Mechanisms of Aging-Related Impairment of Brown Adipocyte Development and Function

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Key Words

Brown adipocytes · Aging · Stem/progenitor cells

Abstract

Aging is one of the primary risk factors for the development of obesity, a pathology that develops due to an imbalance of increased energy consumption over reduced expenditure. Brown adipocytes are responsible for thermogenesis and could therefore counter obesity by increasing energy expenditure. It is by now well established that humans possess thermogenesis-competent brown adipocytes throughout life, and recent findings indicate that brown fat is actively involved in metabolic control and body weight regulation in adults. Aging is accompanied by a loss of classical brown adipocytes as well as the brown-like adipocytes found in white adipose tissue, suggesting that loss of their energy-expenditure capacity might contribute to an obesity-prone phenotype with increased age. We here discuss the hypothesis that the age-related loss of brown adipocyte regenerative capacity is a result of dysfunctional stem/progenitor cells. The possible molecular mechanisms that lead to an age-related decline in brown adipogenic stem/progenitor cell function include cell-autonomous and external effects. General loss of mitochondrial biogenesis and function has repeatedly been linked to age-related perturbation of metabolic processes. We also discuss the possibility that alterations in neuronal

control by the sympathetic nervous system may contribute to impaired regeneration and thermogenesis in aged brown adipocytes. Finally, age-related changes of endocrine signals have been proposed to exacerbate the loss of brown adipose tissue. In conclusion, age-induced impairment of brown adipogenic stem/progenitor cell function could contribute to the loss of brown adipocyte regeneration, thereby promoting the development of obesity and other metabolic disorders with age.

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Introduction

Obesity develops when energy intake exceeds energy expenditure. In 2008, 1.4 billion adults worldwide were overweight, i.e. their BMI was greater than 25, while 200 million men and 300 million women were classified as obese (BMI >30) [1]. Depending on ethnicity and gender, body fat content peaks between 40 and 70 years of age, thus showing a clear association with increasing age [2]. After reaching its peak, body fat content remains stable or declines only slightly until the onset of the frailty syndrome, which occurs at a very old age. Adipose tissue can be divided into 2 functionally distinct categories: white adipose tissue (WAT) and brown adipose tissue (BAT). The primary function of WAT is to store lipids. It is char-

acterized by a low abundance of mitochondria and a large unilocular fat droplet. In contrast to this, BAT possesses the unique ability to dissipate large quantities of energy in a process known as thermogenesis. The capacity to convert excess nutrient energy into heat depends on the expression of uncoupling protein (UCP)-1 in the inner mitochondrial membrane where it dissolves the mitochondrial proton gradient, thereby uncoupling respiration from ATP production [3]. BAT is characterized by dense sympathetic innervation, multilocular small lipid droplets, and a high mitochondrial density, all of which are required for optimal thermogenesis.

While it was only recently discovered that adult human BAT is metabolically active [4–9], its presence throughout the human lifespan has been known for several decades [10]. It is interesting to note that some of these studies have revealed a negative correlation between age and the occurrence of metabolically active BAT [5, 6, 10]. In mice, and very likely also in humans, 2 distinct populations of brown adipocytes occur within the body. The classical BAT, preformed during embryogenesis, is located in the interscapular and deeper neck regions of mice and humans [11]. The second type of UCP1-expressing adipocytes can be found interspersed within WAT and skeletal muscle [12, 13], and these adipocytes have been termed either beige or brite (brown-in-white) adipocytes [14, 15]. Although this is still a matter of debate in the field, the recruitment of beige/brite adipocytes under *in vivo* conditions, a process known as browning, is believed to occur mostly via transdifferentiation of mature white adipocytes [16]. Interestingly, it was shown recently that a subset of WAT-resident progenitors, selected by expression of the surface markers CD137 and TMEM26, have a higher potential to become beige/brite adipocytes. These observations suggest that only a subset of all adipose progenitors in white fat could give rise to browning-competent white adipocytes [17].

Exposure to cold has been known to increase browning by stimulation through the sympathetic nervous system, specifically via binding of the neurotransmitter norepinephrine to the β_3 -adrenergic receptor [16]. Additionally, a number of secreted factors that promote browning alone or in conjunction with sympathetic stimulation have been reviewed elsewhere in more detail [18].

The metabolic significance of BAT in human energy balance and obesity has only recently been established. In one study, repeated exposure of human study participants to mild cold resulted in increased energy expenditure, and a significant correlation was observed between increased cold-induced activity of BAT and reduction of

body fat content [19]. Interestingly, BAT activity also appears to correlate with genetically determined sensitivity to develop metabolic diseases such as type 2 diabetes. Individuals of South Asian descent, an ethnic group with a high prevalence of type 2 diabetes, displayed reduced total BAT volume compared to Caucasian study participants. The authors suggested that the higher capacity for nonshivering thermogenesis through BAT might confer resistance to metabolic perturbations in Caucasians compared to the South Asian study participants [20]. While these findings are of great interest, further analyses will be needed to determine whether changes in human brown fat mass and activity are causally linked to the incidence of diabetes under different physiological conditions.

Mechanisms of Age-Related Atrophy of BAT: Impairment of Regeneration

Given that aging is associated with increased adiposity, it is tempting to speculate that the age-related decline of BAT may exacerbate this effect. Aging has been established as a strong negative determinant of human BAT mass and activity [5, 10, 21]. While it is not fully established which type of BAT is more prevalent in adult humans, it seems likely that aging negatively affects the formation of both types of brown fat, *i.e.* classical as well as beige/brite adipocytes, since essentially all anatomical locations examined show an age-dependent loss of brown adipocytes [10]. In rodents, increased age similarly leads to a reduction of both types of BAT. Classical BAT displays a functional decline that is accompanied by reduced expression of the key functional marker UCP1 [22]. Similarly, the browning of WAT decreases with age [23]. Caloric restriction, a dietary regimen known to extend overall life expectancy, ameliorates these effects in classical BAT, although this could at least in part be due to an overall age-related decline in total DNA content, which was used for normalization in this study [24]. Likewise, caloric restriction partially restored the expression of beige adipocyte markers in senescent subcutaneous WAT (scWAT) [23].

Throughout life, adipocytes regenerate from a pool of mesenchymal progenitor cells that are capable of self-renewal, expansive proliferation, and subsequent differentiation into mature adipocytes. In WAT, this process is blunted with increased age and could be linked to dysfunctional white adipocyte stem/progenitor cells [25]. It seems likely that a similar process occurs in BAT: the in-

duction of proliferation and UCP1 protein accumulation in response to cold exposure has been shown to be essentially absent in aged classical BAT [26]. Interestingly, the authors hypothesized that alterations of extracellular trophic factors may be involved in this functional decline since cells collected from young and old BAT no longer displayed a proliferative defect when grown under in vitro culture conditions [27]. While this is somewhat in contrast to findings reported from WAT where aging was associated with a defect in progenitor proliferation and differentiation both in vivo and in vitro [25], similar mechanisms may impair the age-related regeneration of brown and white fat.

In WAT, different stages of adipogenic progenitors have recently been described using flow cytometric approaches. These studies suggest that the pool of progenitors residing within adipose tissues is heterogeneous and consists of adipogenic progenitors with different degrees of maturity. Specifically, adipogenic progenitors commonly express the cell surface markers stem cell antigen (Sca)-1, cluster of differentiation (CD)-29, and CD34 and are negative for the endothelial and hematopoietic lineage (Lin) markers CD31 and CD45 (Lin⁻:CD29⁺:CD34⁺:Sca1⁺). These cells are highly adipogenic when cultured but are unable to reconstitute functional adipose tissue after transplantation [13, 28]. Further analysis by flow cytometry revealed that a small subpopulation of cells expressing CD24 in addition to the markers mentioned can be found in WAT (Lin⁻:CD29⁺:CD34⁺:Sca1⁺:CD24⁺). This population can reconstitute a fully functional adipose tissue depot when transplanted into lipodystrophic mice and thus represents a more stem cell-like population [28]. Interestingly, this population of cells is also known to replenish the more abundant population of Lin⁻:CD29⁺:CD34⁺:Sca1⁺ cells that do not express CD24 [29]. A very similar population of stem/progenitor cells has also been described in classical BAT (surface marker configuration: CD45⁻:CD29⁺:Sca1⁺:CD144⁺:CD24⁺) although the regenerative capacities have not been tested in vivo [30]. While the negative effects of age on heterogeneous populations of adipose tissue-resident progenitors are well established, it is not currently known whether this is also linked to the ability of stem/progenitor cells to undergo the maturation steps from a stem cell-like progenitor to a fully committed preadipocyte-like progenitor. This process of disturbed progenitor cell maturation could contribute to the reduced regeneration of brown adipocytes in aged individuals.

For brite/beige adipogenesis, the situation is somewhat more complex since these adipocytes mostly trans-

differentiate from preexisting mature white adipocytes [16]. As reported by Wu et al. [17], a distinct subpopulation of WAT-resident progenitors expressing the surface markers CD137 and TMEM26 has a greater potential to give rise to adipocytes with the ability to transdifferentiate into brite/beige adipocytes. The reduced formation of brite/beige adipocytes could therefore be due to either a loss of this progenitor cell population or a defective ability to proliferate and differentiate. Alternatively, it remains to be determined whether an age-related reduction of a specific endocrine signal that promotes browning is responsible for the loss of brite adipocytes with age, which would be consistent with the observation that changes in trophic factors regulate proliferation in aged classical BAT [27].

In summary, a defect at the level of stem and/or progenitor cells could contribute to dysfunctional regeneration of brite/beige and classical brown adipocytes in aged individuals. This could be related to cell-intrinsic differences and/or currently unknown changes in the distribution of different stem/progenitor cell maturation stages within classical BAT and WAT (as the source of brite/beige adipocytes). As noted before, aging also leads to a proliferative defect in BAT that is at least partially caused by changes in external instructive signals [27]. These findings taken together suggest that the microenvironment, also known as the stem cell niche, plays a key role in stem/progenitor cell maintenance and regeneration of brown adipocytes (fig. 1). Here we summarize and discuss the potential mechanisms that have been proposed to explain the loss of brown fat during the aging process and how this could relate to stem/progenitor cell function. Among these, (1) a generalized defect of mitochondrial biogenesis and bioenergetics, (2) neuronal changes in sympathetic tone, adrenergic sensing, or neuropeptide levels, and (3) changes in the endo-/para-/autocrine microenvironment have been proposed (fig. 1).

Impairment of Mitochondrial Biogenesis and Bioenergetics

The mitochondrion is an important component of brown fat thermogenesis. UCP1, uniquely expressed in BAT, is localized to the inner mitochondrial membrane where it dissipates the electrochemical energy stored within the proton gradient as heat instead of converting it to ATP. A general phenomenon that correlates with increasing age is a progressive decline of mitochondrial

Fig. 1. Impairment of brown adipogenic stem/progenitor cell function reduces the regenerative potential of BAT. The underlying mechanisms include cell-autonomous defects, such as reduced mitochondrial function, or changes in the endocrine balance of the microenvironment, such as hormonal and proinflammatory signals, or a combination of both, such as altered input from the sympathetic nervous system or locally reduced adrenergic sensitivity of the stem/progenitor cells.

function that is thought to contribute to numerous pathologies including blindness, deafness, movement disorders, dementias, cardiomyopathy, myopathy, and renal dysfunction [31]. This impairment could be caused by a combination of several molecular mechanisms, i.e. a functional decline of mitochondrial oxidative phosphorylation that leads to reduced fuel oxidation and increased lipotoxic and glucotoxic reactions, or increased levels of reactive oxygen species (ROS) that lead to an accumulation of oxidative damage with age [31, 32]. Both processes could lead to the accumulation of mutations in mitochondrial DNA (mtDNA) which in turn encodes for several subunits of the complexes I, III, and IV of the mitochondrial respiratory chain [31]. The free radical theory of aging proposes that an increase in ROS leads to oxidative damage to proteins and lipids which in turn leads to a vicious cycle of augmented ROS production, further exacerbating the oxidative damage which may

also affect the rate of somatic mtDNA mutations [32]. Mitochondrial electron transport is the main source of ROS, making the mitochondrial network the most immediate target of oxidative damage that can lead to mitochondrial dysfunction. On the other hand, several studies clearly refute the current model that ROS may be involved in the senescence-related decline of mitochondrial function [33]. It has also been shown that mitochondrial ROS per se are not entirely detrimental as they may act as positive regulators of life expectancy through a positive feedback mechanism known as mitochondrial hormesis (mitohormesis) [34]. Exposure to antioxidants quenched ROS production, thereby inhibiting the beneficial effects of nutrient restriction or exercise on life expectancy or insulin sensitivity, respectively [34].

In summary, a decline in mitochondrial energy metabolism and the accumulation of mtDNA mutations are important contributors to human aging. This decline in mitochondrial function has also been reported in BAT and is thus congruent with similar observations in other tissues [24]. The role of oxidative stress in this process has been a matter of debate, and in the context of the predominantly uncoupled respiration in BAT it is unclear whether ROS feature a prominent role in the aging of brown adipocytes.

Reduced Sensitivity to Sympathetic Tone May Diminish BAT Formation with Age

The recruitment of brown adipocytes and thermogenic activity through BAT requires β_3 -adrenergic receptor stimulation [4]. Compared to WAT, BAT is characterized by a high degree of sympathetic innervation. Efferent fibers release norepinephrine that in turn binds to the adipocyte-specific β_3 -adrenergic receptor, a GTP-binding protein (G protein)-coupled receptor, on the plasma membrane. This process enhances BAT thermogenic activity and capacity due to activation of the stimulating subunit of the β_3 -adrenergic receptor, i.e. the G protein G_s , resulting in adenylate cyclase activation. Subsequently, adenylate cyclase catalyzes the conversion of ATP to cyclic AMP (cAMP), thereby activating protein kinase A (PKA). This kinase phosphorylates several protein substrates that promote a concerted induction of UCP1 gene expression and activity, lipolysis, and other processes required for thermogenesis.

In humans, the responsiveness of brown adipocytes to cold is attenuated with increased age: when exposed to an ambient temperature of 19°C for 2 h, 17 of 32 young

subjects and only 2 of 24 older subjects exhibited an increase in radiolabeled glucose uptake into supraclavicular and paraspinal adipose tissue [6]. The fact that BAT prevalence is markedly decreased with age could at least in part be due to changes in sympathetic activation. In a recent study by Yoneshiro et al., it could be shown that single-nucleotide polymorphisms in the genes encoding the β_3 -adrenergic receptor and UCP1 contribute to the age-related decline in BAT activity in humans [35]. Similarly, aging attenuates cold- and norepinephrine-induced nonshivering thermogenesis in rodents [22]. However, this defect appears to be unrelated to changes in sympathetic tone as it has been demonstrated that the ability to sense cold and the sympathetic tone are unchanged or even greater in aged mice before and after a cold challenge [36]. Importantly, reduced cold-induced thermogenesis did not correlate with norepinephrine turnover rates that were elevated in aged males but significantly reduced in older female rats [22]. A similar effect has been proposed in humans, where aging-related chronic activation of the sympathetic nervous system is observed while local sensing within adipose tissue may be impaired [37]. Moreover, aging leads to a reduced density of β -adrenergic receptors in classical BAT [38]. These findings taken together suggest that there is no primary involvement of changed sympathetic tone in the functional decline of senescent BAT, whereas local impairment of β_3 -adrenergic signaling may result in reduced thermogenic function [27]. Local changes in adrenergic sensing may affect the recruitment of brite adipocytes in scWAT [23]. The decrease in UCP1 expression in scWAT is accompanied by a lower expression of β_3 -adrenergic receptors and the transcription factor CREB (cAMP response element-binding protein), a downstream component of adrenergic signaling. In contrast to this, the enzyme monoamine oxidase A (MAO-A) that degrades endogenous catecholamines like norepinephrine is expressed at higher levels in senescent scWAT [23]. Given that the overall sympathetic tone increases, it seems unlikely that the sympathetic input to white fat declines with age. These findings further suggest that the local sensing mechanism is impaired and could thus contribute to the reduced browning of white fat depots.

An alternative theory is that one or several of the known browning molecules may prime cells to increase their sensitivity to sympathetic stimulation. Several recent studies have identified a role of neuropeptides in the control of BAT mass and activity that may implicate these factors in the loss-of-function of aged brown adipocytes. Neurons found in the brain regions involved in regula-

tion of the sympathetic outflow to BAT express the melanocortin 4 receptor (MC4R). The melanocortin system is an important component in the regulation of sympathetic nervous system-mediated BAT thermogenesis. MC4R activation is blocked by the agouti-related peptide (AgRP) which is cosynthesized with neuropeptide Y (NPY). Overexpression of NPY reduces the expression of UCP1 in BAT and thermogenesis while increasing food intake in rats [39]. The expression of this orexigenic neuropeptide is suppressed by leptin in young rats. However, with increased age the negative correlation between leptin and NPY levels is lost, suggesting an impairment of the suppression of hypothalamic NPY due to leptin that could also alter the quality of the sympathetic input to aged BAT [40]. The neuropeptide hormone orexin may act directly on committed brown preadipocytes through orexin receptor-1 which could lead to stimulation of a brown adipogenic gene program. Interestingly, orexin restored the thermogenic defect of senescent mice, and genetic ablation of orexin-producing neurons accelerated the BAT dysfunction of aged animals [41].

In summary, the present state of knowledge suggests that sympathetic tone is unchanged or even increases with age. Some studies have demonstrated that an impaired ability to sense norepinephrine within the adipose tissue depots may contribute to the loss of brown adipocytes. Moreover, the age-related changes in neuropeptide levels such as orexin or NPY could affect brown adipocyte metabolism and energy expenditure locally or modify the quality of sympathetic input to adipose tissue with age.

Aging-Related Changes in Endocrine Control of BAT Formation

It has recently been proposed that the age-related decline in the thermogenic activity of human BAT is a result of an imbalance between the inhibitory effects of glucocorticoids and the thermogenesis-promoting effects of sex hormones [42]. In this line, it could be demonstrated that glucocorticoids such as dexamethasone diminish the norepinephrine-induced expression of UCP1 [43]. Conversely, both androgens and estrogens have beneficial effects on brown adipocyte formation and function [44]. Hence, in the young, a generally inhibitory effect of glucocorticoids on BAT may be opposed by relatively high levels of these sex hormones. While sex hormone levels decline with age, glucocorticoid levels remain relatively stable [42]. As a consequence of this endocrine switch, brown adipocytes assume a white-adipocyte-like

phenotype due to reduced energy expenditure, a process that may exacerbate age-related obesity. Additionally, it was demonstrated that female rats are better at maintaining their thermogenic capacity with increased age compared to male animals. This effect correlates with the serum levels of sex hormones and the thyroid hormone T3, which could, at least in part, help to explain the sexual dimorphism of the functional decline of BAT with age [45].

Furthermore, the production of inflammatory cytokines increases with age, a process that has also been termed inflamm-aging [46]. For instance, the expression of tumor necrosis factor- α (TNF- α) is elevated in WAT-resident preadipocytes from old rats in comparison to young rats [47]. An increased level of TNF- α is known to reduce brown adipogenic differentiation and promote insulin resistance in BAT. This effect involves the inhibitory hyperphosphorylation of serine residues of insulin receptor substrate 2 (IRS-2) by p38 mitogen-activated protein kinase (MAPK) or alternatively reduced tyrosine phosphorylation by activation of protein-Tyr phosphatase 1B (PTP1B). Moreover, TNF- α induces apoptosis of multilocular brown adipocytes, inhibits the expression of β_3 -adrenergic receptor and UCP1, and decreases the thermogenic response of brown adipocytes [48, 49]. In summary, hormonal changes may exacerbate the loss of function of aged BAT through several mechanisms that include an endocrine switch involving sex hormones and glucocorticoids as well as inflammatory cytokines. They could also contribute to the proliferative defect observed in old classical BAT after cold exposure [27]. The underlying molecular mechanisms of these processes remain to be elucidated in more detail to determine the effects of these signals on stem/progenitor cell function.

Conclusion

The negative effects of age on brown adipocytes are indisputable. We here discuss the hypothesis that impaired regeneration, and thus a defect in brown adipogenic progenitor cells, is an important parameter in this apparent loss of thermogenic capacity. The evidence discussed here suggests that cell autonomous as well as external signals contribute to this process. Senescence may reduce mitochondrial biogenesis which in turn impairs the formation of thermogenic brown adipocytes. Additionally, while there is no change or even an increase in sympathetic tone to peripheral tissues, the ability to sense and respond to sympathetic stimulation may be blunted in BAT progenitors. Finally, changes in external factors such as endocrine signals and the composition of the microenvironment could exert an important inhibitory effect on brown adipogenesis that may further aggravate the deterioration of brown adipogenic precursor cells. Further studies are required to help identify targets to prevent or even reverse the loss of brown adipocytes and thus reduce the aging-related onset of overweight and other metabolic complications.

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Disclosure Statement

The authors declare no conflicts of interest.

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