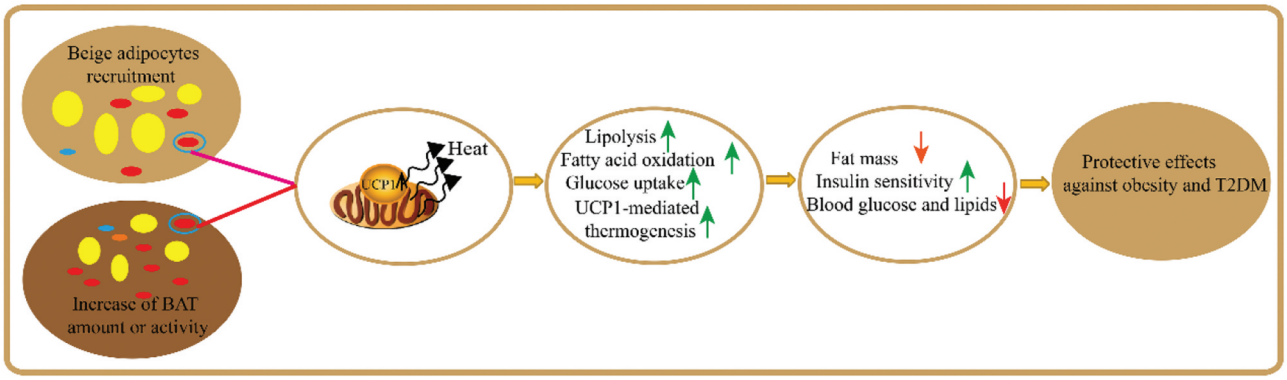


Figure 2. Protective effects of beige and brown adipose tissue against obesity and T2DM. The recruitment of beige adipocytes and the increase of BAT amount and/or BAT activity can promote the expression of UCP1 and increase heat production. Overexpression of UCP1 protein will enhance glucose uptake, fatty acid oxidation and lipolysis of both beige and brown adipocytes, which leads to increased insulin sensitivity and reduction in blood glucose, blood lipids and fat mass, thus preventing T2DM. Note: Upward pointing green arrows and downward pointing red arrows indicate 'increase' and 'decrease', respectively.

Effect of browning agents on obesity and T2DM

To further insight into the mechanism of brown and beige adipose tissue on obesity and T2DM, the effect of browning agents on obesity and T2DM is discussed in the following paragraphs (Figure 3 and Figure 4)

Exercise

Exercise benefits improvement of glucose homoeostasis and offers protection against metabolic disorders such as obesity and diabetes [31]. It has been found that exercise training improves insulin resistance, enhances glucose disposal and muscle glycogen storage by increasing the expression of GLUT4 [32]. In sports training, in addition to using glucose as an energy source, skeletal muscles also use part of lipids as an energy source to accelerate the intake and utilization of free fatty acids (FFA) [33]. In addition, exercise has been reported to induce browning of WAT and enhance energy consumption by promoting hypothalamic brain-derived neurotrophic factor (BDNF) expression [34]. Recently, it has been proved that several novel factors can induce browning of WAT. During the exercise, PGC1-α expression in mouse muscles stimulated the expression of fibronectin type III domain containing 5 (FNDC5), which is cleaved and identified hormone known as irisin [35]. Irisin has been shown to promote the recruitment of brown adipocytes in the WAT of mice [35]. As a circulating factor, meteorin-like (Metrnl) induced in muscles after exercise increases energy expenditure and improves glucose tolerance and the expression of genes associated with beige fat thermogenesis by stimulating an eosinophil and promoting alternative activation of adipose tissue macrophages [31].

In a human experimental study, compared with lean sedentary men, male endurance training athletes had lower activity of BAT; meanwhile, there was no significant difference in the expression of the classical brown adipocyte marker gene in subcutaneous WAT between the two groups [36]. It is speculated that the reason of the aforementioned findings may be that exercise training activates thermogenesis of skeletal muscles, thereby reducing the metabolic activity of BAT in humans. It seems that thermogenic processes of BAT and muscle do not occur simultaneously during exercise. In another human experiment, subcutaneous adipocytes became smaller and fatty acid composition changed after long-term exercise, which was probably related to the preferential mobilization of palmitic acid and oleic acid, and the preferential retention of stearic acid and linoleic acid [37]. The decrease of palmitic acid and oleic acid content in adipose tissue after exercise may be considered as a favourable change. Moreover, lactic acid, the product of anaerobic exercise, is likely to induce browning of WAT [38]. In rodents, exercise seemed to be a weak stimulus for BAT thermogenesis. Surprisingly, exercise increases mitochondrial biogenesis and activity in both subcutaneous WAT and visceral WAT, it also increases the expression of the brown adipocyte marker UCP1 in both adipose tissue depots, although these effects are much more pronounced in subcutaneous WAT [39–41]. This is inconsistent with the results of human experiments, which may be related to species differences, different locations of adipose tissues in humans and animals, and different experimental conditions.

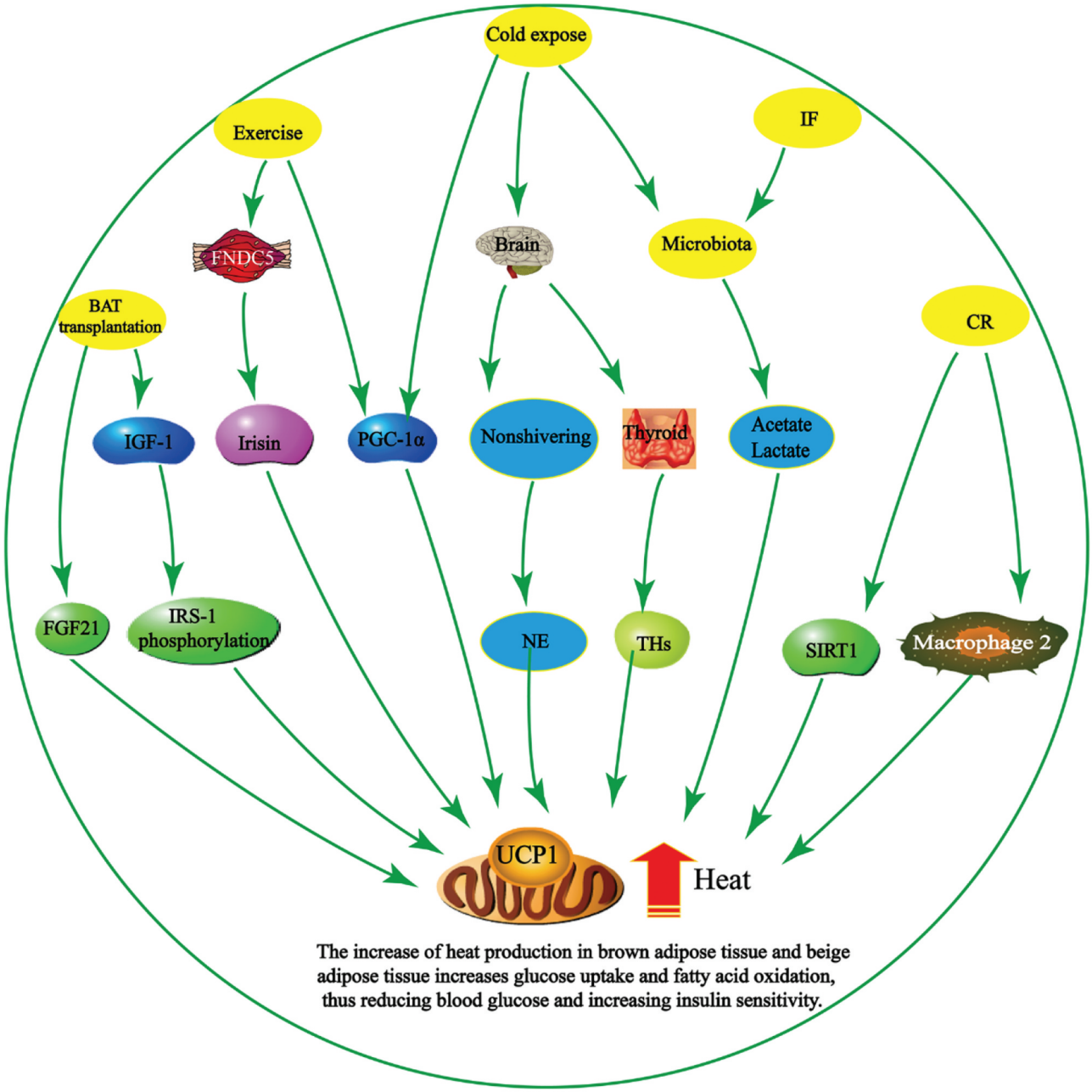


Figure 3. Detailed mechanisms of anti-obesity and type 2 diabetes with browning agents such as exercise, cold exposure, and brown fat transplantation. Note: The green arrow (□) indicates activation or promotion.

Cold exposure

Cold can activate the sympathetic nervous system (SNS) and stimulate the release of norepinephrine (NE) [42]. NE binds to and activates the β-adrenergic receptors (β-ARs) in adipocytes, which contributes to the lipolysis of triglycerides in white and brown adipocytes [43]. Activated β-ARs interact with GTP binding protein GS of heterotrimer to activate adenylate cyclase, thereby increasing the level of cAMP in cells and activating cAMP-dependent protein kinase A (PKA) [42]. In fat cells phosphorylated PKA modifies lipid droplet binding proteins (such as lipid droplet binding proteins A and B) and hormone-sensitive lipases, thus leading to the release of FFA and glycerol, which are exported into the circulation and used as fuel for other tissues [44,45]. The released fatty acids can activate the UCP1 protein and act as a substrate for UCP1-mediated thermogenesis [21]. Furthermore, these fatty acids also conduce to regulating transcription control through peroxisome-proliferator-activated receptors α (PPAR α) and PPAR γ [46]. PPAR γ is of great significance in the differentiation of adipocytes in both WAT and BAT [47,48]. It

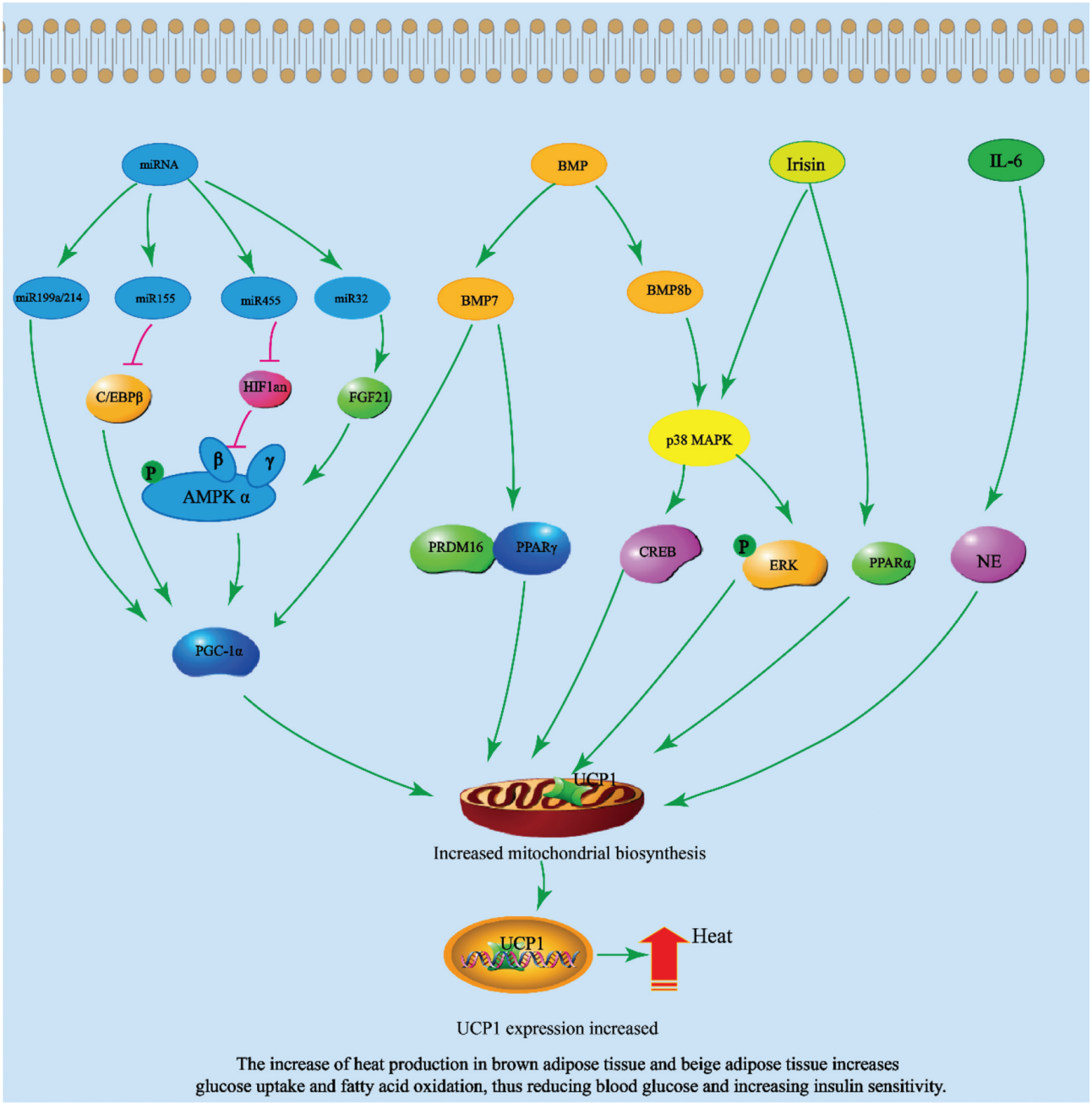


Figure 4. Detailed mechanisms of anti-obesity and T2DM with endogenous browning agents (miRNA, BMP, Irisin and IL-6). Note: The green arrow (□) indicates activation or promotion, and Inverted 'T' () indicates inhibition.

has been confirmed that PPAR γ is conducive to improving the mitochondrial biosynthesis and oxidation function of beige adipocytes in *vivo* and in *vitro* by regulating PGC-1α, thus promoting the browning of WAT [49–51]. PGC-1α seems to be necessary for cold exposure to induce beige adipocyte recruitment and survival in WAT [52].

BAT activity is sustained by the SNS. Both acute and chronic cold exposure enhance energy expenditure and thermogenesis of brown and beige adipose tissues [53,54]. It has been found that BAT activated by cold exposure contributes to enhancing the uptake of triglyceride-rich lipoproteins (TRLs) through lipoprotein lipase (LPL) and transmembrane receptor CD36, thereby accelerating the removal of serum triglycerides [55]. In addition, cold exposure can also increase glucose uptake and insulin sensitivity by enhancing the expression of glucose transporter protein 4 (GLUT4) [56]. Cold exposure can activate beige and brown adipose tissue, and promote the release of factors that induce the browning of WAT in both myocardium and skeletal muscle [57]. For example, the release of

atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) from cardiomyocytes after cold exposure can enhance the expression of both PGC-1α and UCP1, as well as mitochondrial biosynthesis and energy expenditure in WAT [57]. It has been shown that ANP increases BAT mitochondrial activity in a p38 mitogen-activated protein kinase (p38 MAPK)-dependent manner [58]. BNP can increase the expression of UCP1 and PGC-1α in WAT and BAT, as well as increase respiration and energy consumption [58].

BAT transplantation

BAT is a metabolically active organ that participates in maintaining energy homoeostasis. Therefore, functional BAT transplantation may bring therapeutic benefits in treating diabetes, obesity and other related metabolic diseases. It has been shown that BAT transplantation can significantly prevent weight gain, reduce the total fat weight and enhance oxygen consumption in obese mice, thereby ameliorating IR and liver steatosis [59]. It has been found that when the BAT used for transplantation was obtained from interleukin-6 (IL-6)-knockout mice, high-fat diet-induced recipient mice did not reverse insulin resistance, indicating that IL-6 could serve as the secreted factor at least partially mediating the beneficial effects observed in the BAT transplantation studies [60]. It has been confirmed that BAT transplantation can heighten the level of circulating adiponectin, increase expression of β-AR and fatty acid oxidation-related genes in WAT [61]. BAT transplantation models successfully demonstrated the improvements in glucose metabolism and insulin sensitivity, as well as reductions in body mass and decreased adiposity in transplant recipients [62]. BAT transplantation enhanced the uptake of glucose by endogenous BAT, WAT and myocardium, surprisingly, not by skeletal muscles [62]. Also, it has been shown that transplantation of embryonic BAT can correct type 1 diabetes mellitus (T1DM) in streptozotocin-treated mice and reverse symptoms of clinical diabetes such as polyuria, polydipsia and polyphagia [63]. Further research found that plasma insulin-like growth factor-1 (IGF-1) was the first hormone to increase after BAT transplantation in nonobese diabetic mice [64]. As with streptozotocin-treated mice, euglycemia was independent of insulin. Also, it has been suggested that IGF-1 may directly reduce blood glucose through the activation of insulin receptor. Altogether, these findings support the potential for insulin-independent reversal of T1DM with BAT transplantation and suggest IGF-1 as a potential mediator in the resulting equilibrium [64].

Gut microbiota

Gut microbiota is established with the development of the host, and its composition is affected by various factors such as obesity and pregnancy [65,66]. Some changes of gut microbiota composition are associated with T2DM and obesity. Gut microbiota can affect the metabolism and insulin sensitivity of the host, and it is a significant regulator of host metabolic homoeostasis and energy balance. In recent years, a close relationship between gut microbiota and thermogenesis process of adipose tissues has been found [67]. Gut microbiota can activate BAT and promote browning of WAT. It has been shown that the water extract of *Caulis Spatholobi* can ameliorate obesity through activating BAT and modulating the composition of gut microbiota, which induced an increase of anti-obesity and anti-diabetes related bacteria genus [68]. In a cold environment, the expression of UCP1 protein in BAT and subcutaneous WAT of mice treated with antibiotics was reduced, resulting in cold intolerance [69]. However, intragastric administration of butyrate heightened the thermogenic ability of mice treated with antibiotics and reversed this defect [69]. The above-mentioned results indicate that the depletion of microbiota weakens the adaptive thermogenesis of BAT and subcutaneous WAT, and that gut microbiota contributes to the up-regulation of thermogenesis in cold environments, which may be mediated by butyrate. Gut microbiota transplantation technology has a vital effect on remodelling gut microbial composition. Interestingly, it has been demonstrated that a cold-conditioned gut microbiome does not influence adaptive thermogenesis [70]. Based on the above results, it is speculated that cold exposure may increase the content of short-chain fatty acids produced by gut microbiota, thereby increasing adaptive thermogenesis, but a cold-conditioned gut microbiome does not influence adaptive thermogenesis. The latest research demonstrated that resveratrol improved glucose homoeostasis by enhancing BAT activation and browning of WAT. In addition, when the gut microbiota of mice fed with resveratrol was transplanted into sterile mice, the glucose intolerance of sterile mice was improved. The research found that one of the underlying mechanisms might partially be mediated by the gut microbiota-bile acid-Takeda G-protein coupled receptor 5 (TGR5)/UCP1 pathway [71]. Moreover, resveratrol can regulate the composition of gut microbiota by regulating the 'gut microbiota adipose tissue' axis, thus increasing energy consumption and regulating glucose and lipid metabolism to combat obesity [72]. Therefore, regulating the intestinal flora to activate BAT and induce

WAT browning is an effective strategy for protective effects against obesity and T2DM.

Intermittent fasting and caloric restriction

Intermittent fasting (IF) is an effective weight control strategy, but its mechanism is still unclear. A new study demonstrated that isocaloric IF improves glucose tolerance and postprandial insulin levels, and increases incretin expression in leptin-deficient ob/ob mice, indicating that isocaloric IF is effective in ameliorating nutrient-stimulated insulin secretion [73]. It has been found that IF can selectively stimulate the development of beige adipocytes in WAT, and significantly improve obesity, insulin resistance and liver steatosis by regulating intestinal microbial composition to increase the biosynthesis of acetate and lactate [74]. Interestingly, it has been reported that acetate and lactate are inducers of WAT browning [75,76].

Caloric restriction (CR) is another negative energy balance state, which can stimulate selective WAT glucose uptake, enhance the expression of type 2 immune response and sirtuin type1 (SIRT1), and promote the browning of WAT [77]. Further research demonstrated that these metabolic changes were mediated by increased eosinophil infiltration, type 2 cytokine signalling, and M2 macrophage polarization in adipose tissues of CR of animals [77]. Type 2 cytokines are necessary for browning of WAT and metabolic improvement during CR. Research showed that reducing energy intake weakened insulin-mediated lipolysis and affected the ratio of NAD^+/NADH and AMP/ATP [78]. The increased ratio of NAD^+/NADH activated the SIRT signalling pathway, thereby remodelling adipocyte phenotype [78]. SIRT1 suppresses white adipocyte development mediated by PPAR γ, and enhances lipolysis and energy expenditure [79]. Enforced expression of SIRT3 in HIB1B brown adipocytes can enhance the expression of UCP1, PGC-1α and a series of mitochondria-related genes, and play a decisive role in the adaptive thermogenesis of BAT [80]. Similarly, SIRT5 is necessary for the differentiation of brown adipocytes, and increases the expression of BAT marker genes through an indirect effect consisting of histone modifications [81].

Noncoding RNAs

Noncoding RNAs (ncRNAs) are transcripts lacking protein-coding capabilities, but they are of prominent significance in silencing the gene expression connected with complex gene regulatory networks [82,83]. According to the nucleotide length, ncRNAs are subsequently classified into long ncRNAs (lncRNAs) (>200 nucleotides) and small ncRNAs (sncRNAs) (<200 nucleotides). sncRNAs include microRNA (miRNA), small nuclear RNA (snRNA), circular RNA (circRNA), among others [84]. miRNAs are endogenous RNA molecules approximately 21–23 nucleotides in length, which play a prominent role in the regulation of gene expression [85].

It has been reported that miRNAs play a bidirectional role in activating brown and beige adipose tissues [84]. On the one hand, miRNAs play a positive regulatory role in activating BAT and inducing browning of WAT. Among the positive regulators, miR-455 is a new regulator that activates BAT. Under the condition of cold and the browning inducer bone morphogenesis proteins 7 (BMP7), miR-455 specifically expressed in BAT to regulate the differentiation and thermogenesis of brown adipocytes. In addition, adipose-specific miR-455 transgenic mice displayed browning of subcutaneous WAT after cold exposure [86]. miR-455 activates AMP-activated protein kinase (AMPK) α1 by targeting hypoxia-inducible factor 1α subunit inhibitor (HIF1an), which can promote the formation of beige fat cells and mitochondrial heat production [86]. Cold exposure can enhance expression of miR-32 in BAT, which is related to BAT specific super enhancer. By directly inhibiting its downstream target gene Tob1, miR-32 activates p38 MAPK signalling to promote fibroblast growth factor 21 (FGF21) expression and increase serum FGF21 level, thereby inducing browning of WAT [87]. Besides, miR-26 [88], miR-30b/c [89] and miR-196a [90] also play central roles in inducing browning of WAT.

On the other hand, miRNAs play a negative role in activating BAT and inducing browning of WAT. As a key negative regulator of brown and beige fat development and heat production, the overexpression of miR-199a/214 can inhibit the differentiation, thermogenic gene expression and mitochondrial respiration of brown adipocytes, while the knockout of miR-199a/214 can increase thermogenic gene expression and mitochondrial function of brown adipocytes [91]. Further research found that miR-199a/214 directly targeted PRDM16 and PGC-1α to inhibit the differentiation of brown fat cells and the development of beige fat [91]. miR-155 prevents the browning of WAT by inhibiting the expression of UCP1, Cidea, PPAR γ [92]. However, inhibition of miR-155 expression enhances brown adipocyte differentiation [92]. Similarly, miR-327 targets fibroblast growth factor 10 (FGF10) gene to prevent brown adipocyte differentiation and thermogenesis [93]. Besides, overexpression of miR-19b [94], miR-34a

[95] and miR-133 [96] can also suppress brown adipocyte activity and beige adipocyte recruitment.

Bone morphogenetic proteins

As a class of multifunctional growth factors, bone morphogenesis proteins (BMPs) belong to the superfamily of transforming growth factor β (TGFβ). They were initially considered to be significant factors in promoting bone formation, but they were later identified to participate in the development of adipose tissue, intestine, brain, etc. [97]. Some members of the BMP family are of great importance in maintaining energy homoeostasis and participating in the early stage of adipogenesis. It was found that BMP2, 4, 6, 7 and 9 effectively induced mesenchymal stem cells to form adipocytes in *vitro* and in *vivo* [98]. In addition, BMP4, BMP7 and BMP8 can promote the differentiation of white adipocytes to brown adipocytes. Overexpression of BMP4 gene enhances the number of white adipocytes with similar characteristics as brown adipocytes and ameliorates insulin sensitivity [99]. BMP7 participates in the whole process of brown adipocyte development and acts a pivotal part in inducing the browning of WAT. BMP7 increases the expression of the transcriptional regulators PRDM16, PGC-1α, PPARγ and C/EBPs and promotes thermogenesis through p38 MAPK and PGC-1α dependent pathways [100]. BMP8b is another member of the BMP family, which can directly regulate thermogenesis. In mature BAT, BMP8b enhances the response to norepinephrine by increasing p38 MAPK/cAMP response element binding protein (CREB) signal transduction and lipase activity [101].

Irisin

In recent years, irisin as an underlying novel target for the treatment of obesity and insulin resistance, has attracted great interest and attention in the scientific community. Irisin expression can be induced by exercise, it increases energy consumption and ameliorates insulin resistance induced by high-fat diet [102]. Irisin binds to unknown receptors of white adipocytes, thereby increasing both PPARα and UCP1 expression, and inducing the browning of WAT [103]. Irisin can also enhance lipolysis through the cAMP-PKA-HSL /perilipin signalling pathway [104]. In addition, irisin can promote browning of WAT through p38 MAPK and extracellular regulated protein kinase (ERK) signalling pathway, thereby promoting islet β cell proliferation and ameliorating glucose tolerance [105]. Irisin enhances the energy consumption and thermogenesis of both skeletal muscles and BAT [106]. In conclusion, irisin can increase insulin sensitivity in skeletal muscle and adipose tissue, ameliorate the disorders of glucose and lipid metabolism and the function of pancreatic beta cells.

Fibroblast growth factor 21

Fibroblast growth factor 21 (FGF21) is a member of FGF family, it is expressed in liver, WAT, BAT, skeletal muscle and pancreas, and plays a prominent part in energy balance, glucose and lipid metabolism [107]. FGF21 is considered to be a promising agent for the treatment of T2DM and obesity. Clinical trials have reported that FGF21 has beneficial effects on lipid and bile acid metabolism, with clinical improvement in dyslipidemia, steatosis, weight loss and liver damage [108]. It has been found that FGF21 can enhance energy consumption by regulating the expression of thermogenic genes in adipose tissues such as BAT and subcutaneous WAT, thus reducing blood glucose and lipid levels and ameliorating glucose tolerance and insulin resistance in obese and diabetic mice [109–111]. Under long-term cold exposure, FGF21 expression in both BAT and subcutaneous WAT of mice increased significantly [112,113]. Under cold exposure conditions, FGF21 significantly enhances PGC-1α protein levels, and enables PGC-1α to participate in thermogenesis of adipose tissue induced by FGF21 [114]. FGF21 can activate BAT and induce browning of WAT through autocrine and paracrine actions [115]. FGF21 has been shown to increase PGC-1α protein expression and mitochondrial activity of 3T3L1 cells through the AMPK-SIRT1-PGC-1α signalling pathway, thereby enhancing mitochondrial function and increasing oxygen consumption [116]. In addition, FGF21 stimulates islet autophagy by inhibiting AMPK mTOR signalling pathway, which further consolidates the evidence that FGF21 is a drug therapeutic target for obesity and related T2DM [117].

Thyroid hormones

Thyroid hormones (THs) have a vital effect on thermoregulation. The interaction of THs and sympathetic nervous system can increase the expression of UCP1 protein in BAT, thus increasing thermogenesis of BAT [118]. It has been confirmed that THs can regulate BAT and induce browning of WAT [119]. In mice, BAT activity was decreased by hypothyroidism; by contrast, it was increased by hyperthyroidism [120]. Multi-compartment adipocytes and thermogenic gene expression were found in the WAT of mice with hyperthyroidism and hypothyroidism. It was found

that hyperthyroidism and hypothyroidism may promote WAT thermogenesis through enhanced adrenergic signalling or compensation for impaired BAT function [120]. MAPK kinase 6 (MKK6) is highly expressed in WAT of obese individuals. However, MKK6 deletion increases energy expenditure and thermogenic capacity of WAT, protecting mice against diet-induced obesity and development of diabetes [121]. The underlying mechanism may be that deletion of MKK6 increases triiodothyronine (T3)-stimulated UCP1 expression in adipocytes, thereby increasing their thermogenic capacity [121]. In addition, coordinated glucagon and thyroid hormone actions synergize to correct hyperlipidaemia, steatohepatitis, atherosclerosis, glucose intolerance and obesity in mice with impaired metabolism [122].

Interleukin-6

Interleukin-6 (IL-6) is a cytokine secreted by skeletal muscle, T helper cells, white adipocytes and brown adipocytes [123–125]. IL-6 is an independent predictor of T2DM and is considered to be involved in the development of inflammation, insulin resistance and beta-cell dysfunction [126]. Also, increased plasma IL-6 levels have been observed in obese patients [127]. However, the release of central IL-6 can suppress weight gain and visceral obesity, and also enhance UCP1 protein expression in BAT by stimulating the sympathetic nervous system [127]. It has been reported that during acute cold exposure, IL-6 in the serum of mice increased significantly, which can maintain the core body temperature. The abovementioned results indicate that IL-6 may partly act on the central nervous system [128]. It has been found that IL-6 lost fat weight by promoting fat lysis and inducing browning of WAT [129]. Besides, burn promoted browning of WAT and increased lipolysis in mice, and IL-6 was significant regulator in this process [130]. IL-6 can be secreted when skeletal muscles respond to exercise and exerts insulin-sensitizing effects. It was found that BAT transplantation ameliorated glucose tolerance and insulin sensitivity, reduced body weight, increased FGF21 levels, and completely reversed insulin resistance in high fat diet-induced obese mice [60]. Importantly, when obtaining BAT for transplantation from IL-6-knockout mice, the improved metabolic profile was lost, which clearly demonstrated that IL-6 participated in the process by which BAT regulates glucose homoeostasis and insulin sensitivity [60].

Macrophage polarization

In adipose tissue, a pro-inflammatory response promotes insulin resistance, while an anti-inflammatory response enhances insulin sensitivity. The degree of inflammation of adipose tissue depends on the dynamic interaction between various immune cells. According to their immune and metabolic functions, the immune cells in fat can be divided into two categories: neutrophils, mast cells, M1 macrophages, etc., which mainly promote inflammation and reduce insulin sensitivity and glucose metabolism; the other category includes regulatory T cells, alternatively activated (M2) macrophages, eosinophils, etc., which mainly exert anti-inflammatory effects and can promote tissue repair and improve insulin sensitivity [131–134]. Macrophages can be used as regulators to activate BAT and promote browning of WAT, and regulate the thermogenesis process, so as to link the immune response with thermogenesis and energy consumption. Under cold exposure, IL-4 selectively activates M2 macrophages in adipose tissue, and the activated M2 macrophages secrete catecholamines to increase thermogenic gene expression in BAT and promote lipolysis in WAT [135]. Furthermore, it was found that the browning of WAT caused by burn was related to the increase of macrophage infiltration, and more M2 macrophages were observed in the fat of burn patients. Similarly, the increase in M2 macrophages was also observed in the fat of burn mice [136]. Macrophages in burn stressed subcutaneous WAT undergo alternate activation to enhance tyrosine hydroxylase expression and promote catecholamine production [136]. Therefore, macrophages with anti-inflammatory effects contribute to inducing browning of WAT, and enhance systemic thermogenesis and energy expenditure.

Current status of research on browning agents for the treatment of obesity and T2DM

Based on the great plasticity of adipose tissue in thermogenesis, activating BAT and inducing browning of WAT has become a new way of treating obesity and T2DM. The development of drugs that activate BAT and/or induce browning of WAT has become a research hotspot in recent years. Commonly used drugs include: β_3 adrenergic receptor agonists (Mirabegron), thiazolidinediones (Rosiglitazone), Glucagon-like peptide-1 receptor agonists (GLP-1RAs) (Liraglutide) and Berberine etc. (Figure 5).

By activating β-adrenergic receptor, β_3-adrenergic receptor agonists can reduce the weight of both subcutaneous and epididymal WAT, promote the

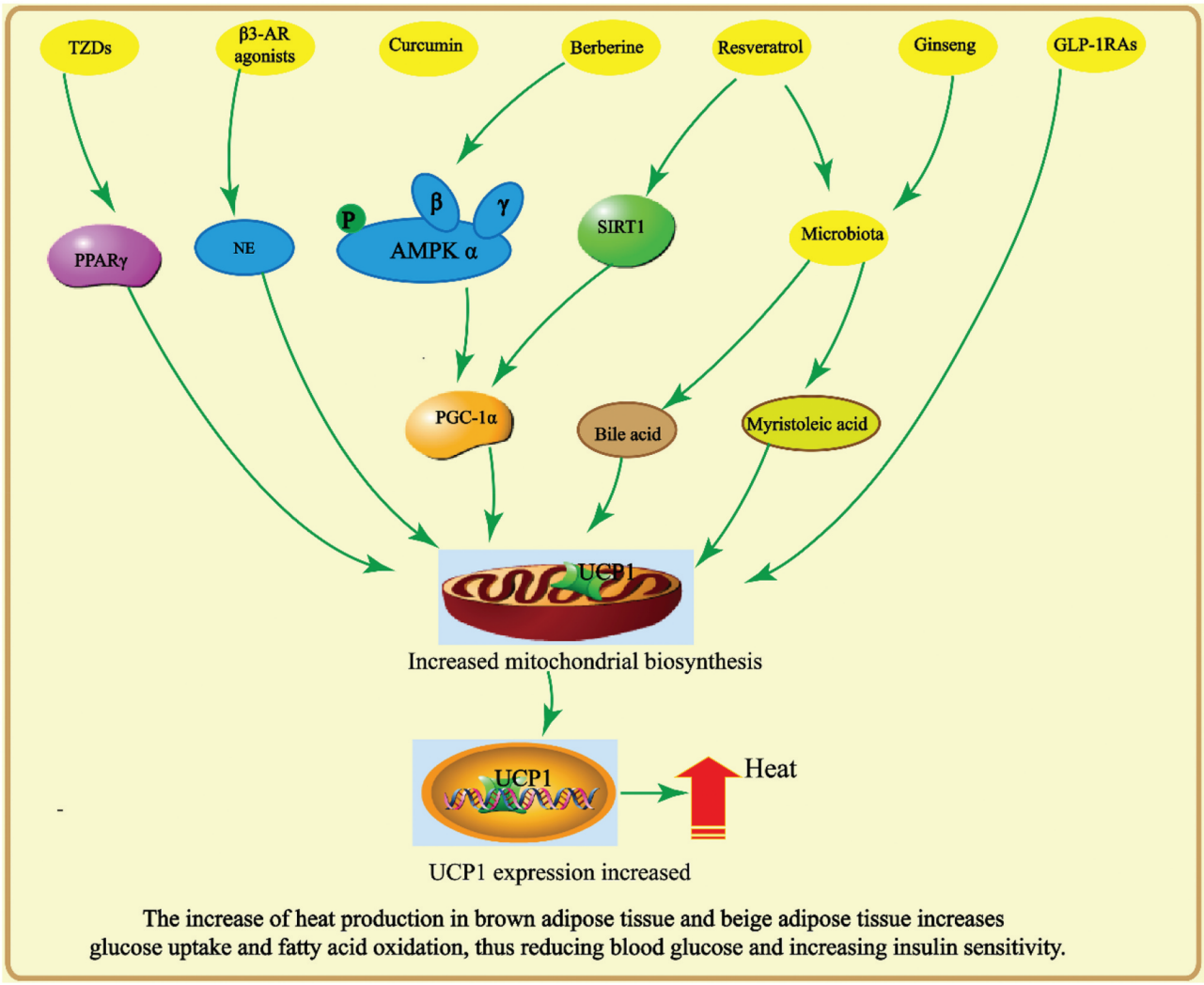


Figure 5. Detailed mechanisms of anti-obesity and T2DM with browning agents such as β3 AR agonists, TZDs, GLP-1RAs and Berberine etc. Note: The green arrow (□) indicates activation or promotion.

expression of UCP1 gene in subcutaneous WAT and BAT, increase thermogenesis and insulin sensitivity, and effectively ameliorate hyperglycaemia in diabetic animal models [137–139]. Unfortunately, β_3-adrenergic receptor agonist drugs (L-796,568) failed in human trials [140]. Importantly, mirabegron, a new β_3-adrenergic receptor agonist, acutely stimulates human BAT thermogenesis and increases resting metabolic rate [141]. Rosiglitazone, as a PPARγ agonist, can heighten the expression of mitochondrion thermogenic gene and UCP1 in 3T3-L1 adipocytes, increase the calcium inflow and glucose uptake mediated by α1-adrenoceptor in the subcutaneous groin and epididymal WAT of mice, and promote browning of WAT in mice [142,143]. As a GLP-1RAs, liraglutide can reduce visceral fat weight and enhance fatty acid oxidation. In addition, liraglutide can increase the expression of BAT marker genes in subcutaneous WAT of diabetic rats, promote the browning of subcutaneous WAT and ameliorate metabolic abnormalities in rats [144]. Retinoic acid plays an important role in adipocyte differentiation, adaptive thermogenesis and fatty acid oxidation. It can reduce body weight, improve insulin sensitivity and promote browning of WAT [145].

Ginseng extract can inhibit the obesity of db/db mice, and its mechanism is related to the induction of *Enterococcus faecalis*, which can produce an unsaturated long-chain fatty acid, myristoleic acid (MA), which can reduce adiposity by BAT activation and beige adipocyte formation [146]. The ethanol extract of *Platycodon* grandiflorum root can increase the intake of fatty acid in epididymal WAT by inducing the expression of related transporters, resulting in decreased blood FFA concentrations. Moreover, the ethanol extract of *Platycodon* grandiflorum root can increase the expression of browning related genes, the

level of overall transcriptome (Bmp4, Ucp3, Sirt3, etc.) and enzyme activity of carnitine palmitoyltransferase, thereby enhancing energy expenditure and promoting the browning of epididymal WAT and energy consumption [147]. Berberine is the main bioactive component of *Coptis chinensis*, a traditional Chinese herbal medicine, which has a significant effect on regulating glucose and cholesterol levels, anti-obesity and anti-diabetic [148,149]. It was found that berberine enhanced the activity of BAT and the expression of the thermogenic protein in subcutaneous WAT of leptin receptor-deficient db/db mice through the AMPK signalling pathway [150]. Resveratrol, a kind of natural polyphenol substance with strong activity, has a high content in many traditional Chinese medicines such as *Polygonum cuspidatum* and *Veratrum*. It has been demonstrated that resveratrol can reduce lipogenesis, improve the rate of lipolysis, increase the expression of both PGC-1α protein and UCP1 protein, and promote the browning of WAT by activating the SIRT1 signalling pathway in adipose tissue [151]. *Pentamethylquercetin* can heighten mitochondrial biogenesis and upregulate UCP-1 expression in WAT of high-fat-diet fed mice, and it contributes to promoting the browning of WAT [152]. Curcumin has been shown to enhance the expression of β_3-adrenergic receptor gene in subcutaneous WAT of mice, thereby increasing plasma levels of noradrenaline and thermogenesis [153]. Genipin as a product of gardenoside hydrolysed by β-glucosidase, ameliorated diet-induced obesity via promoting lipid mobilization and browning of WAT in rats [154]. Nicotine is the main bioactive substance in tobacco, which can affect food intake and energy consumption. Moreover, it can induce browning of WAT through central action, which depends on the κ opioid receptor secreted by the lateral hypothalamus [155]. In addition, menthol [156], cardamom [157], allicin [158] and capsaicin [159] can also promote browning of WAT to counteract obesity and T2DM.

Conclusion and prospects

In the past decade, scientists have learnt new information about the origin of BAT and WAT, and gained new insights about the plasticity of adipocytes. Activated BAT plays a prominent role in resisting cold and maintaining body temperature, meanwhile, it has the effects of ameliorating glucose homoeostasis and insulin sensitivity. Under the stimulation of certain conditions, based on the plasticity of WAT, beige adipocytes with similar characteristics and functions as those of brown adipocytes can originate from WAT. Beige and brown adipocyte-mediated thermogenesis may be a significant factor in determining human energy balance. Activating BAT and inducing WAT browning-mediated thermogenesis is an attractive strategy for improving glucose homoeostasis and insulin resistance, which can be used as a new option for the treatment of obesity and T2DM. However, in parallel with these exciting developments, there are still many important issues to be addressed. It has been found that both UCP1-dependent and UCP1-independent mechanisms play a role in diet-induced thermogenesis [160]. Beige adipocytes of abdominal fat are mostly UCP1 negative but possess thermogenic capacity associated with a futile creatine cycle [161]. Importantly, Kazak et al. found that creatine enhances energy expenditure through stimulation of mitochondrial ATP turnover, and supports cold- and adrenergic-mediated adaptive thermogenesis [162,163]. This requires us to further study the related thermogenic mechanism of thermogenic fat. As most of the conclusions are based on animal or in vitro studies, the specific mechanisms underlying the browning of WAT in humans need to be better elucidated. Enhanced BAT metabolic rate will require more oxygen and energy, which may increase the load on other organs of the body. In addition, although some drugs (CL 316,243, rosiglitazone, etc.) have shown efficacy in activating BAT and inducing browning of WAT, their use is not devoid of possible serious side effects. How to control BAT activation and browning of WAT, as well as a series of problems such as safety, efficiency and specificity are all unanswered questions that need to be addressed.

Traditional Chinese medicine has the characteristics of multi-component, multi-target and high safety. At present, some of the most promising browning agents (plant extracts, Chinese medicine) to activate BAT and induce browning of WAT, including resveratrol, berberine, and curcumin, have made great progress in the experimental stage. For example, resveratrol in *Polygonum cuspidatum* can enhance the rate of fat decomposition, mitochondrial heat production and UCP1 protein expression by activating the SIRT1 signalling pathway. In addition, resveratrol can also activate BAT and induce browning of WAT through gut microbiota-bile acid-TGR5/UCP1 pathway to ameliorate glucose homoeostasis. Berberine activates WAT and induces browning of WAT by activating AMPK signalling pathway. But their overall role in metabolism and other aspects of physiology needs further study. Therefore, it is imperative for scientists to discover safe and effective Chinese medicine with the function of activating BAT and/or induce browning of WAT to treat obesity and T2DM. With the development of

mechanisms research of BAT activation and browning of WAT induced by safe and effective Chinese medicine, a new avenue for the treatment of obesity and T2DM will be built, and the prospects of Chinese medicine in the treatment of diabetes will be much brighter and broader.

Abbreviations

White adipose tissue (WAT), Brown adipose tissue (BAT), Beige adipocytes, Type 2 diabetes mellitus (T2DM), Type 1 diabetes mellitus (T1DM), Uncoupling protein 1 (UCP1), Peroxisome-proliferator-activated receptor γ coactivator-1α (PGC-1α), Peroxisome proliferator-activated receptors γ (PPAR γ), Positive regulatory domain-containing 16 (PRDM16), Fibronectin type III domain containing 5 (FNDC5), cAMP-dependent protein kinase A (PKA), p38 mitogen-activated protein kinase (p38 MAPK), Extracellular regulated protein kinases (ERK), Hypoxia-inducible factor 1, α subunit inhibitor (HIF1an), AMP-activated protein kinase (AMPK), Fibroblast growth factor 10 (FGF10), Fibroblast growth factor 21 (FGF21), CCAAT/enhancer-binding protein β (C/EBPβ), Insulin-like growth factor-1 (IGF-1), Insulin receptor substrate 1 (IRS1), Takeda G-protein coupled receptor 5 (TGR5), Norepinephrine (NE), Sirtuin1 (SIRT1), Bone morphogenesis proteins (BMPs), Thyroid hormones (THs), Intermittent fasting (IF), Caloric restriction (CR), Interleukin-6 (IL-6), MAPK kinase 6 (MKK6), β-adrenergic receptor agonists (β-ARs agonists), Thiazolidinediones (TZDs), Glucagon-like peptide-1 receptor agonists (GLP-1RAs).

Authors' contributions

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Disclosure statement

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