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Метаболические особенности и терапевтический потенциал бурой и «бежевой» жировой ткани

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По данным Международной Диабетической Федерации (IDF), в России 10,9 млн больных страдают сахарным диабетом (СД), тогда как зарегистрировано всего около 4 млн пациентов; у 11,9 млн человек имеется нарушенная толерантность к глюкозе и нарушенная гликемия натощак [1].
Одним из наиболее весомых факторов риска развития СД 2 типа (СД2) является ожирение, которое усиливает имеющуюся инсулинорезистентность (ИР). Последняя является основным патогенетическим звеном СД2.
По современным представлениям, существует три типа жировой ткани: белая (white adipose tissue, WAT), бурая (brown adipose tissue, BAT) и «бежевая», последние две обладают термогенной функцией. По результатам проведенных исследований выяснены основные этапы развития адипоцитов, однако единой точки зрения на образование «бежевых» адипоцитов не получено. На данный момент активно изучается биология BAT и «бежевой» жировой ткани. Так, выявлены основные транскрипционные факторы/сигнальные пути/гормоны, способствующие развитию и активации данных тканей. Наиболее обсуждаемыми гормонами являются ирисин и фактор роста фибробластов 21 (FGF21). Выяснено положительное влияние BAT и «бежевой» жировой ткани на углеводный, липидный и энергетический обмены. Основными методами визуализации BAT являются ПЭТ-КТ с ^{18}фтордезоксиглюкозой (^{18}FDG) и МР-спектроскопия.
В условиях эпидемии ожирения и ассоциированных с ним заболеваний (в том числе СД2), повышается интерес к изучению адипогенеза и возможностей влияния на данный процесс. BAT и «бежевая» жировая ткань могут быть мишенью для разработки препаратов против ожирения и СД2.
Ключевые слова: *инсулинорезистентность; сахарный диабет 2 типа; бурая жировая ткань; «бежевая» жировая ткань*

Metabolic characteristics and therapeutic potential of brown and 'beige' adipose tissues

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According to the International Diabetes Federation, 10.9 million people have diabetes mellitus (DM) in Russia; however, only up to 4 million are registered. In addition, 11.9 million people have impaired glucose tolerance and impaired fasting glucose levels [1].
One of the significant risk factors for type 2 DM (T2DM) is obesity, which increases insulin resistance (IR). IR is the major pathogenetic link to T2DM.
According to current concepts, there are three types of adipose tissue: white adipose tissue (WAT), brown adipose tissue (BAT) and 'beige', of which the last two types have a thermogenic function. Some research results have revealed the main stages in the development of adipocytes; however, there is no general consensus regarding the development of 'beige' adipocytes. Furthermore, the biology of BAT and 'beige' adipose tissue is currently being intensively investigated, and some key transcription factors, signalling pathways and hormones that promote the development and activation of these tissues have been identified. The most discussed hormones are irisin and fibroblast growth factor 21, which have established positive effects on BAT and 'beige' adipose tissue with regard to carbohydrate, lipid and energy metabolism. The primary imaging techniques used to investigate BAT are PET-CT with 18F-fluorodeoxyglucose and magnetic resonance spectroscopy.
With respect to the current obesity epidemic and associated diseases, including T2DM, there is a growing interest in investigating adipogenesis and the possibility of altering this process. BAT and 'beige' adipose tissue may be targets for developing drugs directed against obesity and T2DM.
Keywords: *Insulin resistance; type 2 diabetes; brown adipose tissue; 'beige' adipose tissue*

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Obesity is a chronic relapsing disease that is an epidemic worldwide; it is also closely associated with the development of various metabolic diseases. Obesity and overweight occur due to an imbalance between caloric intake and energy expenditure.

Adipose tissue is an important metabolic organ that has been traditionally classified into two types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT and BAT have different anatomical locations, morphological structures and functions. Both these adipose tissue types are involved in maintaining the energy balance. WAT primarily stores energy in the form of triglycerides, whereas BAT generates heat during cold- or diet-induced thermogenesis. A new type of adipose tissue, beige (beige/brite; brown in white), has been recently discovered. It is noteworthy that beige adipocytes respond to stimuli, such as cold and others, by increasing the expression of thermogenic markers.

In 1551, the Swedish naturalist Conrad Gessner first described BAT in the interscapular regions in marmots. Since the 1960s, BAT has been regarded as a thermogenic organ [2]. BAT uses chemical energy to produce heat as a protective mechanism during exposure to cold or during excessive food intake. BAT biology gained impetus several years ago because of its therapeutic potential, owing to its positive metabolic effects on obesity and associated diseases. The main issue that is currently being discussed is the possibility of activating or increasing the mass of BAT in adults.

BAT evolved to generate heat to protect animals (mammals) from hypothermia. This phenomenon, called non-shivering thermogenesis, is particularly essential during hibernation and infancy. Recent research on BAT biology demonstrated that it can play a significant role in controlling energy homeostasis and thus can provide a new direction for developing drugs for treating obesity.

Obesity results from chronic excess caloric intake that is greater than energy expenditure. All anti-obesity drugs are designed for reducing energy intake by suppressing appetite or by inhibiting intestinal fat absorption. However, these types of drugs are often associated with side effects, such as depression or dyspepsia, which has necessitated developing alternative drugs. Because BAT has a significant ability to generate heat using uncoupling protein 1 (UCP1, thermogenin) in mitochondria, the effect on BAT-associated thermogenesis may lead to a new direction for increasing energy expenditure.

During recent years, significant results have been obtained from investigation on BAT biology. First, positron emission tomography-computed tomography (PET-CT) with fluorine-18-deoxyglucose (18FDG) examinations, typically used in oncology, revealed active BAT in adults. The amount of BAT is inversely proportional to the body mass index (BMI), which increased the likelihood that the differences in the amount or activity of the thermogenic function of BAT can promote/inhibit weight loss [3]. The presence of BAT was associated with a low total body fat content and a low risk of type 2 diabetes mellitus (T2DM). Second, studies using animal models resulted in a better understanding of adipogenesis stages. These findings indicated that humans and rodents have 2 types of UCP1-positive thermogenic adipocytes of different origins; classic brown adipocytes and so-called beige adipocytes, with the latter located among white adipocytes. Third, some transcriptional regulators and signalling molecules were identified that promoted brown and beige adipogenesis and enabled the generation of BAT and beige adipocytes *in vivo*.

UCP1 and its role in non-shivering thermogenesis

Despite large amounts of mitochondria and a high level of cellular respiration, brown adipocytes have a markedly lower ability to synthesize ATP. Most cells that lack UCP produce ATP using ATP synthase, which uses the proton gradient across the inner mitochondrial membrane. In comparison, brown adipocytes express a markedly lower level of ATP synthase and, conversely, use UCP1 that weakens the proton gradient by separating cellular respiration from mitochondrial ATP synthesis, thereby promoting thermogenesis [4]. Although other members of the UCP family, including UCP2 and UCP3, have structures homologous to those of UCP1, they do not provide adaptive thermogenesis *in vivo*. Consequently, UCP1 plays a primary role in non-shivering thermogenesis [5].

UCP1 expression does not necessarily reflect the thermogenic activity of brown adipocytes. When at rest, the activity of thermogenin is usually suppressed by purine di- and triphosphate nucleotides. Purine nucleotides, primarily ATP, bind to UCP1 on the cytosolic side and prevent proton transport. Free fatty acids (FFAs) are UCP1 activators. Kirichok et al. used a whole-cell patch-clamp technique and found that UCP1 was the fatty acid anion/H+ symporter. They also found that although UCP1 was not active due to ATP inhibition, long-chain fatty acids resulted in the opposite effect by binding to the cytoplasmic side of UCP1, which abrogated the inhibition of UCP1 activity [6]. Furthermore, one long-chain fatty acid molecule binds to the UCP1 protein, acting as a substrate for the transport of one hydrogen ion per transport cycle. Although anions of long-chain fatty acids compete with ATP for binding to UCP1, it is unlikely that they bind to the same UCP1 surface because of the differences in their structures. Thus, more detailed investigations are required on the structural characteristics of UCP1 for possibly activating non-shivering thermogenesis.

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The FFA-associated control of UCP1 activity is fundamental from a physiological point of view. FFAs are end products of cold stimulation or hypernutrition. In response to these two physiological stimuli, norepinephrine is released from sympathetic nerve terminals and affects adrenergic receptors (ARs), primarily β3-AR in BAT. This activates adenylate cyclase and increases intracellular cAMP levels, which then activate cAMP-dependent protein kinases. A protein kinase phosphorylates hormone sensitive lipases and protein-binding lipid droplets (e.g. perilins), leading to hydrolysis of triglycerides in lipid droplets of BAT. Moreover, FFAs are either formed by cAMP-induced lipolysis or by capture from circulation and are subsequently used as substrates for β-oxidation in brown adipocytes. They are also a substrate for H+ transport by UCP1. To activate UCP1, FFA levels should be approximately 100 times higher than ATP levels, as shown by Fedorenko [6], implying that UCP1 activity is inhibited under normal physiological conditions.

Although transgenic UCP1 expression maintains the mitochondria of brown adipocytes in an active uncoupled state *in vivo*, enhanced UCP1 expression may be cytotoxic for adipocytes and cause BAT atrophy. Efforts made in the 1930s to use chemical uncouplers as anti-obesity drugs, such as 2,4-dinitrophenol, were not successful [7]. Based on new data, unravelling the structural determinants by which ATP or free fatty acids and other metabolites control UCP1 activity may develop strategies for inventing a new class of anti-obesity drugs.

More than 30 years ago, researchers suggested that BAT played a primary role not only in cold-induced adaptive thermogenesis but also in diet-induced thermogenesis when energy expenditures increased in response to a particular diet that protected animals from obesity. Additional research using genetically modified mouse models demonstrated abnormalities in non-shivering BAT thermogenesis that resulted in obesity and IR. UCP1 knockout mice have a unique characteristic: obesity under normal temperature conditions [8].

Numerous studies using animal models have primarily attempted to attribute changes in the energy balance to changes in UCP1 transcription in adipose tissues. Although UCP1 is the major factor determining thermogenesis by BAT, there are several other factors influencing this phenomenon, including disturbed oxidative phosphorylation, fatty acid absorption and further metabolism and mitochondrial biogenesis. The UCP1 mRNA level does not always reflect the level and activity of UCP1 [9]. For example, UCP1 transcripts are induced rather rapidly after treatment with cAMP (2–4 h) or PPARγ agonists; however, this rapid increase in UCP1 levels can be followed by a rapid return to basal levels upon termination of the associated stimulus. The above-mentioned stimulating factors cause a slow but steady increase in the UCP1 protein level, which persist for several days. The half-life of UCP1 mRNA is approximately 2.7 h, whereas that of UCP1 protein is 5–7 days *in vivo*. Therefore, the biological significance of a change in UCP1 transcript levels for BAT function should be evaluated in conjunction with other variables.

Origin of thermogenic adipocytes

Mesenchymal cells are the starting point for adipogenesis, from which two progenitor pools are formed: Myf5-positive and -negative cells. Myf5-positive cells are a source of brown preadipocytes and myocytes, from which mature cells are formed. White and beige adipocytes develop from Myf5-negative progenitors [10].

To date, it remains unclear whether beige adipocytes originate from already existing white adipocytes or are formed *de novo* from progenitor cells [11]. These phenomena are referred to as browning (Fig. 1). Cinti et al. have demonstrated that large unilocular white adipocytes are converted to beige adipocytes when exposed to cold and β-AR agonists; however, recent studies have provided findings inconsistent with this. Wang et al. developed a system for labelling mature adipocytes and, after exposure to low temperatures, revealed newly formed beige adipocytes [12].

Several key transcriptional regulators, including PRDM16 and C/EBPβ, control the formation of either BAT or skeletal myocytes. Experiments have demonstrated that, indeed, presumed brown adipocytes with PRDM16 or C/EBPβ ablation are converted to cells with the phenotype and expression of selective markers of skeletal myocytes, such as myogenin and others [14]. In contrast, myogenin knockout mice did not exhibit the differentiation of skeletal muscles but had an increased BAT depot in the interscapular area. Altogether, these data are consistent with the hypothesis that brown adipocytes and skeletal myocytes have a common ancestor.

Beige cells, a second type of thermogenic UCP1-positive adipocytes, are found as clusters in subcutaneous adipose tissues of adult animals after long-term exposure to cold, β-AR agonists or PPARγ agonists or after exercise. This type of thermogenic adipocyte has many biochemical and morphological characteristics of classic brown adipocytes, including multiple lipid droplets, large amounts of mitochondria and UCP1 expression. Nevertheless, beige adipocytes arise from Myf5-negative cells; thus, their origin is different from that of brown adipocytes. A recent study demonstrated that platelet-derived growth factor receptor α-positive progenitor cells from abdominal subcutaneous fat can convert to UCP1-positive adipocytes in response to stimulation with β3-AR agonists *in vivo* [15]. In mice, approximately 62% adipocytes in the inguinal region are formed from Myf5-positive cells, indicating considerable

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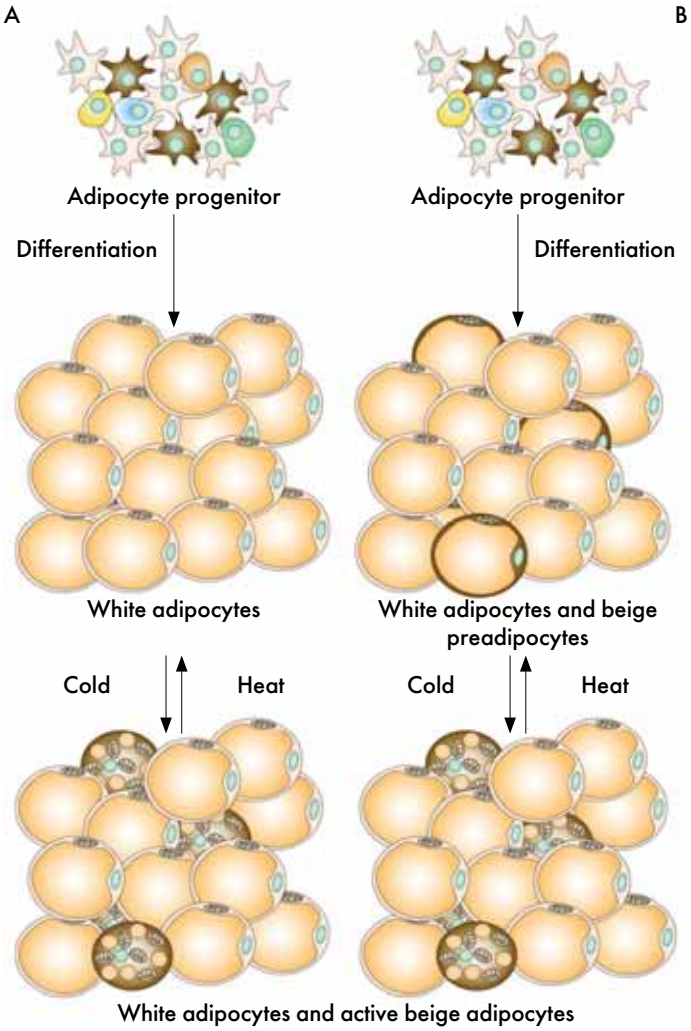

Figure 1. Development of beige adipocytes. A. Formation from white adipocytes on exposure to cold; B. Formation from adipocyte progenitors. Adapted from M. Rosenwald; and C. Wolfrum. The origin and definition of brite versus white and classical brown adipocytes [13]

heterogeneity of adipogenic progenitors in subcutaneous adipose tissues [16].

Spiegelman et al. have recently isolated a clonal population of beige cells by immortalizing the stromal-vascular fraction of mouse subcutaneous fat . The molecular characteristics of these beige cells markedly differed from those of white adipocytes. Nevertheless, almost all adipocytes from subcutaneous adipose tissues can become UCP1-positive cells in mice exposed to cold or treated with a β3-AR agonist for a long period of time. Furthermore, all subcutaneous fat preadipocytes can express markers of brown/beige cells, including UCP1, after prolonged exposure to PPARγ agonists, even after exposure at post-mitotic stages. Therefore, the plasticity between white and beige adipocytes may exist at the progenitor stage.

The two types of thermogenic adipocytes also differ in their levels of gene expression. Although beige cells and classic brown adipocytes share a number of genetic markers, such as *UCP1*, *PGC1a*, *Cidea* and *PRDM16*, these cell types also express specific markers that probably reflect their origins [17]. For example, beige adipocytes do not express the myocyte-specific genes (*Zic1, Lhx8* or *Epstl1*) but do express the unique genes (*Cited1, Tmem26, CD137* or *Tbx1*).

Role of beige adipocytes in energy metabolism

Assessing the contribution of beige adipocytes only to non-shivering thermogenesis appears to be difficult, and experimental studies have provided contradictory results. The total amount of UCP1 protein in beige adipocytes is quite low at approximately 10% of the amount of this protein in brown adipocytes. In contrast, using bone morphogenetic protein (BMP) receptor type 1A knockout mice that had a nearly complete loss of BAT and activation of browning, it was shown that disturbances in non-shivering thermogenesis (tendency for hypothermia) were detected after short-term (48 h) exposure to cold, while body temperature was maintained at a normal level after prolonged exposure (11 days). This indicates that beige adipocytes can greatly promote non-shivering thermogenesis and, thus, energy expenditure, at least in rodents. It was also demonstrated that mice with an increased amount of beige fat tissues were protected from diet-induced obesity. For example, Fabp4-PRDM16 transgenic mice with stimulation for browning and an unchanged UCP1 level in BAT had increased thermogenesis, a limited increase in body weight and improved glucose tolerance with a high-fat diet.

Location of adipose tissues

Anatomical locations of adipose tissue types are different. WAT is located throughout the body and is subdivided into two common types: visceral and subcutaneous. Visceral tissue is found around vital organs and has a specific protective function. Depending on the location, visceral WAT is classified into mesenteric, retroperitoneal, perigonadal or omental adipose tissues. In humans, subcutaneous WAT is predominantly located in the thigh and gluteal regions and provides thermogenic protection.

BAT is located in the neck area and supraclavicular, paravertebral, axillary, mediastinal, pericardial, perirenal and periadrenal, trachea-oesophageal, intercostal and mesenteric regions as confirmed by histological methods and PET-CT examinations [10].

Beige adipocytes were found for the first time among white adipocytes. However, to the best of our knowledge, only few studies have determined the localization of beige adipocytes. Superficial adipose tissues of the neck in adults have been found to have characteristic genetic markers of beige adipose tissues [18].

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Cytological structures of different adipocyte types

Brown adipocytes have a polygonal shape, numerous lipid droplets, a large amount of mitochondria (comparable to that in cardiomyocytes) and a centrally located nucleus. White adipocytes have a round shape, large lipid droplets and a nucleus shifted toward the periphery. The morphology of beige adipocytes depends on their state; in the basal state, they have the characteristics of white adipocytes, while in the activated state, they have the characteristics of brown adipocytes [11].

The characteristics of adipocyte cytological structures define their functional differences. The major characteristic of brown and beige adipocytes (during their activation period) is their ability to non-shivering thermogenesis.

Modern imaging techniques

BAT has been previously described in infants and hibernating animals. In humans, BAT tends to decrease with age. The discovery of active BAT in healthy adults renewed interest in its study. The development of new imaging technologies, particularly PET-CT [19] with 18FDG, enabled visualization and quantification of active brown adipose tissues in adults.

The use of PET-CT for studying BAT in humans began with a 67-year-old woman who had a tumour in her right epiphrenic region. PET-CT with 18FDG revealed a lesion with the density corresponding to that of adipose tissues, but with a higher 18FDG capture rate compared with subcutaneous or visceral fat depot. Based on a histological examination, hibernoma was diagnosed after the tumour had been removed. The presence of brown adipocytes was confirmed by an immunohistochemical analysis [20].

PET-CT with 18FDG showed that, quantitatively, there were more brown adipose tissues in females than in males at a 2:1 ratio and was often visualized in people aged up to 50 years with normal body weights and without any carbohydrate metabolism disturbances. PET-CT with 18FDG is the "gold standard" and has a high specificity and sensitivity. However, it is noteworthy that this method is invasive, imposes a significant radiation load, depends on environmental factors (room temperature, season, the amount of clothing on the patient), visualizes only active brown adipose tissues and a specialist's evaluation may be quite subjective [21].

Studies on BAT imaging are currently underway using MRI, which is based on the differences in the structures and vascularization of BAT and WAT [22]. Because of the limited experience of using MRI, its advantages have not been completely assessed. Nevertheless, certain advantages are noteworthy. MRI is performed using ionizing radiation with no isotopes, has a minimal risk to a patient's health, can be used for all ages, including children, is related to non-invasive examination methods and does not require special conditions (e.g. maintaining a certain temperature).

Managing the development and functions of thermogenic adipocytes

Transcriptional regulators involved in development of brown and beige adipocytes

PPARγ and C/EBPS are the major transcription factors that control the differentiation of adipocytes. Genetic ablation of PPARγ results in a complete loss of white and brown adipocyte differentiation. C/EBPα is required only for the formation of white fat, suggesting that there are other members of the C/EBP family that are involved in brown adipocyte formation. C/EBPβ expression levels are higher in brown adipose cells than in white adipose cells. Brown adipocyte differentiation requires PPARγ, whereas PPARγ ectopic expression by fibroblasts or mesenchymal cells induces the formation of white adipocytes, suggesting a multi-factorial effect on brown adipogenesis.

PGC-1α and its modulators

The PPARγ co-activator-1α (PGC-1α) was originally identified in brown adipocytes as a cold-induced transcriptional PPARγ co-activator. PGC-1α is an essential regulator of mitochondrial biogenesis and oxidative metabolism in many cell types, including brown adipocytes and myocytes. Ectopic PGC-1α expression by white adipose cells induces the expression of mitochondrial and thermogenic genes. However, PGC-1α ablation does not affect brown adipocyte differentiation, indicating that PGC-1α is an optional factor for brown adipogenesis.

Furthermore, PGC-1α activates the transcription of *UCP1* by activating both PPARγ and thyroid hormone receptors (THRs). Iodothyronine deiodinase type 2 (DIO2) enzyme activates THR, resulting in the formation of triiodothyronine (T3) from thyroxine (T4) in brown adipocytes [23].

PGC-1α expression levels in BAT correlate with the activity of non-shivering thermogenesis. In humans, PGC-1α mRNA expression is 2–15 times higher in BAT than in WAT [24].

Preadipocytes isolated from the supraclavicular region and stimulated with a number of PPARγ agonists have higher PGC-1α expression. Further, their UCP1 level is comparable with that of typical brown adipocytes.

Several modulators of PGC-1α expression or activity have been identified. RIP140, SRC2/TIF2/GRIP1, retinoblastoma protein and p107 all inhibit the transcriptional activity of PGC-1α.

PRDM16

PRDM16 is a protein with a zinc finger domain, a molecular weight of 140 kDa and is expressed at a high level in

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BAT. The ectopic expression of PRDM16 in white adipocyte progenitors or myoblasts induces browning. PRDM16 increases the transcriptional activities of PGC-1α, PPARγ and C/EBPs. Furthermore, a PRDM16 transcription complex includes C-terminal binding protein 1 (CtBP-1) and 2 (CtBP-2); this direct interaction promotes the selective suppression of genes in white adipocytes [25]. Transplanting fibroblasts that expressed PRDM16 and C/EBPβ in mice resulted in ectopic adipogenesis that had morphological and biochemical characteristics of thermogenic adipocytes.

Recent studies identified several regulators of *PRDM16* and *C/EBPβ*. For example, early B cell factor 2 (EBF2) activates PRDM16 transcription and initiates the genetic program for browning in myoblasts and white adipose cells. Plac8 is an activator of *C/EBPβ* transcription and promotes the differentiation of brown fat. Transducin-like enhancer of split 3 (TLE3) has a retroactive effect as it suppresses the formation of brown adipocytes by antagonizing the *PRDM16* function. In addition to these transcriptional regulators, several miRNAs, including miR-133, miR-193B and miR-365, affect *PRDM16* and negatively affect the development of BAT. miR-196a activates *C/EBPβ* and induces beige adipogenesis by directly suppressing the homeobox C8 (*Hoxc8*) gene [26].

The stability of PRDM16 protein is controlled by PPARγ agonists. For example, thiazolidinedione induces beige adipogenesis in white adipose tissues. Ohno et al. [27] have found that PPARγ agonists, particularly rosiglitazone, caused browning by extending the half-life of the PRDM16 protein.

ForkheadboxC2 (Foxc2) is a transcription factor of the Forkhead family that is exclusively expressed in human and mouse adipose tissues. Foxc2 transgenic expression in white adipose tissues induces the formation of beige adipocytes with an increasing number of mitochondria and enhanced expression of thermogenic genes, including *UCP1* and *PGC-1α* [28]. Foxc2 transgenic mice gain less weight when fed a high-fat diet and do not develop obesity, insulin resistance or hypertriglyceridemia [29].

Many other nuclear factors that regulate the formation of beige adipocytes have been found. For example, the sexual receptor-1 co-activator induces the formation of beige adipocytes, while the binding transcription factor-2 inhibits their formation. An increased expression of twist-related protein 1 (TWIST-1) in white adipose tissues triggers browning.

Signalling pathways that control the development and activation of brown/beige adipocytes

β-AR

The β-AR signalling pathway is the predominant pathway involved in both thermogenesis in BAT and the development of brown and beige adipocytes.

Norepinephrine is released from sympathetic nerve terminals and binds to β-ARs, increasing intracellular cAMP levels, further leading to the activation of protein kinase A (PKA) through binding to cAMP and p38MAPK. P38MAPK phosphorylation indirectly contributes to increased expressions of UCP1 and PGC-1α. Of the three β-AR subtypes, β1-, β2- and β3-AR, β1-AR plays an important role in the proliferation of brown adipocytes in response to norepinephrine, whereas β3-AR promotes the thermogenic function of mature brown adipocytes and induces beige adipocyte formation [30]. β2-ARs are found in the blood circulation, and their functions include changes in the blood supply to BAT. In β3-AR knockout mice, the cold-induced formation of beige adipocytes is markedly reduced, while the development of brown adipocytes remains unchanged. Propranolol treatment of human brown preadipocytes resulted in approximately 50% reduction in UCP1 mRNA expression [10].

Nitric oxide signalling pathway

Nitric oxide (NO) is a short-lived signalling molecule that is synthesized by endothelial cells and other cell types. Cyclic guanosine 3′,5′-monophosphate (cGMP) is formed by NO-sensitive guanylate cyclase and activates cGMP-dependent protein kinase (PKG). Treating brown adipocytes with cGMP induces the expression of UCP1 and mitochondrial biogenesis in a PKG-dependent manner [31]. Further, cGMP signalling induces browning.

Transient receptor potential vanilloid signalling pathway

Capsinoids activate transient receptor potential vanilloid 1 in the gastrointestinal tract and lead to thermogenesis in BAT of humans and rodents [4].

Phosphoinositol-3-kinase signalling pathway (involving PTEN)

Ortega-Molina et al. [32] demonstrated that PTEN had a positive effect on the thermogenic function of BAT by blocking the phosphoinositol-3-kinase (PI3K) signalling pathway. Pharmacological PI3K inhibitors increase BAT thermogenesis and, correspondingly, energy expenditure.

Hormones that control brown and beige adipogenesis

BMPs

These hormones belong to the superfamily of transforming growth factor-β (TGF-β). Treating fibroblasts or adipocyte precursors in culture with BMP7 induces the expression of regulators of brown and beige adipogenesis, particularly PRDM16. Another member of the BMP family, BMP4, is involved in browning of subcutaneous adipose tissues in mice. BMP8b affects mature brown adipocytes and the hypothalamus, improving BAT ther-

mogenesis but does not affect the differentiation of brown adipocytes.

Several members of the TGF-β superfamily, including GDF-8 (myostatin), TGF-β1 and activins, adversely affect brown adipogenesis and non-shivering thermogenesis.

Fibroblast growth factors

Unlike most fibroblast growth factors (FGFs), which act in an autocrine or a paracrine manner, FGF19 (FGF15 in mice), FGF21 and FGF23 are endocrine forms of FGFs. Endocrine FGFs bind to the cell surface co-receptors α-Klotho and/or β-Klotho. Transgenic FGF19 expression in mice increases their metabolic rate and reduces their mass of adipose tissues, partly by activating non-shivering thermogenesis. In neonatal mice, FGF21 produced in the liver circulates in the blood; its level is highly elevated at birth and activates non-shivering thermogenesis in BAT. FGF21 promotes browning by increasing PGC-1α levels in adipose tissues. In mice, a negative effect of chronically elevated levels of circulating FGF21 was noted on bone metabolism (bone loss) [33]. However, FGF21 formed in adipose tissues acts locally and does not affect FGF21 circulating levels. Therefore, FGF21 induction in adipose tissues appears promising and may contribute toward treating obesity and insulin resistance (IR) without exerting a systemic effect, particularly on bone tissues.

According to studies conducted on animal models, FGF21 improves insulin sensitivity, carbohydrate and lipid metabolism and preserves the function of beta cells. Furthermore, unlike most FGF-family proteins, FGF21 has no proliferative and tumorigenic effects. FGF21, by binding to a complex of β-Klotho–FGF receptors, stimulates insulin-independent glucose uptake by adipocytes because of GLUT1 induction through the sequential activation of transcription factors. FGF21 can increase the mass of BAT and beige adipose tissues, which may contribute to a better control of carbohydrate metabolism.

A direct correlation between FGF21 levels and carbohydrate metabolism disturbances has been observed, explained by its compensatory increase in response to IR. Thus, FGF21 levels increased in insulin-resistant conditions (IGT and T2DM) but decreased in T1DM and LADA [34].

FGF21 is expressed by human skeletal muscles in response to insulin stimulation. FGF21 is probably insulin-associated myokine [35].

FGF21 significantly increases glucose uptake by skeletal muscles, which has a beneficial effect on carbohydrate metabolism. However, several other studies in humans have indicated that FGF21 inhibited lipolysis in adipocytes, demonstrating that this antilipolytic effect may be another mechanism by which FGF21 improves insulin sensitivity [36].

Irisin

A myokine has been recently identified whose precursor was the membrane FNDC5 protein. Physical activity (physical exercise for endurance) or PGC-1α overexpression induced the expression of FNDC5 by skeletal muscles and increased the levels of the circulating fraction of irisin.

Treating adipocytes with irisin or gene therapy with adenoviral vectors with mouse liver FNDC5 initiates browning of white adipose tissues and protects animals from diet-induced obesity. Furthermore, irisin binds to the Fc fragment of human IgG in the CD137+ population of preadipocytes and promotes the formation of beige adipocytes [17].

Irisin was originally discovered as a myokine [37], although it has also been found that the gene encoding irisin, FNDC5, is expressed in WAT in humans, which may increase the possibility for enhancing thermogenesis via a number of autocrine mechanisms. In one study, it was found, using a real-time polymerase chain reaction (PCR), that *FNDC5* expression reduced in patients wh were obese and had T2DM. *FNDC5* expression in visceral and subcutaneous WAT was positively correlated with BAT marker levels (PRDM16 and UCP1) in humans. However, the mechanism underlying the autocrine regulation of browning remains unclear (Fig. 2).

Cardiac natriuretic peptides

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are released from the heart and are important endocrine regulators of water homeostasis and haemodynamics. NP effects are mediated by NP-receptor A (NPRA), while another type, NPRC, binds to ANP and BNP and eliminates these from the circulation. Collins et al. demonstrated that cold exposure increased the levels of circulating NPs and NPRA expression in adipose tissues . Administering BNP to mice and treating human adipocytes with NP induces the thermogenic program in BAT, mitochondrial biogenesis and respiration uncoupling. Furthermore, the WAT of NPRC-deficient mice contains a higher number of beige adipocytes. Because high levels of circulating NPs are associated with cardiac failure, it is necessary to define the therapeutic window when these peptides can increase energy expenditure without negatively affecting the cardiovascular system or other organs/tissues.

Prostaglandins

Chronic cold exposure induces the expression of the cyclooxygenase-2 (COX2) gene and enhances the release of prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2) in WAT. Transgenic COX2 expression in white adipose tissues and PGI2 treatment of adipocyte progenitors induces the expression of genes selective for thermogenic adipocytes,

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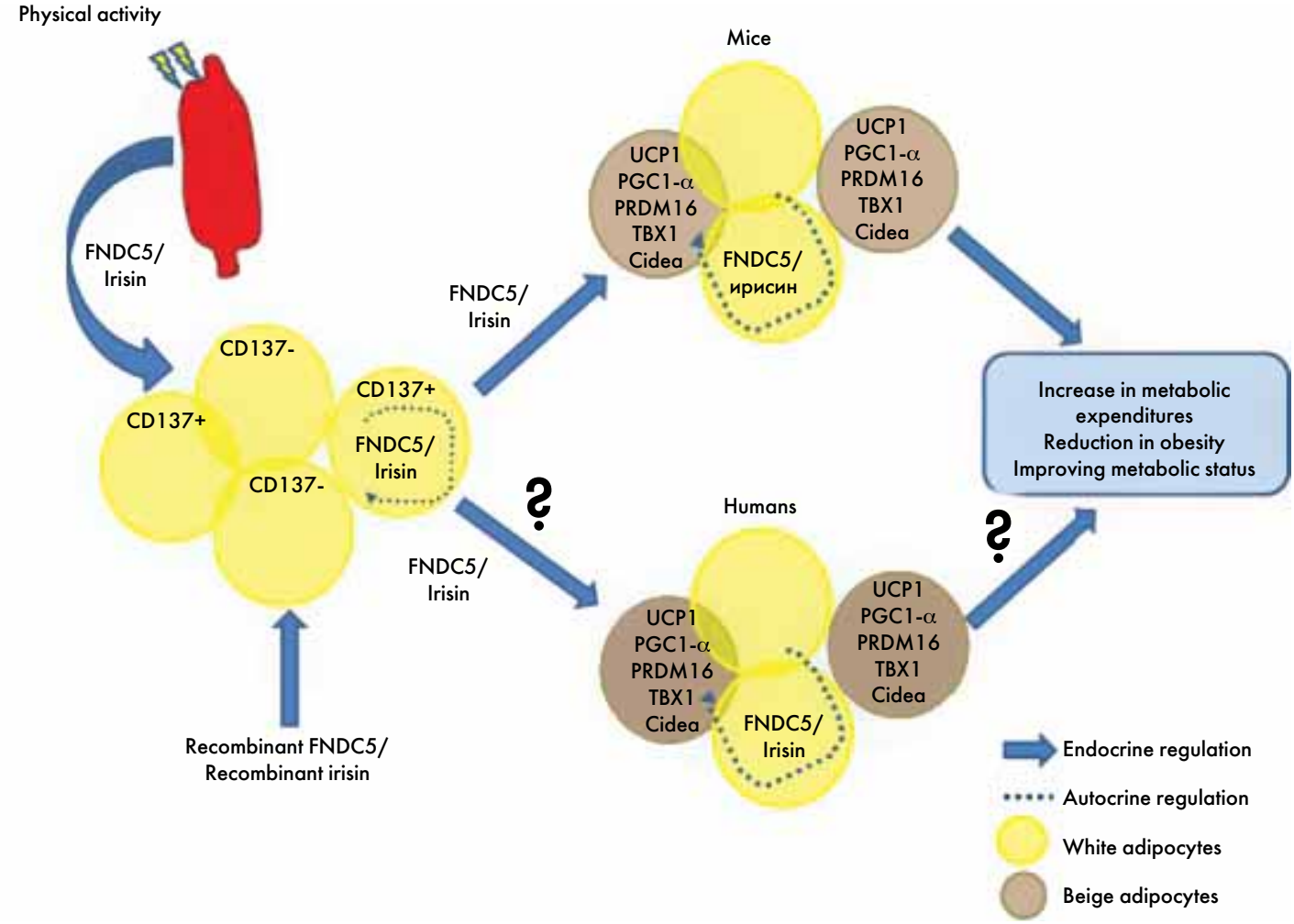

Figure 2. Effects of FNDC5/irisin on browning [adapted from V.A. Irving, C.D. Still, G. Argyropoulos. Does IRISIN Have a BRITE Future as a Therapeutic Agent in Humans?] [37]

such as UCP1 and Cidea. Conversely, COX2 gene ablation or pharmacological inhibition of cyclooxygenase activity worsens browning in white adipose tissues [38].

Influence of organs/tissues on BAT and beige adipose tissues

Orexin, synthesized by the hypothalamus, and catecholamines, produced by the adrenal medulla, are potent activators of brown adipocyte formation and non-shivering thermogenesis. Moreover, catecholamine hypersecretion in patients with pheochromocytoma has been shown to initiate WAT browning, and a feedback response between abdominal obesity and plasma catecholamine levels was found [39]. Activated macrophages in adipose tissues also secrete catecholamines, regulating adaptive thermogenesis. Skeletal muscles secrete both positive and negative regulators of browning, irisin and TGF-β. Natriuretic peptides activate thermogenesis in BAT and browning of white adipose tissues. Bile acids and FGF21 released from the liver are also important mediators of brown and beige adipogenesis.

Therapeutic potentials of BAT and beige adipose tissues

The range of therapeutic effects of the activation of BAT and beige adipose tissues may be more extensive than simply generating a negative energy balance using non-shivering thermogenesis. These metabolic effects are related to increased glucose and lipid uptake for oxidation that results in hypolipidemic and hypoglycemic manifestations [40]. Considering the reduction in glucolipotoxicity, it can be assumed that metabolic stress and, correspondingly, damage to β-cells in the pancreas and peripheral IR will be reduced.

Glucose and lipid uptake occurs due to an increased activity and number of glucose transporters and lipoprotein lipase because of the influence of the sympathetic nervous system on ARs of BAT.

The ectopic expression of UCP1 in epididymal fat improves leptin and insulin sensitivity in mice. Further, transplanting BAT into the intra-abdominal region markedly improves insulin sensitivity [41].

Despite its small amount, BAT can have a disproportionately large metabolic effect. Studies have shown that the

rate of glucose uptake per gram of BAT in patients with normal BMI values at room temperature was similar to that of skeletal muscles and that the rate of glucose uptake by BAT at low temperatures exceeded the rate of glucose uptake by skeletal muscles upon stimulation with insulin. The average weight of BAT in a healthy adult is approximately 50 g.

Based on the differences in cold-induced thermogenesis between BAT-positive and -negative people (based on PET-CT with 18FDG findings), it was found that BAT-dependent energy expenditure was approximately 200–400 kcal/day at low temperatures; under normal temperature conditions, this was much lower. Despite this, small differences of 10 kcal/day can only lead to a significant reduction in fat mass. A 10 kcal/day reduction is known to be equivalent to 1.1 g of fat per day and 4 kg of fat per 10 years [4]. Upon the activation of BAT, this reduction will be much higher.

Therefore, it seems important to search for agents that can activate brown and/or beige adipose tissues, which may be a prerequisite for developing new drugs for treating obesity and T2DM.

Considering the wide range of medical hypoglycaemic therapies, investigating effects of medicinal agents on functions of brown and beige adipose tissues is necessary. For example, a study on the effects of glucagon-like peptide-1 (GLP-1), glucagon and oxyntomodulin was conducted using animal models [42]. After injecting these peptides into mouse ventricles, their body weight decreased and thermogenesis in BAT was induced. However, injectable forms of GLP-1, which are widely used in modern practice, were not considered as agents that could affect the activation of brown and beige adipose tissues in adult patients with T2DM. Moreover, no studies have been conducted to clarify any changes in BAT and beige adipose tissues in adults with various disorders of carbohydrate metabolism after bariatric surgery, which would also be interesting.

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