® Postepy Hig Med Dosw (online), 2018; 72: 499-511
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 08.06.2017
Accepted: 05.01.2018
Published: 04.06.2018

Physiologically-induced adipocyte browning

Fizjologicznie uwarunkowane brązowienie adipocytów

Oskar Wojciech Wiśniewski[1], Malwina Malinowska[1], Magdalena Gibas-Dorna[2]

[1]Poznan University of Medical Sciences, Poland
[2]Department of Physiology, Poznan University of Medical Sciences, Poland

Summary

The data consistently suggests that expansion and activation of beige/brite adipose tissue may possibly serve as a novel cure for obesity and obesity-related complications. Interestingly, besides well-known agents affecting adipocyte transformation, such as cold-induced sympathetic stimulation, the vast majority of biological systems (e.g. cardiovascular, endocrine, immune, musculoskeletal and central nervous system) also play a role in an adipose tissue modelling and maintaining energy homeostasis. Therefore, we decided to describe in detail the browning of the adipose tissue with a wide range of physiological factors associated with this process and to present the significant distinctions between "classical" brown and beige/brite adipocytes.

Here, we review the current state of knowledge about browning phenomenon with regard to obesity prevention and/or management.

Keywords: **brown adipose tissue • beige/brite adipocytes • browning**

GICID 01.3001.0012.0757
DOI: 10.5604/01.3001.0012.0757
Word count: 8141
Tables: 1
Figures: 1
References: 78

Author's address: Oskar Wojciech Wiśniewski, Department of Physiology, Poznan University of Medical Sciences, Swiecickiego 6, 60-781 Poznan, Poland; e-mail: wisniewski.oskar@outlook.com

INTRODUCTION

Worldwide, there has been a dramatic increase in the prevalence of obesity and overweight in the last decades. According to World Health Organization (WHO), the number of people suffering from obesity has more than doubled since 1980 and exceeded 600 million of adults in 2014 [70]. Therefore, numerous studies have focused on analyzing the metabolic properties of adipose tissue, in order to combat obesity and obesity-related diseases, such as diabetes type 2, hypertension and dyslipidemia.

Human adipose tissue consists of two anatomically and functionally distinct types, white and brown adipocytes. White adipose tissue (WAT) is involved in energy storage and endocrine function, whereas brown adipose tissue (BAT) dissipates energy, generates heat and plays a role in the secretion of particular adipokines (also known as "batokines") [31,67]. Brown adipocytes also have been shown to appear in white fat depots and these adipocytes are often called beige, or brite (brown-in-white) fat cells.

The aim of this paper was to characterize beige adipocytes and the adipocyte browning process, and to review the physiological factors activating or inhibiting the browning phenomenon. In addition, we focused on the beneficial effects of the browning process on adiposity, hyperlipidaemia, insulin resistance and vascular diseases.

COMPARISON OF VARIOUS ADIPOSE TISSUE TYPES

White adipose tissue vs brown adipose tissue

Traditionally, adipose tissue is divided into white adipose tissue (WAT) and brown adipose tissue (BAT). Adipocytes containing one large lipid droplet and only a narrow hem of cytoplasm (unilocular morphology) are known as white, whereas fat cells, which are abundant in mitochondria and consist of numerous, but smaller lipid droplets (multilocular morphology) are named brown fat cells [59,71]. Almost ten years ago, an emerging type of adipocytes was distinguished and determined as beige/brite (brown-in-white) fat cells (also known as brown-like, inducible brown, recruitable brown or white adipose brown adipocytes) [28,29]. Beige fat cell clusters appear among WAT depots in response to various stimuli, such as cold exposure or chronic β3-adrenergic stimulation [42,58] and are derived from undifferentiated progenitor cells or mature white adipocytes [40,59,64] (read more in sections: Adipocyte browning and Browning activators). This recently isolated type of adipose tissue is acknowledged as a subtype of BAT, which differs from "classical" brown and white adipocytes.

Although all adipocytes are innervated by sensory and sympathetic nerves [3], the density of nerve fibers is not identical within the different types of adipose tissue. WAT is significantly less innervated than beige and "classical" BAT [29,71]. Moreover, WAT depots contain cells that are not equally supplied with nerve fibers and this fact may possibly determine whether an adipocyte is able to undergo the process of browning triggered, among others, by sympathetic drive [3,59].

In addition, there is a remarkable difference in the function of various adipose tissue types. Depending on the body's demands, white adipose tissue acts as a potent buffer maintaining constant level of fatty acids in the circulation by storing lipids in the form of triglycerides and esterified form of cholesterol, and also releasing free fatty acids [48,65], whereas the main function of beige and "classical" BAT is non-shivering thermogenesis, a process that counteracts the reduction of core body temperature in response to cold exposure. Both brown and beige fat cells contain a large number of mitochondria that express high levels of thermogenic proteins, such as uncoupling protein 1 (UCP1) [9,60,71]. UCP1 increases inner membrane permeability in mitochondria and allows for the dissipation of electrochemical gradient in the form of generated heat via proton leak.

Additionally, all three types of fat cells (WAT, BAT and beige adipocytes) may serve as an active endocrine tissue [28,31] and play an important role in the insulation of internal organs [48]. Interestingly, the presented types of adipocytes have different origins, which is precisely described below (section: Browning process).

Beige/brite adipose tissue vs „classical" brown adipose tissue

Both "classical" brown and beige adipocytes are characterized by multilocular morphology, high mitochondrial content and alike oxidative control [1,63]. In addition, the two types of BAT show overlapping gene expression patterns and a few common gene-related hallmarks [58]. Both tissues express UCP1, cell death-inducing DFFA-like effector A (CIDE-A), peroxisome proliferator-activated receptor γ coactivator-1-α (PGC1α) and other BAT markers [28,50]. The hallmarks specific only to the beige adipocytes include the following: tumor necrosis factor receptor superfamily member 9 (TNFRSF 9, also recognized as a CD 137), T-Box 1 (Tbx1) and Transmembrane Protein 26 (Tmem26) [58,59,72], whereas LIM homeobox 8 (LHX8) and in particular Zic family member 1 (ZIC1) expression are characteristic for "classical" BAT [31,42].

Importantly, it has been reported that in contrast to "classical" BAT, beige adipose tissue thermogenic profile is not only inducible, but also reversible [28,59]. In the basal state, the concentration of UCP1 in beige adipose tissue and uncoupled respiration rate are significantly lower than in "classical" brown fat [72]. Amounts of UCP1 become comparable between the tissues only in maximal response to various thermogenic stimuli [28], which are described later in this paper (section: Browning activators). Furthermore, in the presence of 3',5'-cyclic adenosine monophosphate (cAMP) in conditioned medium uncoupled respiration in murine beige adipocytes equals or even exceeds the uncoupled respiration rate of "classical" BAT [72]. This observation suggests that both types of BAT have similar thermogenic capacities and uncoupled respiration in beige fat cells is more cAMP-responsive than in "classical" BAT [28,72]. Nevertheless, the maximal rate of respiration is significantly higher in "classical" BAT-derived mitochondria as compared with beige-derived ones, indicating that the classical BAT still predominates in thermogenesis [63].

As the beige adipocytes stimulation is completed, their morphology and gene expression profile changes. Murine *in vitro* experiments showed that the former beige fat cells are re-converted and start to imitate white adipocytes (read more in section: Browning process) with inhibited thermogenic activity [59]. Thus, constant sympathetic stimulation is essential to maintain beige adipocyte phenotype and thermogenic function [16].

Another aspect that needs to be highlighted refers to the distinct localization of various BAT types. Recent studies

of murine models have documented that beige adipocytes are predominantly found in subcutaneous inguinal and retroperitoneal fat depots [24,28,58] and are hardly detectable in perigonadal and epididymal fat [24,58]. Taking into consideration that human BAT is composed of fat cells, which are much more similar to beige than "classical" brown fat cells in mice [59,72], supraclavicular fat is mainly acknowledged as the beige adipose tissue depot in humans [31], while interscapular, perirenal and cervical regions are recognized as "classical" BAT depots [31,42]. In addition, beige adipocytes content decreases in favour of classical brown fat cells as one moves deeper within the neck and back [58].

However, in response to various specific stimuli, beige adipocytes can emerge within most WAT depots [28], particularly in subcutaneous adipose tissue, which is highly prone to browning [72].

BROWNING PROCESS

Various types of adipose tissue originate from distinct cell lineages. "Classical" brown adipocytes, as well as myocytes, arise from mesodermal precursors in dermomyotome, which transiently express specific transcription factors: myogenic factor 5 (MYF5) and paired box 7 (PAX7) [29,40,42]. Beige and white fat cells originate from a MYF5-PAX7- [29,40] cellular lineage (residing in adipose tissue vascular surroundings and endothelium) [42,64], which, in turn, are characterized by the expression of platelet derived growth factor receptor α (PDGFRα) and early B-cell factor 2 (EBF2) [1,28,64]. In addition, apart from specific bi-potential progenitor cell-mediated biogenesis, beige fat cells can arise from mature white adipocytes through a direct interconversion called transdifferentiation [59].

Hence, an adipocyte browning process (also known as beiging or britening) is a phenomenon of undifferentiated precursor cells or already existing white fat cells acquiring beige adipose tissue phenotype. During the course of browning, morphological changes precede the onset of UCP1 expression in vivid mice [59]. As soon as beiging process starts, adipose tissue undergoes a dynamic remodelling, which manifests in expanded capillarization and tyrosine hydroxylase-positive nerve fibers innervation [31].

Adipocyte browning process is partially driven by progesterone receptor domain–containing 16 (PRDM16) [1,28], a transcription factor known as a "key driver of brown fat cell fate" [28], mainly involved in the up-regulation of certain BAT-specific genes, such as UCP1, PGC1α, CIDE-A and the others, as demonstrated by *in vivo* and *in vitro* murine studies [62]. In addition, the integral mechanism provoking the entire browning is mitochondrial biogenesis, a process activated by PGC1α, nuclear respiratory factors 1 and 2 (NRF1 and NRF2, respectively) and mitochondrial transcription factor A (TFAM) [1].

Interestingly, murine *in vivo* and *in vitro* studies revealed that the transdifferentiation of white adipocytes into the beige ones is reversible and the opposite whitening process may also occur [28,31,59]. One of the mechanisms required for beige-to-white conversion is mitophagy (mitochondria-selective autophagy), which is presumably mediated by UCP1 or PTEN-induced putative kinase 1 (PINK1)-Parkin pathway and inhibited by protein kinase A (PKA) [1]. The negative influence of PKA on mitophagy is also consistent with previous results from *in vitro* murine experiments, in which termination of β3-adrenergic signalling with subsequent decline in PKA activity facilitated beige adipocytes to regain white adipocyte phenotype [31,59].

After being whitened, beige fat cells are not directly apoptotically removed, but function as white adipocytes until their normal depletion due to the tissue turnover, and may undergo re-browning process once again [28,31,59]. Browning and whitening of adipocytes are recurring processes.

BROWNING ACTIVATORS

As mentioned before, beige adipose tissue can emerge only in response to certain inducers. Apart from well-known cold and/or sympathetic stimulation, there is a wide range of factors activating the browning process. This paper presents only endogenous activators that can act under physiological conditions, excluding, for example, nutrients or other exogenous factors/compounds that may influence the browning process. Unless otherwise noted, all data included in this section (Browning activators) was obtained on the basis of numerous *in vivo* murine studies.

COLD EXPOSURE

Sympathetic nervous system activity

As peripheral thermoreceptors sense a significant decline in environmental temperature, this information is instantly relayed through thin Aδ nerve fibers to the thermoregulatory region of central nervous system (CNS). Following cold exposure, the posterior component of hypothalamus is activated. This, in turn, leads to an increase in sympathetic nervous system (SNS) drive and vasoconstriction in order to maintain constant core body temperature [49]. Since fatty tissue receives sympathetic innervation [3], stimulation of adipose β3-receptors is also present. As a result, intracellular concentration of cAMP and PKA activity are elevated [6]. Finally, adipocytes undergo a browning process with augmented UCP1 and PGC1α expression and energy expenditure [28,29,49].

Immune system

Recently, it has been shown that some of the immune cells (eosinophils, group 2 innate lymphoid cells (ILC2),

M2 macrophages and mast cells) may induce beige fat biogenesis and limit obesity [9,23,29].

For example, Meteorin-like protein (Metrnl), as a circulating factor induced in adipose tissue upon cold exposure, enhances the production of eosinophil-derived Th2 cytokines (IL-4 and IL-13) [29,54]. This, in turn, activates anti-inflammatory M2 macrophages and facilitates their accumulation in fat depots [29,40,54]. As shown in murine *in vivo* studies and experiments *in vitro* on primary human monocytes, so induced macrophages intensify the expression of their enzymes, which are relevant to the catecholamine biosynthesis, including the most crucial: tyrosine hydroxylase (TH) [29,40,49]. Produced catecholamines promote adipocyte browning through β3-adrenergic receptor activation. Additionally, these processes could be enhanced by cold-induced adiponectin, as it multiplies M2 macrophages proliferation and expression of UCP1, which has been proven by murine *in vitro* reasearch [29].

Group 2 innate lymphoid cells (ILC2) are responsible for adipocyte britening in two ways. Firstly, they produce a specific set of interleukins (IL-5, IL-13) initiating type 2 immune responses [29,31,40], which lead to an alternative activation of M2 macrophages with the consequences illustrated above. Secondly, ILC2 are capable of autonomous browning of murine adipose tissue *in vitro* and *in vivo* [9,29]. In this case, the main mediators are methionine-enkephalin (MetEnk) peptides, which enhance UCP1 expression, acting directly on adipocytes [9,29,31]. Both actions of ILC2, together with their activation and accumulation within WAT, are induced by thymic stromal lymphopoietin (TSLP), IL-25 [40] and IL-33 [9,31,40].

Another pathway triggering browning, common for activated eosinophils and induced ILC2, involves IL-13 (produced by both, eosinophils and ILC2) and eosinophil-derived IL-4. These cytokines stimulate signalling via the IL-4Rα in PDGFRα+ bipotential adipocyte precursors potentiating adipocyte browning by themselves [29,31,40] or when the other triggering factor occurs.

Eventually, an *in vitro* murine study confirmed that mast cells also participate in the browning process due to cold-induced degranulation, when their secretions (histamine and IL-4) boost UCP1 expression [23].

Fibroblast growth factor 21

Fibroblast growth factor 21 (FGF21), a hormone produced within fat depots or by the liver [24,28,33], is acknowledged as another meaningful adipose tissue browning activator. The biosynthesis of FGF21 is elevated upon cold, β3-stimulation or suckling [24,28,41]. A key function of this protein is significant surge of PGC1α concentration, which, in turn, promotes mitochondrial biogenesis [1] and adipocyte browning [24]. In addition, FGF21 may also contribute to the increase in other compounds indicative of the browning process, such as UCP1. The action of FGF21 is mediated by a subset of cell surface FGF receptors (FGFRs), which are classical tyrosine kinases that require β-klotho co-receptor to form an active complex FGF21-β-klotho-FGFR [24].

Follistatin

Although follistatin (FST) is commonly recognized as an elemental follicle-stimulating hormone (FSH) inhibitor, it is also involved in cell differentiation, including cold-induced adipocyte britening [8]. *In vivo* and *in vitro* murine studies revealed that the paracrine action of adipose-derived FST is mediated by p38 mitogen-activated protein kinase (MAPK)/ERK1/2 signalling and manifests in increased expression of "classical" and beige BAT hallmarks, such as UCP1, PGC1α, PRDM 16, CIDE-A and CD137 [8,66]. In addition, FST facilitates adipocyte britening by counteracting transforming growth factor β (TGFβ) pathway, which is dominant inhibitor of britening phenomenon. Furthermore, FST may act synergistically with other adipocyte britening triggers, for example, by sensitizing fat cells to β3-adrenergic stimulation [66].

Natriuretic peptides

Recent *in vivo* murine and *in vitro* human studies have brought evidence that cardiac hormones, ANP (atrial natriuretic peptide) and BNP (B-type natriuretic peptide), are involved in the stimulation of adipocyte browning, and their concentration also elevates upon cold exposure [6,28]. Their function resembles SNS stimulation, as they act directly on fat cells and lead to phosphorylation of identical intracellular enzymes, such as p38α mitogen-activated protein kinase (MAPK). However, as opposed to SNS activation, a second messenger produced in response to natriuretic peptides (NPs) is cyclic guanosine monophosphate (cGMP), which activates protein kinase G (PKG). Nevertheless, SNS activation and NPs exert an additive effect on adipocyte browning [6]. Besides p38α MAPK activation, NPs contribute to the accumulation of PGC1α. Taken together, these molecular changes provoke increased UCP1 expression and energy expenditure, as well as mitochondriogenesis.

Notably, the browning process can also be influenced by genetic variations of NPs receptors. Available evidence, based on *in vivo* and *in vitro* murine experiments, indicates that up-regulated natriuretic peptide receptor A (NPRA) promotes adipocyte browning, whereas the activation of natriuretic peptide receptor C (NPRC) exerts inhibitory effect, as it serves as a clearance receptor, reducing NPs plasma concentration [6].

Thyroid hormones

Thyroid hormones (THs), thyroxine (T4) and biologically active triiodothyronine (T3), are well-known agents induced by cold exposure. Their increased secre-

tion is one of the most important mechanisms activated in non-shivering thermogenesis. THs evoke calorigenic effect via up-regulation of Na^+/K^+-ATPase activity and they strongly participate in adipocyte browning process. Murine *in vivo* and *in vitro* studies, as well as *in vitro* experiments on human multipotent adipose-derived stem cells (hMADS), collectively indicate that T3, acting directly on nuclear thyroid hormone response elements (TREs) via thyroid hormone receptor β (TRβ), regulates expression of browning-associated genes [39,45,50]. In response to T3, augmented expression of CCAAT/enhancer binding protein (C/EBP-α), CIDE-A, NRF1, PGC1α and UCP1 is observed [39,44]. As a result, mitochondriogenesis with concomitant enhanced energy expenditure occurs. In addition, the elevated content of mitochondrial carnitine palmitoyltransferase-1b (CPT-1b) increases the ability to oxidise free fatty acids (FFA), leading to increased oxidative capacity [39].

Concentration of THs is precisely regulated by three different types of iodothyronine deiodinases (DIO1, DIO2 and DIO3), enzymes able to convert T4 into T3 or reverse T3 (rT3). With respect to adipocyte browning, experiments on hMADS and rats (*in vivo*) present the crucial role of adipose DIO2, which elevates intracellular T3 level [39,50]. Overexpression of DIO2 may be induced following cold exposure, additionally increasing T3 concentration [39]. Interestingly, DIO2 activity could also be augmented in a cAMP-dependent manner via Takeda G-protein-coupled receptor 5 (TGR 5) directly by bile acids (in murine and human cell cultures) [69] or indirectly through activation of intestinal farnesoid X receptor (in mice experiments *in vivo*) [22].

Considering that thyroid hormones display multiple pleiotropic effects, their influence on other browning activators should not be surprising. In a study of hypothyroid patients long-lasting decreased function of the thyroid gland coexisted with lowered plasma irisin concentration; however, no correlations between irisin and fT3 or fT4 levels were found [78]. The explanation of this phenomenon is most likely associated with inverse relationship, observed in hypothyroidism, between creatine kinase (CK; a marker of muscle damage) and irisin, a potent browning activator produced by both muscles and adipose tissue (read more in the chapter: Irisin). Chronic deficiency of THs leads to muscle degradation with increased CK levels and lowered plasma irisin [7,78].

Taken together, T3 and indirectly T4 contribute to the promotion of adipocyte browning and related systemic changes, such as elevated body temperature, insulin sensitivity and weight loss [44].

Vascular endothelial growth factor A

Adipocyte-derived vascular endothelial growth factor A (VEGF-A) is the next cold-inducible agent, which promotes fat cells browning [28,31,52]. VEGF-A may directly induce browning of WAT, but the precise mechanism is not fully understood [52]. On the other hand, VEGF-A serves as an angiogenic factor promoting blood vessel expansion and branching within BAT. Cold exposure stimulates VEGF-A production, which, in turn, through the VEGF-R2 signalling pathway, evokes angiogenesis that is essential for browning [28,31,52].

Although each of the mentioned above factors may function as an independent trigger for adipocyte browning, the vast majority of them usually work under physiological conditions as downstream mechanisms of cold stimulation; therefore, all of them were described with respect to the effects of cold exposure.

Simplified mechanisms of action for all presented cold-induced browning activators and its mutual relationships are presented in Fig. 1.

EXERCISE

Irisin

One of the most crucial exercise-induced adipocyte browning activators is irisin, a novel adipo-myokine [53,57] formed by proteolytic cleavage of C-terminal fragment of a membrane protein called fibronectin type III domain-containing protein 5 (FNDC5) [7,72]. Based on recent *in vivo* and *in vitro* murine studies, exercise stimulates an increase in the expression of PGC1α, a pivotal irisin stimulator. This, in turn, elevates irisin plasma concentration [7,53]. Detailed mechanism of irisin inducing an effect on the browning process is still under investigation. Some reports indicate that its action is partially mediated by an enhanced peroxisome proliferator-activated receptor α (PPARα) expression and manifests in multiplication in UCP1 and PRDM16 biosynthesis [7,72].

Secretion of irisin is also indirectly up-regulated in response to cold exposure in humans [41]. When body temperature decreases, a few preventive mechanisms are activated, including the most efficacious shivering thermogenesis. As a result of rapid and frequent muscle contractions, irisin plasma concentration rises significantly.

Although there is no doubt that irisin participates in murine browning process, not all reports regarding irisin presence and its role in humans are consistent. Working *in vitro* on human *FNDC5* gene encoding the precursor for irisin, Raschke and co-workers have found that in humans no or very low translation of FNDC5 mRNA into precursor protein occurs [55]. They concluded that FNDC5 gene appears to be a transcribed pseudogene with low translation efficiency, which explains the small release of irisin from the primary human myotubes. Moreover, they observed no effect of recombinant FNDC5 and irisin on the britening of primary human adipocytes.

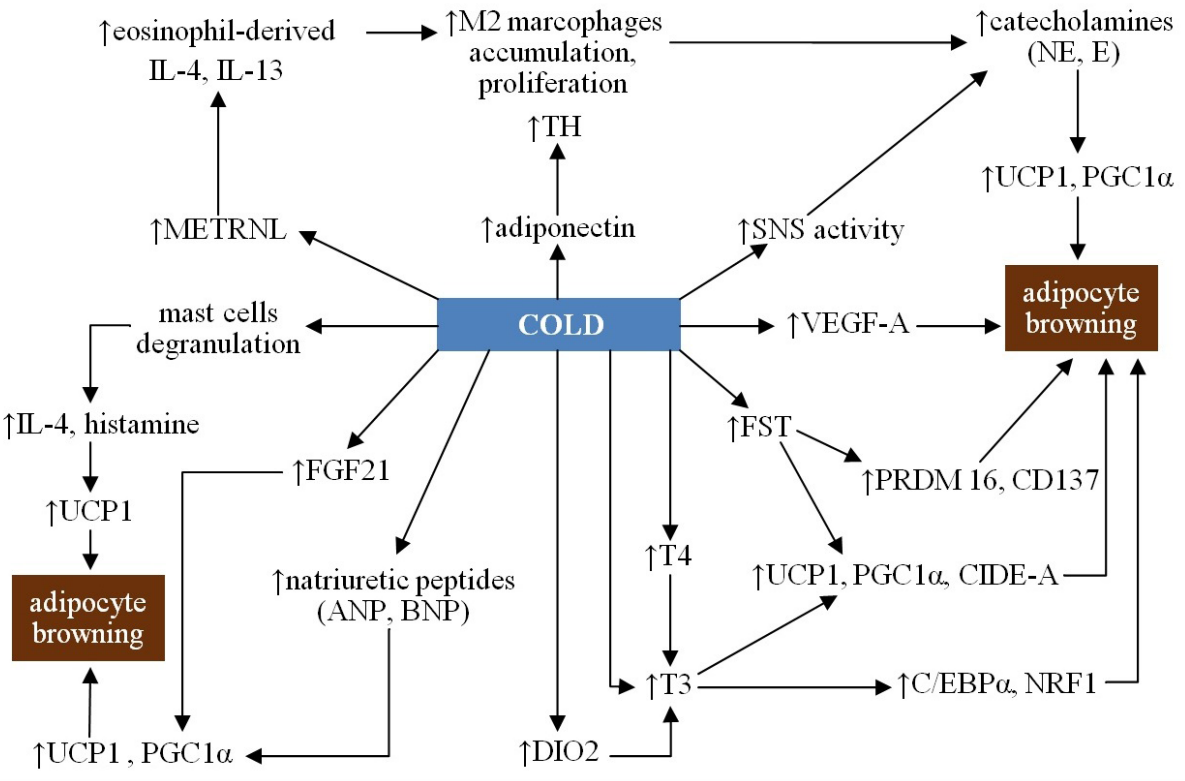

Fig. 1. Downstream mechanisms of cold-induced adipocyte browning (precise description in the text above);
ANP – atrial natriuretic peptide, BNP – B-type natriuretic peptide, C/EBP-α – CCAAT/enhancer binding protein, CD 137 – tumor necrosis factor receptor superfamily member 9, CIDE-A – cell death-inducing DFFA-like effector A, DIO2 – type 2 iodothyronine deiodinase, E – epinephrine, FGF21 – fibroblast growth factor 21, FST – follistatin, IL-4 – interleukin-4, IL-13 – interleukin-13, METRNL – Meteorin-like protein, NE – norepinephrine, NRF1 – nuclear respiratory factor 1, PGC1α – peroxisome proliferator-activated receptor γ coactivator-1-α, PRDM16 – progesterone receptor domain–containing 16, SNS – sympathetic nervous system, T3 – triiodothyronine, T4 – thyroxine, TH – tyrosine hydroxylase, UCP1 – uncoupling protein 1, VEGF-A – vascular endothelial growth factor A

Apelin

The next sample of exercise-induced adipocyte beiging refers to adipose tissue derived hormone, apelin, which may also increase in response to cold exposure [67]. Activated apelin receptors (APJ receptors) simultaneously induce PI3K/Akt and AMPK pathways in human and murine brown preadipocytes, leading to augmented expression of UCP1, PGC1α, PRDM 16 and other BAT-related genes. Apelin also stimulates metabolic activity of adipocytes. The observed intensification of mitochondriogenesis and increased energy expenditure are all indicative of completed adipocyte beiging. In addition, apelin is believed to counteract the inhibitory effect of tumor necrosis factor alpha (TNFα) on adipocyte beiging in murine cell cultures (read more in section: Browning inhibitors).

Interleukin-6

It has been demonstrated that interleukin-6 (IL-6) released by contracting muscles may promote the exercise-associated adipocyte browning process. As shown in murine studies, exercise induces large quantities of IL-6, which are required for the increased expression of UCP1 mRNA in WAT; however, the precise mechanism of this effect remains unknown [36]. It is suggested that UCP1 biosynthesis may be partially driven by the activation of signal transducer and activator of transcription 3 (STAT3).

Exercise metabolites

β-aminoisobutyric acid (BAIBA), β-hydroxybutyrate and lactate, as intermediate metabolites of physical activity, may positively influence adipocyte britening [12,21,56].

Recently, it has been shown that β-hydroxybutyrate and lactate up-regulate UCP1 and CIDE-A in murine and human adipose cell cultures, as well as in mice *in vivo* [12]. The action of these metabolites reflects their ability to change cellular oxidation/reduction potential and UCP1 biosynthesis. Induced by both, β-hydroxybutyrate and lactate, britening of murine adipocytes *in vitro* requires an active peroxisome proliferator-activated receptor γ (PPARγ) signalling and is PPARα-independent process. According to the current *in vitro* murine studies, the

functioning of β-hydroxybutyrate and lactate is strictly regulated by adipose monocarboxylate transporters (MCTs), which participate in β-hydroxybutyrate and lactate transmembrane transfer. Interestingly, apart from exercise, cold exposure also contributes to increased MCTs expression and plasma lactate increment, observed in vivid mice.

The action of BAIBA (investigated with the use of *in vivo* and *in vitro* murine models, and human adipose tissue *in vitro* examination) approximates to β-hydroxybutyrate and lactate activity, as it manifests in UCP1 and CIDE-A overexpression [56]. However, there is a significant difference concerning signal transduction. While β-hydroxybutyrate and lactate use PPARγ pathway, the action of BAIBA is mediated by PPARα signalling. In addition, exercise-enhanced PGC1α regulates BAIBA generation, which additionally stimulates PGC1α biosynthesis, thereby creating a positive loop.

Exercise induces also browning-associated immune system pathways. Beyond cold exposure, physical activity contributes to Meteorin-like (METRNL) concentration surge, which was precisely described in animal *in vitro* and human *in vivo* studies [29,54]. METRNL, in turn, stimulates adipocyte browning process, triggering the release of interleukins (IL-4, IL-13) from eosinophils and indirectly macrophages-derived catecholamines.

Finally, follistatin is recognized as a factor promoting adipocyte beiging as a result of exercise and its plasma levels in humans and mice *in vivo* positively correlate with both duration and intensity of physical effort [27].

OTHER ACTIVATORS

Adrenomedullin 2

Adrenomedullin 2 (ADM2) is a hormone primarily recognized as a cardiovascular protective factor and its direct action can be mediated via a variety of signalling pathways [47,77]. As soon as ADM2 binds to calcitonin receptor-like receptor (CRLR), it forms specific composites with receptor activity-modifying proteins (RAMPs). Regarding *in vitro* studies on primary rat adipocytes, CRLR-RAMP1 complex promotes adipocyte browning, inducing PKA and p38α MAPK [47]. On the other hand, ADM2 may also exert its browning effect through AMPK phosporylation [77]. Taken together, activated intracellular kinases contribute to elevated UCP1, PGC1α and PRDM 16 expression and energy expenditure, all indicative of browning phenomenon [47,77]. Similar results were also observed in studies on primary human adipocytes and mice *in vivo* [47].

In addition, according to murine *in vivo* studies, ADM2 may affect immune system mechanisms stimulating M2 macrophages proliferation with concomitant catecholamines synthesis and adipose tissue browning [47].

Bone morphogenetic proteins 4 and 7

Recent *in vitro* studies have shown that bone morphogenetic proteins 4 and 7 (BMP4 and BMP7) affect human undifferentiated adipocyte progenitors [20]. In response to BMP 4 and BMP7 activation of SMAD1/5/8 signalling occurs, leading to uncompleted adipose tissue browning, characterized by elevated UCP1 expression with no other hallmarks of britening, such as significantly increased mitochondrial content or basal respiration rate. Furthermore, there are also gaps in browning-associated genes expression profile, as PGC1α, NRF1 and TFAM are not stimulated. Nevertheless, both proteins (BMP4 and BMP7) should be considered as browning activators, as they may synergistically induce other browning stimulators.

Farnesoid X receptor

Farnesoid X receptor (FXR) is one of the most frequently investigated bile acid activated receptors. Administration of FXR agonist (fexaramine) to mice stimulates the mechanisms of adipocyte browning driven by FXR [22]. Among various downstream targets of FXR, fibroblast growth factor 15 (FGF 15) is the crucial one in this phenomenon. FGF 15 triggers a remarkable reduction of bile acids pool and simultaneously contributes to a relative increase of chenodeoxycholic acid derivatives, such as litocholic acid. It is assumed that this alteration enables adipocyte browning and three involved mechanisms have been described so far.

Firstly, adipocyte browning process is stimulated by the up-regulation of β3-adrenergic receptor gene expression and an increase in blood catecholamines (read more in section: Browning activators; chapters: Sympathetic nervous system activity and Immune system) [22]. Secondly, a decline in inflammatory cytokines that inhibit the process of browning (e.g. tumor necrosis factor alpha, TNFα), plays a role in FXR-related adipocyte britening (read more in section: Browning inhibitors; chapter: Obesity). Finally, the overexpression of DIO2 mediated by TGR5 results in the elevation of T3 and induction of adipocyte browning process (read more in section: Browning activators; chapter: Thyroid hormones) [22,69]. Taken together, the activation of distinct FXR-dependent pathways eventually leads to augmented UCP1 and PGC1α expression, both indicative of adipocyte browning [22].

In addition, recent murine research carried out with BAR502, a mutual agonist of FXR and TGR5, also confirmed the browning effect of bile acids; however, the exact mechanism remains to be fully elucidated [11].

Lysine-specific demethylase 1

Lysine-specific demethylase 1 (LSD1) is a flavin adenine dinucleotide-dependent enzyme, which controls the adipocyte browning process through epigenetic modifications [61]. Forming complexes with various trans-

criptional factors, such as PRDM16 or NRF1 [19,43], LSD1 selectively removes mono- and dimethyl groups from lysine 4 or lysine 9 of histone H3 (H3K4 or H3K9) [18]. Demethylation of H3K4 results in repression of adipocyte browning inhibitors [19,43,75], whereas demethylation of H3K9 leads to the up-regulation of thermogenic genes [19,43,61]. Both these effects efficiently participate in promotion of adipocyte browning process, as demonstrated in *in vivo* and *in vitro* murine studies.

LSD1, acting together with PRDM16, C/EBP-α, CoREST and other molecules on H3K4, down-regulates the expression of WAT-specific genes and disturbs Wnt signalling [15,19,75], which is referred to as browning inhibitor [46]. In addition, LSD1 affects glucocorticoid synthesis by a significant decrease in the expression of hydroxysteroid 11-β-dehydrogenase isozyme 1 (HSD11B1), which, in turn, reduces browning-inhibitory effect of active glucocorticoids [43,75].

LSD1, as a potent stimulator of browning, may influence target genes not only as a corepressor, but also as a coactivator [19,43]. Acting together with Zfp 516 or Nrf1 on H3K9, LSD1 potentiates transcription of BAT hallmarks, such as UCP1, PGC1α and PRDM16 [18,19,61]. Additionally, LSD1 sensitises adipose β3 receptors to adrenergic stimulation, which, in turn, stimulates browning [18].

Although mechanistic data on LSD1 is still inconsistent, authors agree that LSD1 is an important browning activator, which could be recruited by cold exposure [18,61].

Neural control

At the central level browning of WAT is regulated by certain hypothalamic regions, including thermoregulatory center in preoptic hypothalamus (as precisely described above), ventromedial hypothalamic nucleus (VMH), arcuate nucleus (ARC), dorsomedial hypothalamic nucleus (DMH) and paraventricular hypothalamic nucleus (PVH). They integrate several stimuli in order to control the process of browning through the sympathetic nervous system and different mechanisms are responsible for their stimulation.

VMH is activated in response to glucagon-like peptide-1 (GLP-1), a hormone released by enteroendocrine cells to signal satiety, delay gastric emptying and avert postprandial hyperglycaemia [4]. Brain GLP-1 receptors (GLP-1R) signalling is mediated via 5'AMP-activated protein kinase (AMPK) pathway.

ARC includes two antagonistic groups of neurons liable to maintain balance between hunger and satiety, which might be induced by leptin in a phosphatidyinositol 3-kinase (PI3K)/protein kinase B-dependent manner [17]. In addition, this pathway could be enhanced by insulin, which acts synergistically with leptin, leading to browning on a larger scale with substantial boost in energy expenditure.

DMH enables SNS-dependent adipocyte beiging selectively in inguinal WAT (IWAT) and dorsomedial subcutaneous WAT, however only when neuropeptide Y (NPY) gene expression within the DMH decreases [3]. In this case, DMH increases the expression of PPARγ and Pgc-1α mRNA, both involved in thermogenesis in rats.

Adipocyte browning and increased energy expenditure also occur as a result of the induction of hypothalamic brain-derived neurotrophic factor (BDNF) expression in response to environmental stimuli linked with adrenergic activation. BDNF acts within PVH, a region containing CRH and TRH neurons which have a thermogenic property via the SNS [10,31].

Taken together, the thermoregulatory center, ARC, VMH, DMH and PVH are all considered to be able to intensify the expression of adipose UCP1, PRDM 16, PGC1α, TMEM 26, CD137 and neural TH [3,4,10,17], which are indicative of WAT browning due to the remarkable increase in SNS activity. However, detailed mechanisms, defining how the activation of specific CNS regions impacts enhanced SNS drive, remain unknown and require further research.

Parathyroid hormone and parathyroid hormone-related protein

Recently, a powerful browning-positive effect of parathyroid hormone (PTH) and parathyroid hormone--related protein (PTHrP) was observed in murine models (in- and ex-vivo experiments) [34,35]. It has been found that full-length PTH, as well as PTH polypeptides made up of 34 N-terminal amino acids and PTHrP, promote UCP1, PGC1α and DIO2 genes expression. Up-regulation of these genes was also confirmed in patients suffering from primary hyperparathyroidism suggesting similar effect of PTH on human adipose tissue [34]. Mechanistically, action of both, PTH and PTHrP, is mediated by the same receptor known as PTH/PTHrP receptor and results in stimulation of PKA pathway [34,35].

Interestingly, dynamic induction of adipocyte browning process caused by the overexpression of PTH (e.g. in chronic kidney disease) or PTHrP (e.g. in cancer) seems to play a significant role in the development of cachexia, as the caloric-consuming thermogenic function of beige adipocytes may contribute to negative energy balance [34,35].

Prostaglandin E2

Prostaglandin E2 (PGE2) is an eicosanoid mostly considered as a potent vasodilator, endogenous pyrogen, as well as a fertilization and labour participant. Its production is initiated in almost all tissues by active cyclooxygenase 1 (COX-1), cyclooxygenase 2 (COX-2) and terminal prostaglandin E synthases [26]. Recent *in vitro* research revealed that in human WAT, PGE2 may induce adipo-

cyte white-to-beige transdifferentiation only in mature cells, but not in adipocyte progenitors [26], whereas in mice the effect is observed mostly in preadipocytes [25]. PGE2 participates in the differentiation of WAT by increasing expression of UCP1 and PRDM 16, genes indicative of browning process [25,26].

BROWNING INHIBITORS

Emerging studies on WAT browning have been primarily focused on understanding inducing mechanisms that transform WAT to energy-dissipating tissue. Data on browning inhibition is scarce and only few natural inhibitors are described in the literature.

Ageing

Recent research provided evidence that adipocyte browning process is remarkably suppressed in aged mice [64]. Interestingly, the reason this phenomenon is not solely associated with age-related chronic inflammation has previously been considered. Currently, it is assumed that depletion of browning ability is also determined by a decline in the number of PDGFRα+ progenitor cells, which proceeds with age [64]. Adipose tissue depots characterized by a decrease in PDGFRα+ population show interfered browning capacity upon β3-adrenergic stimulation, despite unchanged β3-adrenergic receptors (β3-AR) density. Furthermore, an extreme reduction (80% loss) in UCP1 expression is observed in the course of ageing. Finally, the prevalence of multilocular adipocytes among fat depots diminishes with age. Taken together, ageing may be regarded as an adipocyte browning inhibitor, as it impairs differentiation, functioning and depletes reserves of beige adipose tissue.

Nevertheless, low-grade inflammation is still considered as one of the potential mechanisms behind reduced UCP1 expression and inhibition of browning. For example, tumor necrosis factor alpha (TNFα), an inflammatory cytokine which plasma concentration rises with age [51], contributes to the disruption of adipocyte browning in response to β3-adrenergic stimulation. It disturbs β3-AR downstream signalling, irrespectively of β3-AR expression and supresses UCP1 expression [60]. Hence, age-dependent impairment of browning capacity has an association with both reduced PDGFRα+ population and action of inflammatory cytokines, such as TNF-α.

Glucocorticoids

Findings from mice and human studies have documented a potent suppressive effect of glucocorticoids (GCs) on adipocyte browning process [37]. Acting on the nuclear glucocorticoid receptor, GCs activate glucocorticoid response element in promoter region of miR-27b gene, thereby leading to miR-27b overexpression. miR-27b, in turn, interferes with 3′ UTR sequence of PRDM16 mRNA, silencing both transcription and translation of PRDM16. As a result, the expression of PRDM16 and PRDM16-dependent genes, such as UCP1 or CIDE-A, is significantly down-regulated. Thus, GCs may impair adipocyte browning via potentiation of miR-27b expression.

Obesity

The role of low-grade chronic inflammation in the inhibition of adipocyte browning is critical in obese mice. Hypertrophied adipocytes are capable of secreting a wide range of inflammatory cytokines, including monocyte chemoattractant protein-1 (MCP1) [60]. MCP1 provokes macrophages accumulation within fat depots. The infiltrated macrophages are then immediately activated in a classic way, transforming into M1 macrophages, which secrete inflammatory cytokines along with TNFα [13,60]. TNFα suppresses the induction of thermogenic adipocytes *in vivo* and *in vitro*, affecting β3-AR downstream signalling pathway and UCP1 expression, and the mechanisms controlling this suppression are independent of mitochondrial biogenesis [60].

Taken together, obesity serves as a potent inhibitor of adipocyte browning process, thereby creating the vicious circle: the greater expansion of WAT, the lower formation of beige adipocyte, thermogenesis and energy expenditure with concomitant increase in body fat mass.

Inhibition of adipocyte browning process is also associated with the activity of TGF-β/Smad3 or Wnt signalling pathways. Mechanistically, their common feature includes repression of BAT-specific genes, such as UCP1, PGC1α and CIDE-A, as demonstrated in *in vivo* and *in vitro* murine studies [46,73].

CLINICAL SIGNIFICANCE

Importantly, adipocyte browning process brings significant metabolic benefits that may help fight the growing worldwide problem of obesity and obesity-related diseases, such as atherosclerosis (arteriosclerotic vascular disease, ASVD), diabetes mellitus type 2, dyslipidemia and hypertension (HT). Here we present the current results of numerous *in vivo* murine studies with regard to physiological/pathophysiological background for developing new initiatives to advance prevention and treatment research regarding obesity, hypertension, diabetes type II, and atherosclerosis.

Completed browning of WAT manifests in the expansion of beige adipocyte population, which entails further changes. Considering thermogenic properties of brite adipocytes, adipocyte browning contributes to a remarkable increase in basal and uncoupled respiration [6,20,67]. Of note, generated heat increases the body core temperature [22,44], causing vasodilation and decrease in blood pressure, thus preventing arterial hypertension (HT). In addition, completed browning process promotes oxidative respiration and stimulates

Table 1. Clinical benefits of adipocyte browning process

Clinical effects of adipocyte browning process				
↓ ADIPOSITY	↓ ATHEROSCLEROSIS	↓ DIABETES TYPE 2	↓ DYSLIPIDEMIA	↓ HYPERTENSION
Mechanism:				
↑ energy expenditure	↓ secretion of inflammatory cytokines	↑ insulin sensitivity	↑ LPL activity and related lipid profile alterations	↑ temperature-related vasodilation
↑ lipolysis	↑ HDL-C (anti-oxidative potential)	↑ peripheral glucose uptake	↑ HDL-C	↓ vascular smooth muscle tension
↓ appetite	↑ adiponectin (cardioprotective effect)	↓ blood glucose level	↓ TG, LDL-C and TC	↓ vascular myocytes proliferation
	↓ vascular myocytes proliferation			↑ HDL-C (anti-oxidative potential)
				↑ adiponectin (cardioprotective effect)
				↑ natriuresis

HDL-C - high-density lipoprotein-cholesterol, LDL-C - low-density lipoprotein-cholesterol, LPL - lipoprotein lipase, TC - total cholesterol, TG - triglycerides.

qualitative changes in respiration substrates in favor of lipids, as demonstrated by elevated oxygen consumption rate (OCR) and decreased respiratory exchange ratio (RER) [6,39,49]. These changes are associated with intensified energy expenditure [7,11,35], which is one of the three major changes related to browning (increased beige adipocyte population, decreased number of white fat cells and enhanced energy expenditure).

Increased energy expenditure potentiates lipolysis, activating adipose hormone-sensitive lipase (HSL) and providing fuel for adipocyte respiration [14,23]. This, in turn, elevates plasma free fatty acids (FFA) level, enabling beige adipocyte-targeted substrate delivery. Clearly, lipolysis decreases adipose tissue mass and provokes body weight loss with all associated health benefits [7,22,44]. To maintain optimal thermogenesis the energetic processes requires a readily available fuel supply, which, apart from HSL activation, includes lipoprotein lipase (LPL) activity and up-regulated function of LDL-receptor (LDL-R) [2,5]. LPL and LDL-R, in turn, are capable of changing blood lipid profile into less atherogenic (with increased high-density lipoprotein-cholesterol (HDL-C) and declined triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and total cholesterol (TC)), thereby preventing dyslipidemia and atherogenesis. Finally, increased basal metabolic rate also affects glucose metabolism, stimulating its peripheral uptake with concomitant decrease of its blood concentration [44,77].

In addition to weight loss itself, many other benefits, beyond the reduction of WAT mass, are observed. Firstly, shrinkage of WAT may result in lower secretion of various inflammatory cytokines and, together with declined glucose blood level and augmented glucose uptake, may improve insulin sensitivity [22,31,44], thereby counteracting diabetes type 2. Secondly, reduced low-grade inflammation (due to body weight loss) may restore physiologic profile of secreted adipokines, such as adiponectin or leptin [22,38]. This, in turn, results in both limited leptin overproduction and leptin resistance [38], which, together with improved insulin sensitivity, may restore normal appetite regulatory mechanism. Furthermore, blood level of adiponectin may rise, providing potent cardioprotective effect and thus, limiting ASVD development and HT severity. Thirdly, considering that WAT secretes significant quantities of angiotensionogen, diminished population of white adipocytes could possibly coexist with lowered local angiotensinogen production and lowered blood pressure [32,74]. In addition, neutralised hyperinsulinemia, via cellular mediators that enhance natriuresis, may also participate in returning arterial blood pressure to normal values [68]. Finally, lowered Ang II, together with restored insulin, may be found to suppress vascular myocytes proliferation and prevent ASVD and HT development and/or progression [30,76].

Clinical benefits of adipocyte browning process with all described above mechanisms are summarized in Table 1.

CONCLUSIONS

This paper gives an overview of adipocyte browning process focusing on a wide range of browning activators and a few known inhibitors.

Because of its clinical importance, browning agents, mechanisms and therapeutic potentials of BAT have gained considerable attention, as they may provide novel strategies combating obesity and obesity-related disorders.

WAT browning contributes to the expansion of beige adipose tissue, characterized by increased basal and uncoupled respiration. This, in turn, leads to accelerated whole-body energy expenditure with concomitant weight loss. Hence, physiologic browning activators might serve as natural and promising factors to correct the energy imbalance that underlies obesity. In addition, even people who understand the difficulty of long-term weight loss often have problems with dietary restrictions and regular physical activity. Therefore, targeting BAT for the treatment of obesity will enable the development of a more convenient intervention.

Another relevant clinical implication is that adipocyte browning significantly contributes to glucose and lipid homeostasis. Firstly, the enlargement of beige adipose tissue depots enhances insulin sensitivity, facilitates peripheral glucose uptake and promotes its utilization. Secondly, beige fat cells are involved in the meaningful improvement of lipid metabolism by promoting free fatty acids β-oxidation. Taken together, active adipocyte browning supports treatment of obesity-related diseases, such as diabetes mellitus type 2, dyslipidemia and hypertension.

REFERENCES

[1] Altshuler-Keylin S., Kajimura S.: Mitochondrial homeostasis in adipose tissue remodeling. Sci. Signal., 2017; 10: eaai9248

[2] Bartelt A., Bruns O.T., Reimer R., Hohenberg H., Ittrich H., Peldschus K., Kaul M.G., Tromsdorf U.I., Weller H., Waurisch C., Eychmüller A., Gordts P.L., Rinninger F., Bruegelmann K., Freund B., et al.: Brown adipose tissue activity controls triglyceride clearance. Nat. Med., 2011; 17: 200-205

[3] Bartness T.J., Ryu V.: Neural control of white, beige and brown adipocytes. Int. J. Obes. Suppl., 2015; 5: S35-S39

[4] Beiroa D., Imbernon M., Gallego R., Senra A., Herranz D., Villarroya F., Serrano M., Fernø J., Salvador J., Escalada J., Dieguez C., Lopez M., Frühbeck G., Nogueiras R.: GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. Diabetes, 2014; 63: 3346-3358

[5] Berbée J.F., Boon M.R., Khedoe P.P., Bartelt A., Schlein C., Worthmann A., Kooijman S., Hoeke G., Mol I.M., John C., Jung C., Vazirpanah N., Brouwers L.P., Gordts P.L., Esko J.D., et al.: Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat. Commun., 2015; 6: 6356

[6] Bordicchia M., Liu D., Amri E.Z., Ailhaud G., Dessì-Fulgheri P., Zhang C., Takahashi N., Sarzani R., Collins S.: Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J. Clin. Invest., 2012; 122: 1022-1036

[7] Boström P., Wu J., Jedrychowski M.P., Korde A., Ye L., Lo J.C., Rasbach K.A., Boström E.A., Choi J.H., Long J.Z., Kajimura S., Zingaretti M.C., Vind B.F., Tu H., Cinti S., et al.: A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature, 2012; 481: 463-468

[8] Braga M., Reddy S.T., Vergnes L., Pervin S., Grijalva V., Stout D., David J., Li X., Tomasian V., Reid C.B., Norris K.C., Devaskar S.U., Reue K., Singh R.: Follistatin promotes adipocyte differentiation, browning, and energy metabolism. J. Lipid Res., 2014; 55: 375-384

[9] Brestoff J.R., Kim B.S., Saenz S.A., Stine R.R., Monticelli L.A., Sonnenberg G.F., Thome J.J., Farber D.L., Lutfy K., Seale P., Artis D.: Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature, 2015; 519: 242-246

[10] Cao L., Choi E.Y., Liu X., Martin A., Wang C., Xu X., During M.J.: White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. Cell Metab., 2011; 14: 324-338

[11] Carino A., Cipriani S., Marchianò S., Biagioli M., Santorelli C., Donini A., Zampella A., Monti M.C., Fiorucci S.: BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. Sci. Rep., 2017; 7: 42801

[12] Carrière A., Jeanson Y., Berger-Müller S., André M., Chenouard V., Arnaud E., Barreau C., Walther R., Galinier A., Wdziekonski B., Villageois P., Louche K., Collas P., Moro C., Dani C., et al.: Browning of white adipose cells by intermediate metabolites: an adaptive mechanism to alleviate redox pressure. Diabetes, 2014; 63: 3253-3265

[13] Castoldi A., Naffah de Souza C., Câmara N.O., Moraes-Vieira P.M.: The macrophage switch in obesity development. Front. Immunol., 2016; 6: 637

[14] Chechi K., van Marken Lichtenbelt W., Richard D.: Brown and beige adipose tissues: phenotype and metabolic potential in mice and men. J. Appl. Physiol. (1985), 2018; 124: 482-496

[15] Chen Y., Kim J., Zhang R., Yang X., Zhang Y., Fang J., Chen Z., Teng L., Chen X., Ge H., Atadja P., Li E., Chen T., Qi W.: Histone demethylase LSD1 promotes adipocyte differentiation through repressing Wnt signaling. Cell Chem. Biol., 2016; 23: 1228-1240

[16] Contreras G.A., Lee Y.H., Mottillo E.P., Granneman J.G.: Inducible brown adipocytes in subcutaneous inguinal white fat: the role of continuous sympathetic stimulation. Am. J. Physiol. Endocrinol. Metab., 2014; 307: E793-E799

[17] Dodd G.T., Decherf S., Loh K., Simonds S.E., Wiede F., Balland E., Merry T.L., Münzberg H., Zhang Z.Y., Kahn B.B., Neel B.G., Bence K.K., Andrews Z.B., Cowley M.A., Tiganis T.: Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell, 2015; 160: 88-104

[18] Duteil D., Metzger E., Willmann D., Karagianni P., Friedrichs N., Greschik H., Günther T., Buettner R., Talianidis I., Metzger D., Schüle R.: LSD1 promotes oxidative metabolism of white adipose tissue. Nat. Commun., 2014; 5: 4093

[19] Duteil D., Tosic M., Lausecker F., Nenseth H.Z., Müller J.M., Urban S., Willmann D., Petroll K., Messaddeq N., Arrigoni L., Manke T., Kornfeld J.W., Brüning J.C., Zagoriy V., Meret M., et al.: Lsd1 ablation triggers metabolic reprogramming of brown adipose tissue. Cell Rep., 2016; 17: 1008-1021

[20] Elsen M., Raschke S., Tennagels N., Schwahn U., Jelenik T., Roden M., Romacho T., Eckel J.: BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. Am. J. Physiol. Cell Physiol., 2014; 306: C431-C440

[21] Evans M., Cogan K.E., Egan B.: Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. J. Physiol., 2017; 595: 2857-2871

[22] Fang S., Suh J.M., Reilly S.M., Yu E., Osborn O., Lackey D., Yoshihara E., Perino A., Jacinto S., Lukasheva Y., Atkins A.R., Khvat A., Schnabl B., Yu R.T., Brenner D.A., et al.: Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat. Med., 2015; 21: 159-165

[23] Finlin B.S., Zhu B., Confides A.L., Westgate P.M., Harfmann B.D., Dupont-Versteegden E.E., Kern P.A.: Mast cells promote seasonal white adipose beiging in humans. Diabetes, 2017; 66: 1237-1246

[24] Fisher F.M., Kleiner S., Douris N., Fox E.C., Mepani R.J., Verdeguer F., Wu J., Kharitonenkov A., Flier J.S., Maratos-Flier E., Spiegelman B.M.: FGF21 regulates PGC-1α and browning of white adipose tissues in adaptive thermogenesis. Genes Dev., 2012; 26: 271-281

[25] García-Alonso V., López-Vicario C., Titos E., Morán-Salvador E.,

González-Périz A., Rius B., Párrizas M., Werz O., Arroyo V., Clària J.: Coordinate functional regulation between microsomal prostaglandin E synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor γ (PPARγ) in the conversion of white-to-brown adipocytes. J. Biol. Chem., 2013; 288: 28230-28242

[26] García-Alonso V., Titos E., Alcaraz-Quiles J., Rius B., Lopategi A., López-Vicario C., Jakobsson P.J., Delgado S., Lozano J., Clària J.: Prostaglandin E2 exerts multiple regulatory actions on human obese adipose tissue remodeling, inflammation, adaptive thermogenesis and lipolysis. PLoS One, 2016; 11: e0153751

[27] Hansen J., Brandt C., Nielsen A.R., Hojman P., Whitham M., Febbraio M.A., Pedersen B.K., Plomgaard P.: Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. Endocrinology, 2011; 152: 164-171. Erratum in: Endocrinology, 2015; 156: 1200

[28] Harms M., Seale P.: Brown and beige fat: development, function and therapeutic potential. Nat. Med., 2013; 19: 1252-1263

[29] Hui X., Gu P., Zhang J., Nie T., Pan Y., Wu D., Feng T., Zhong C., Wang Y., Lam K.S., Xu A.: Adiponectin enhances cold-induced browning of subcutaneous adipose tissue via promoting M2 macrophage proliferation. Cell Metab., 2015; 22: 279-290

[30] Isenović E.R., Kedees M.H., Tepavcević S., Milosavljević T., Korićanac G., Trpković A., Marche P.: Role of PI3K/AKT, cPLA2 and ERK1/2 signaling pathways in insulin regulation of vascular smooth muscle cells proliferation. Cardiovasc. Hematol. Disord. Drug Targets, 2009; 9: 172-180

[31] Kajimura S., Spiegelman B.M., Seale P.: Brown and beige fat: physiological roles beyond heat generation. Cell Metab., 2015; 22: 546-559

[32] Kalupahana N.S., Moustaid-Moussa N.: The adipose tissue renin-angiotensin system and metabolic disorders: a review of molecular mechanisms. Crit. Rev. Biochem. Mol. Biol., 2012; 47: 379-390

[33] Kharitonenkov A., Shiyanova T.L., Koester A., Ford A.M., Micanovic R., Galbreath E.J., Sandusky G.E., Hammond L.J., Moyers J.S., Owens R.A., Gromada J., Brozinick J.T., Hawkins E.D., Wroblewski V.J., Li D.S., et al.: FGF-21 as a novel metabolic regulator. J. Clin. Invest., 2005; 115: 1627-1635

[34] Kir S., Komaba H., Garcia A.P., Economopoulos K.P., Liu W., Lanske B., Hodin R.A., Spiegelman B.M.: PTH/PTHrP receptor mediates cachexia in models of kidney failure and cancer. Cell Metab., 2016; 23: 315-323

[35] Kir S., White J.P., Kleiner S., Kazak L., Cohen P., Baracos V.E., Spiegelman B.M.: Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature, 2014; 513: 100-104

[36] Knudsen J.G., Murholm M., Carey A.L., Biensø R.S., Basse A.L., Allen T.L., Hidalgo J., Kingwell B.A., Febbraio M.A., Hansen J.B., Pilegaard H.: Role of IL-6 in exercise training- and cold-induced UCP1 expression in subcutaneous white adipose tissue. PLoS One, 2014; 9: e84910

[37] Kong X., Yu J., Bi J., Qi H., Di W., Wu L., Wang L., Zha J., Lv S., Zhang F., Li Y., Hu F., Liu F., Zhou H., Liu J., et al.: Glucocorticoids transcriptionally regulate miR-27b expression promoting body fat accumulation via suppressing the browning of white adipose tissue. Diabetes, 2015; 64: 393-404

[38] Leal Vde O., Mafra D.: Adipokines in obesity. Clin. Chim. Acta, 2013; 419: 87-94

[39] Lee J.Y., Takahashi N., Yasubuchi M., Kim Y.I., Hashizaki H., Kim M.J., Sakamoto T., Goto T., Kawada T.: Triiodothyronine induces UCP-1 expression and mitochondrial biogenesis in human adipocytes. Am. J. Physiol. Cell Physiol., 2012; 302: C463-C472

[40] Lee M.W., Odegaard J.I., Mukundan L., Qiu Y., Molofsky A.B., Nussbaum J.C., Yun K., Locksley R.M., Chawla A.: Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell, 2015; 160: 74-87

[41] Lee P., Linderman J.D., Smith S., Brychta R.J., Wang J., Idelson C., Perron R.M., Werner C.D., Phan G.Q., Kammula U.S., Kebebew E., Pacak K., Chen K.Y., Celi F.S.: Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab., 2014; 19: 302-309

[42] Lidell M.E., Betz M.J., Dahlqvist Leinhard O., Heglind M., Elander L., Slawik M., Mussack T., Nilsson D., Romu T., Nuutila P., Virtanen K.A., Beuschlein F., Persson A., Borga M., Enerbäck S.: Evidence for two types of brown adipose tissue in humans. Nat. Med., 2013; 19: 631-634

[43] Lin J.Z., Farmer S.R.: LSD1-a pivotal epigenetic regulator of brown and beige fat differentiation and homeostasis. Genes Dev., 2016; 30: 1793-1795

[44] Lin J.Z., Martagón A.J., Cimini S.L., Gonzalez D.D., Tinkey D.W., Biter A., Baxter J.D., Webb P., Gustafsson J.Å., Hartig S.M., Phillips K.J.: Pharmacological activation of thyroid hormone receptors elicits a functional conversion of white to brown fat. Cell Rep., 2015; 13: 1528-1537

[45] Lindsey R.C., Mohan S.: Thyroid hormone acting via TRβ induces expression of browning genes in mouse bone marrow adipose tissue. Endocrine, 2017; 56: 109-120

[46] Lo K.A., Ng P.Y., Kabiri Z., Virshup D., Sun L.: Wnt inhibition enhances browning of mouse primary white adipocytes. Adipocyte, 2016; 5: 224-231

[47] Lv Y., Zhang S.Y., Liang X., Zhang H., Xu Z., Liu B., Xu M.J., Jiang C., Shang J., Wang X.: Adrenomedullin 2 enhances beiging in white adipose tissue directly in an adipocyte-autonomous manner and indirectly through activation of M2 macrophages. J. Biol. Chem., 2016; 291: 23390-23402

[48] Murawska-Ciałowicz E.: Tkanka tłuszczowa – charakterystyka morfologiczna i biochemiczna różnych depozytów. Postępy Hig. Med. Dośw., 2017; 71: 466-484

[49] Nguyen K.D., Qiu Y., Cui X., Goh Y.P., Mwangi J., David T., Mukundan L., Brombacher F., Locksley R.M., Chawla A.: Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature, 2011; 480: 104-108

[50] Obregon M.J.: Adipose tissues and thyroid hormones. Front. Physiol., 2014; 5: 479

[51] Paolisso G., Rizzo M.R., Mazziotti G., Tagliamonte M.R., Gambardella A., Rotondi M., Carella C., Giugliano D., Varricchio M., D'Onofrio F.: Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. Am. J. Physiol., 1998; 275: E294-E299

[52] Park J., Kim M., Sun K., An Y.A., Gu X., Scherer P.E.: VEGF-A expressing adipose tissue shows rapid beiging and enhanced survival after transplantation and confers IL-4-independent metabolic improvements. Diabetes, 2017; 66: 1479-1490

[53] Pukajło K., Kolackov K., Łaczmański Ł., Daroszewski J.: Iryzyna – nowy mediator homeostazy energetycznej. Postępy Hig. Med. Dośw., 2015; 69: 233-242

[54] Rao R.R., Long J.Z., White J.P., Svensson K.J., Lou J., Lokurkar I., Jedrychowski M.P., Ruas J.L., Wrann C.D., Lo J.C., Camera D.M., Lachey J., Gygi S., Seehra J., Hawley J.A., et al.: Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell, 2014; 157: 1279-1291

[55] Raschke S., Elsen M., Gassenhuber H., Sommerfeld M., Schwahn U., Brockmann B., Jung R., Wisløff U., Tjønna A.E., Raastad T., Hallén J., Norheim F., Drevon C.A., Romacho T., Eckardt K., et al.: Evidence against a beneficial effect of irisin in humans. PLoS One, 2013; 8: e73680

[56] Roberts L.D., Boström P., O'Sullivan J.F., Schinzel R.T., Lewis G.D., Dejam A., Lee Y.K., Palma M.J., Calhoun S., Georgiadi A., Chen M.H., Ramachandran V.S., Larson M.G., Bouchard C., Rankinen T., et al.: β-Aminoisobutyric acid induces browning of white fat and hepatic β-oxidation and is inversely correlated with cardiometabolic risk factors. Cell Metab., 2014; 19: 96-108

[57] Roca-Rivada A., Castelao C., Senin L.L., Landrove M.O., Baltar J., Belén Crujeiras A., Seoane L.M., Casanueva F.F., Pardo M.: FNDC5/irisin is not only a myokine but also an adipokine. PLoS One, 2013; 8: e60563

[58] Rosen E.D., Spiegelman B.M.: What we talk about when we talk about fat. Cell, 2014; 156: 20-44

[59] Rosenwald M., Perdikari A., Rülicke T., Wolfrum C.: Bi-directional interconversion of brite and white adipocytes. Nat. Cell Biol., 2013; 15: 659-667

[60] Sakamoto T., Nitta T., Maruno K., Yeh Y.S., Kuwata H., Tomita K., Goto T., Takahashi N., Kawada T.: Macrophage infiltration into obese adipose tissues suppresses the induction of UCP1 level in mice. Am. J. Physiol. Endocrinol. Metab., 2016; 310: E676-E687

[61] Sambeat A., Gulyaeva O., Dempersmier J., Tharp K.M., Stahl A., Paul S.M., Sul H.S.: LSD1 Interacts with Zfp516 to promote UCP1 transcription and brown fat program. Cell Rep., 2016; 15: 2536-2549

[62] Seale P., Conroe H.M., Estall J., Kajimura S., Frontini A., Ishibashi J., Cohen P., Cinti S., Spiegelman B.M.: Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin. Invest., 2011; 121: 96-105

[63] Shabalina I.G., Petrovic N., de Jong J.M., Kalinovich A.V., Cannon B., Nedergaard J.: UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep., 2013; 5: 1196-1203

[64] Shin W., Okamatsu-Ogura Y., Machida K., Tsubota A., Nio-Kobayashi J., Kimura K.: Impaired adrenergic agonist-dependent beige adipocyte induction in aged mice. Obesity, 2017; 25: 417-423

[65] Siemińska L.: Adipose tissue. Pathophysiology, distribution, sex differences and the role in inflammation and cancerogenesis. Pol. J. Endocrinol., 2007; 58: 330-342

[66] Singh R., Braga M., Reddy S.T., Lee S.J., Parveen M., Grijalva V., Vergnes L., Pervin S.: Follistatin targets distinct pathways to promote brown adipocyte characteristics in brown and white adipose tissues. Endocrinology, 2017; 158: 1217-1230

[67] Than A., He H.L., Chua S.H., Xu D., Sun L., Leow M.K., Chen P.: Apelin enhances brown adipogenesis and browning of white adipocytes. J. Biol. Chem., 2015; 290: 14679-14691

[68] Tiwari S., Riazi S., Ecelbarger C.A.: Insulin's impact on renal sodium transport and blood pressure in health, obesity, and diabetes. Am. J. Physiol. Renal Physiol., 2007; 293: F974-F984

[69] Watanabe M., Houten S.M., Mataki C., Christoffolete M.A., Kim B.W., Sato H., Messaddeq N., Harney J.W., Ezaki O., Kodama T., Schoonjans K., Bianco A.C., Auwerx J.: Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature, 2006; 439: 484-489

[70] World Health Organization: Fact sheet on obesity and overweight. http://www.who.int/mediacentre/factsheets/fs311/en/ (29.04.2017)

[71] Wójcik B., Górski J.: Brunatna tkanka tłuszczowa u dorosłego człowieka: występowanie i funkcja. Endokrynol. Otył. Zab. Przem. Mat, 2011; 7: 34-40

[72] Wu J., Boström P., Sparks L.M., Ye L., Choi J.H., Giang A.H., Khandekar M., Virtanen K.A., Nuutila P., Schaart G., Huang K., Tu H., van Marken Lichtenbelt W.D., Hoeks J., Enerbäck S., et al.: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell, 2012; 150: 366-376

[73] Yadav H., Quijano C., Kamaraju A.K., Gavrilova O., Malek R., Chen W., Zerfas P., Zhigang D., Wright E.C., Stuelten C., Sun P., Lonning S., Skarulis M., Sumner A.E., Finkel T., et al.: Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling. Cell Metab., 2011; 14: 67-79

[74] Yvan-Charvet L., Massiéra F., Lamandé N., Ailhaud G., Teboul M., Moustaid-Moussa N., Gasc J.M., Quignard-Boulangé A.: Deficiency of angiotensin type 2 receptor rescues obesity but not hypertension induced by overexpression of angiotensinogen in adipose tissue. Endocrinology, 2009; 150: 1421-1428

[75] Zeng X., Jedrychowski M.P., Chen Y., Serag S., Lavery G.G., Gygi S.P., Spiegelman B.M.: Lysine-specific demethylase 1 promotes brown adipose tissue thermogenesis via repressing glucocorticoid activation. Genes Dev., 2016; 30: 1822-1836

[76] Zhang F., Hu Y., Xu Q., Ye S.: Different effects of angiotensin II and angiotensin-(1-7) on vascular smooth muscle cell proliferation and migration. PLoS One, 2010; 5: e12323

[77] Zhang H., Zhang S.Y., Jiang C., Li Y., Xu G., Xu M.J., Wang X.: Intermedin/adrenomedullin 2 polypeptide promotes adipose tissue browning and reduces high-fat diet-induced obesity and insulin resistance in mice. Int. J. Obes., 2016; 40: 852-860

[78] Zybek-Kocik A., Sawicka-Gutaj N., Wrotkowska E., Sowiński J., Ruchała M.: Time-dependent irisin concentration changes in patients affected by overt hypothyroidism. Endokrynol. Pol., 2016; 67: 476-480

The authors have no potential conflicts of interest to declare.