REVIEW

New insights into the secretory functions of brown adipose tissue

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Abstract

In recent years, an important secretory role of brown adipose tissue (BAT) has emerged, which is consistent, to some extent, with the earlier recognition of the important secretory role of white fat. The so-called brown adipokines or 'batokines' may play an autocrine role, which may either be positive or negative, in the thermogenic function of brown adipocytes. Additionally, there is a growing recognition of the signalling molecules released by brown adipocytes that target sympathetic nerve endings (such as neuregulin-4 and S100b protein), vascular cells (e.g., bone morphogenetic protein-8b), and immune cells (e.g., C-X-C motif chemokine ligand-14) to promote the tissue remodelling associated with the adaptive BAT recruitment in response to thermogenic stimuli. Moreover, existing indications of an endocrine role of BAT are being confirmed through the release of brown adipokines acting on other distant tissues and organs; a recent example is the recognition that BAT-secreted fibroblast growth factor-21 and myostatin target the heart and skeletal muscle, respectively. The application of proteomics technologies is aiding the identification of new members of the brown adipocyte secretome, such as the extracellular matrix or complement system components. In summary, BAT can no longer be considered a mere producer of heat in response to environment or dietary challenges; it is also an active secretory tissue releasing brown adipokines with a relevant local and systemic action. The identification of the major brown adipokines and their roles is highly important for the discovery of novel candidates useful in formulating intervention strategies for metabolic diseases.

Key Words

- brown adipose tissue
- brown adipokine
- batokine
- thermogenesis

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Introduction: the evolving research on the BAT secretome

In recent years, extensive research has been conducted to elucidate the secretory role of brown adipose tissue (BAT), including its endocrine role. In certain ways, the history of this research has followed a parallel and delayed path relative to the research of the white adipose tissue (WAT) secretome. For decades, the biological function of WAT was considered to be restricted to fat storage and the release of metabolic foodstuffs when needed; similarly, BAT has been considered to essentially play a role in energy expenditure to support non-shivering thermogenesis. We know that, in brown adipocytes, there is a regulated uncoupling of the mitochondrial respiratory chain relative to ATP synthesis in order to produce heat, owing to the unique presence of the uncoupling protein-1 (UCP1) in brown adipocyte mitochondria, and this makes the brown adipocytes specialised for the consumption of metabolic foodstuffs

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to produce heat. Currently, we also have a comprehensive understanding of the physiological (mainly sympathetic nervous system-mediated) mechanisms of the regulation of BAT as a heat-producing system with regard to thermic stimuli in the environment, and possibly, to dietary stimuli (Cannon & Nedergaard 2004). Several decades ago, mostly fuelled by the discovery of leptin, WAT had begun to be recognised as a source of regulatory proteins targeting distinct organs and tissues. The term 'adipokine' was adopted to describe the myriad of molecules secreted by white fat that have been described to date (Blüher & Mantzoros 2015). However, the potential secretory role of BAT has been intensely investigated only much more recently. Possibly, the low expression of many 'white' adipokines, such as leptin, in brown fat and the low expression of pro-inflammatory cytokines known to be released by WAT (e.g. tumour necrosis factor-α; TNFα) (Cannon & Nedergaard 2004) may have also contributed to a somewhat historically neglected research on the secretory role of BAT. In principle, a rather distinct pattern of factors secreted by BAT, relative to the case for WAT, is expected; given the distinct (and even opposite) role of BAT (energy expenditure) and WAT (energy storage) in energy metabolism, it is unlikely that their secretomes are similar.

Research on the secretory role of BAT has been driven by several observations: first, the much more intense systemic phenotype in mouse models with genetically targeted ablation of BAT (e.g. UCP-DTA mice) relative to those with just thermogenic impairment of BAT (e.g. UCP1-null mice) (Lowell *et al.* 1993); second, the widespread observations that the experimental transplantation of small amounts of BAT lead to relevant effects on systemic metabolism, mainly the improvement in glucose homeostasis and even reduced weight gain, which are hardly explainable by the intrinsic thermogenic activity of the transplant (Villarroya & Giralt 2015, Villarroya *et al.* 2017*a*); third, the identification of genes encoding secreted proteins as a part of the pattern of expression of genes regulated in the BAT in response to thermogenic stimuli. Hence, research during the recent years has led to an increased interest in identifying BAT-secreted factors, especially those capable of acting on organs at a distance and exerting an endocrine effect.

What are brown adipokines or 'batokines'?

The term 'brown adipokines' (in some cases also called 'batokines') has some imprecisions in its use, which are, in fact, rather similar to those associated with the use of the general term 'adipokine'. In some contexts, 'brown adipokine' is used to refer to proteins (or even other non-peptidic types of regulatory factors) released by brown fat 'tissue', whereas others restrict the use of the term to proteins released by the brown adipocyte 'cell type'. This is a biologically relevant distinction, considering the presence of non-brown adipocyte cells in the BAT (e.g. macrophages and other immune cells, vascular cells, nerve endings) which may be relevant sources of secreted factors. To date, no molecule reported to be secreted by BAT has been found to be totally 'brown specific' in the sense that, for example, UCP1 protein expression is. Hence, existing literature often qualifies brown adipokines as secreted factors 'preferentially' released by BAT-versus-WAT and, in some cases, compared to other tissues or organs. Moreover, in many research reports, there is also the assumption that a brown adipokine is a molecule that is released intensely by the BAT when the tissue is thermogenically activated; this makes sense when the secretory function of BAT is associated with the induction of its physiological role as a heat-producing tissue. There are also some non-consensual uses of the term 'brown adipokine' related to the chemical nature of the released molecule. Whether the recognition of small non-coding RNAs (e.g. miRNA-99b) (Thomou *et al.* 2017) as relevant signalling molecules released by BAT and targeting the liver also justifies the use of the term 'brown adipokines' is unclear; the same applies to the identifications of BAT-secreted factors of lipidic nature (Lynes *et al.* 2017) which are expected to be named 'brown lipokines'. Thus, it is clear that for any description of the factors secreted by BAT that are capable of exerting signalling properties, the term 'brown adipokine' would be of limited value without the addition of their cellular source, their regulation in relation to the thermogenic activity of BAT and, of course, their chemical nature. There are several relatively recent review articles that summarise the state-of-the-art knowledge regarding brown adipokines (Villarroya *et al.* 2017*a*,*b*, Lee *et al.* 2019); however, the intense research in this field and growing recognition of novel factors secreted by BAT has aided the identification of novel BAT-secreted factors and/or expanded our understanding of the targets and functions of some already known batokines. A systematic description of the currently identified brown adipokines and, in general, bioactive factors (including brown lipokines and miRNAs) released by brown and beige adipocytes is provided in Supplementary Table 1 (see section on supplementary data given at the end of this article). Herein, we review the most recent significant

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advances in the knowledge of the BAT secretome and its importance for autocrine, paracrine, and endocrine signalling.

What does proteomics tell us about the BAT secretome and identification of new batokines?

Considering the progress in proteomics technology, the direct assessment of the proteins released by brown adipocytes appears as an obvious analytical approach to obtain a thorough knowledge of the brown adipocyte secretome. However, only two recent studies utilising this strategy have been published (Ali Khan *et al.* 2018, Villarroya *et al.* 2019), and these report the identification of the set of protein factors whose expression is significantly induced in the culture medium of murine brown adipocytes acutely exposed to cAMP (71 proteins) or to noradrenaline (280 proteins), which are similar, but not identical, thermogenic stimuli for the brown fat cell. These data were remarkably coincident, and 60% (42 of 71) of the proteins found in the study by Villarroya *et al.* (2019) were also identified in the study by Ali Khan *et al.* (2018). Some of these factors were adipokines that have already been known to be secreted by brown adipocytes, such as adiponectin (Hui *et al.* 2015), angiotensinogen (Campbell & Habener 1987), and chemerin (Hansen *et al.* 2014). Other previously identified brown adipokines such as fibroblast growth factor-21 (FGF21), neuregulin-4 (NRG4) or bone morphogenetic protein-8b (BMP8b) (Villarroya *et al.* 2017*a*) were not found in either of the two studies, and this may be related to the low concentration ranges of these factors and potential limitations of the proteomics technology.

Interestingly, many members of the brown adipocyte secretome commonly identified in the two aforementioned studies were extracellular matrix (ECM) components or regulators (matricellular proteins). Alterations in the formation of a functional ECM have been reported in WAT-related pathological conditions such as obesity. Physiological WAT expansion requires ECM remodelling; reaching a threshold in such capacity when the WAT is maximally overloaded with fat is considered a significant trigger of obesity-associated pathologies (Crewe *et al.* 2017, Datta *et al.* 2018). BAT is a highly plastic tissue, and its long-term thermogenic activation leads to hypertrophic and hyperplastic processes, ultimately resulting in adaptive BAT recruitment (Cannon & Nedergaard 2004). The identification of ECM components (distinct types of collagens) and matricellular proteins (non-structural proteins present in the ECM and having a regulatory role) as members of the secretome of thermogenically activated brown adipocytes possibly highlights the importance of BAT remodelling when the tissue has to be expanded in response to thermogenic activation. The role of the ECM in BAT has been practically unexplored to date; the recent findings based on proteomics analyses clearly indicate the requirement for further research to explore this novel biological process related to the BAT secretome. Hansen (2014), who analysed the mouse brown adipocyte proteome indirectly through a sequence trap genetic analysis, also reported the identification of several ECM components and matricellular proteins such as epidermal growth factor-containing fibulin-like extracellular matrix protein (EFEMP1), collagen α-1 (III) chain (COL3A1), procollagen C-proteinase enhancer protein (PCOLCE) or secreted acidic cysteine-rich glycoprotein (SPARC) as a part of the brown adipocyte secretome in response to noradrenaline.

Proteomics studies have also coincidently identified several components of the complement system in the secretomes of cAMP- and noradrenaline-stimulated brown adipocytes. This was a surprising finding. In addition to complement factor-D (also called adipsin), in fact, one of the first white adipokines to be identified (Cook *et al.* 1987), other factors such as complement C4-B, complement factor-B, and complement C3 have been reported to be a part of the brown adipocyte secretome according to the studies by both Villarroya *et al.* (2019) and Ali Kahn *et al.* (2018). Further research is warranted to ascertain the role of complement system components in the signalling that originates in brown adipocytes upon thermogenic activation.

Finally, it is worth mentioning that if we compile the data obtained from the two direct proteomics analyses of mouse samples and the indirect sequence trap-based analysis, only the following five proteins are commonly found as thermogenic stimulus-induced batokines: two components of the ECM (COL3A1 and PCOLCE), insulin-like growth factor-binding protein-4 (IGFBP4), follistatin-like protein-1 (FSTL1), and chemerin (also named Rarres2, from 'retinoic acid receptor responder-2') (Fig. 1). These factors warrant further investigation as potential batokines, because to date, only chemerin has been specifically studied as an adipokine released by brown adipocytes (Hansen *et al.* 2014).

Recently, Deshmukh *et al.* (2018) have reported a proteomics-based analysis of the brown adipocyte

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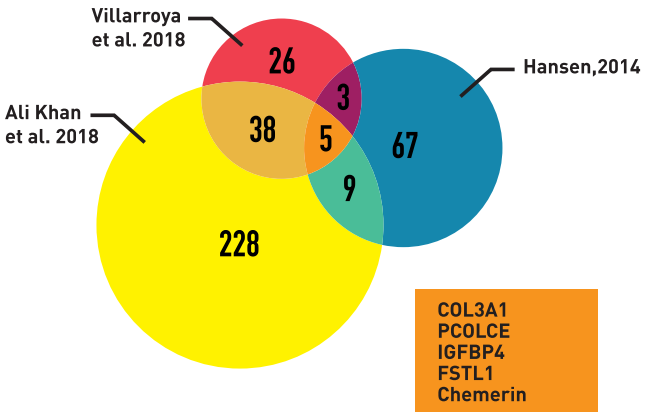

Figure 1
Venn diagram showing the overlap between the proteins secreted by brown adipocytes in culture in response to cAMP (Villarroya *et al.* 2018*a*,*b*) or noradrenaline (Ali Khan *et al.* 2018), based on proteomics analysis, and in response to noradrenaline, based on the signal sequence trap technique (Hansen 2014). The numbers of secreted proteins found in the different studies are shown in the intersections. The box indicates the five proteins secreted by thermogenically stimulated brown adipocytes that were commonly identified in these three studies: collagen α-1 (III) chain (COL3A1), procollagen C-proteinase enhancer protein (PCOLCE), insulin-like growth factor binding protein-4 (IGFBP4), follistatin-like protein-1 (FSTL1), and chemerin.

proteome from human brown adipocytes in response to noradrenaline. ECM components, complement factors and several other proteins reported to be a part of the thermogenically stimulated mouse brown adipocyte secretome were also found in the noradrenaline-stimulated human brown adipocyte secretome (e.g., fibrillin-1, SPARC-like protein 1, progranulin, and transforming growth factor-β receptor-3). These authors also propose that ependymin-related protein 1 (EPDR1) may be a novel human brown adipokine acting on the brown fat activity itself and influencing the overall energy expenditure.

The emerging BAT-to-heart signalling based on FGF21

FGF21 is among the first proposed endocrine signals acting as a batokine, given the strong release of FGF21 by BAT under conditions of thermogenic activation (Hondares *et al.* 2011). Accordingly, tissues and organs known to be sensitive to FGF21 action (e.g. WAT, brain, pancreas) have been considered as potential targets of the FGF21 secreted from the BAT. The heart is one of these potential target tissues, given the reports indicating a strong cardioprotective effect of FGF21 (Planavila *et al.* 2013). However, direct evidence for this was lacking until the study by Ruan and his collaborators, who concluded, as a consequence of a study on the adenosine A2A receptor in BAT, that the FGF21 released by BAT targets the heart (Ruan *et al.* 2018). Brown adipocyte-specific FGF21 knockout impaired the previously observed effects of adenosine A2A receptor agonism in attenuating hypertensive cardiac remodelling. These findings identify an endocrine role of BAT in controlling hypertensive cardiac remodelling though the release of FGF21. However, Thoonen *et al.* (2015) have reported that UCP1-null mice show exaggerated myocardial injury, fibrosis, and adverse cardiac remodelling, as well as decreased survival, in response to experimental cardiac insults. Transplantation of functional BAT reversed the myocardial injury and increased the survival of mice, suggesting a systemic cardioprotective role of functional BAT. However, it is well known that BAT from UCP-1-null mice expresses much higher levels of FGF21 than wild-type mice and is thus a significant source of circulating FGF21 (Keipert *et al.* 2015), suggesting that factors other than FGF21 may contribute to the protective actions of BAT on the heart.

Notably, several studies reporting heart alterations, including signs of diabetic cardiomyopathy, in UCP-DTA mice have been published (Duncan *et al.* 2007, Ilkun *et al.* 2015). The UCP-DTA mouse model is based on the transgenic expression of the diphtheria toxin A chain (DTA) under the control of the UCP1 gene promoter. These mice have genetically ablated BAT and are prone to diet-induced obesity due to impaired energy expenditure (Lowell *et al.* 1993). Cardiac alterations in UCP-DTA mice have been interpreted as a result of the metabolic abnormalities in this mouse model associated with diet-induced obesity. However, the current awareness of the secretory role of BAT suggest that an additional explanation based on the impairment of the release of cardioprotective batokines (FGF21 or others) due to BAT ablation may account for cardiac alterations in the UCP-DTA mice.

Myostatin and BAT-to-muscle signalling

A relevant recent breakthrough in the identification of BAT-originated signalling to other tissues, that is, the endocrine role of BAT, is the recent identification of myostatin as a batokine that targets skeletal muscle. While several batokines have been reported to have the theoretical potential to target muscle, until recently no clear BAT-to-skeletal muscle signalling had been unequivocally identified. As a result of a recent study

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using experimental models of the targeted invalidation of the transcription factor IRF4 in BAT, Rosen and his collaborators (Kong *et al.* 2018) have identified skeletal muscle as an affected target and identified myostatin as a BAT-released factor mediating the BAT-to-muscle signalling.

Previous studies had already identified myostatin as a factor reciprocally associated with the extent of the thermogenic stimulus of BAT and the browning of WAT (Braga *et al.* 2013, Shan *et al.* 2013), and a negative autocrine role of BAT activity had been proposed (Steculorum *et al.* 2016). Myostatin is a potent negative regulator of skeletal muscle growth (Rodriguez *et al.* 2014). Kong *et al.* have found that, under conditions of BAT inactivation such as treatment with thermoneutral temperature, myostatin levels were increased and the exercise capacity of skeletal muscle was lowered. In contrast, experimental activation of BAT lowered the myostatin levels and favoured exercise performance. Surgical BAT ablation blunted these effects on the muscles. In summary, BAT appears to control skeletal muscle function through the secretion of myostatin.

Immune cells, novel targets of batokines: the chemokine C-X-C motif chemokine ligand-14 (CXCL14) as a brown adipokine, and beyond

In recent years, the importance of immune cells infiltrating brown and beige adipose tissues for their recruitment and activation in response to thermogenic requirements has been reported (Villarroya *et al.* 2018*a*,*b*). Multiple reports have indicated that the recruitment of alternatively activated macrophages to BAT and beige adipose tissue is positively associated with thermogenic activation; however, the mechanisms by which macrophages intervene in BAT and/or beige adipose tissue activation remain controversial. The chemokine CXCL14 has been identified as a brown adipokine that is released by brown adipocytes in response to noradrenergic stimulation and leads to the alternative activation and recruitment of macrophages (Cereijo *et al.* 2018). Moreover, the CXCL14 released by BAT appears to influence the recruitment of M2 macrophages to subcutaneous WAT, thus promoting browning. This role of CXCL14 highlights the capacity of batokines to target immune cells. In fact, it is also known that increased pro-inflammatory status of BAT and beige adipose tissues (including the recruitment of pro-inflammatory immune cells) is negatively associated with their thermogenic activity. There are indications that factors secreted by thermogenically active brown adipocytes are capable of targeting pro-inflammatory cells and limit local inflammation. It has been reported that brown adipocytes are able to dampen the inflammatory responses in macrophages (Dowal *et al.* 2017). We have confirmed that the conditioned medium from brown adipocyte cultures represses the pro-inflammatory activity of M1-activated macrophages (Cereijo *et al.* 2018, Campderros *et al.* 2019). However, identifying the molecular actors involved in the signalling from brown/beige adipocyte to immune cells has unveiled a complex scenario. While CXCL14 accounts for the recruitment and activation of M2 macrophages, it does not appear to directly influence pro-inflammatory signalling in M1 polarised macrophages. We recently found that growth-and-differentiation factor-15 (GDF15), however, is secreted by brown adipocytes and exerts anti-inflammatory effects on M1 macrophages. GDF15 is a member of the TGFβ family that is widely recognised as a systemic marker of multiple pathologies, from cardiovascular disease to cancer. Recently, GDF15 has been reported to exert anorexigenic effects through its action at the brainstem and to contribute towards controlling the energy balance (Breit *et al.* 2017, Tsai *et al.* 2018). GDF15 is intensely secreted by brown fat upon exposure to cold conditions and by brown adipocytes after noradrenergic stimulation (Verdeguer *et al.* 2015, Campderros *et al.* 2019) and exerts mostly autocrine effects on macrophages, tuning down their pro-inflammatory responses (Campderros *et al.* 2019). Whether these signalling capacities of brown adipokines towards immune cells are restricted to the local cross-talk between immune cells and adipocytes at adipose depots or have consequences on the systemic immune status of the organism are challenging possibilities that remain to be ascertained in further researches.

Intriguingly, for many years, the cytokine interleukin-6 (IL-6) has been known to be secreted by BAT in response to thermogenic stimuli (e.g. cold environment) and by brown adipocytes in response to noradrenaline (Burýsek & Houstek 1997), and is in fact, one of the molecules considered to be a potential batokine. The importance of the IL-6 secreted by BAT has been evidenced by studies that showed that BAT explants from IL-6-KO mice (devoid of the capacity to secrete IL-6) do not show the capacity of wild-type BAT explants to promote a healthy metabolic response in models of experimental transplantation in mice (Stanford *et al.* 2013). Recently, the secretion of IL-6 by beige adipocytes has been reported to have positive autocrine actions upon the browning process, at least *in vitro* (Kristóf *et al.* 2019). Although it has not been

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directly addressed, it is possible that the reported effects of IL-6-induced M2 macrophages activation (Mauer *et al.* 2014) may play a role in the positive effects of BAT-secreted IL-6. The recognition of a differential role of IL-6 in the past few years, whereby it acts as a cytokine or as a myokine (Pal *et al.* 2014), is suggestive of the necessity to further explore the physiological role of IL-6 when it is specifically released by the BAT.

BMP8b, a key brown adipokine for the adaptive remodelling of brown and beige adipose tissues to thermogenic demands

In 2012, BMP8b, a member of the Bmp protein family, was identified as a brown adipokine released by brown adipocytes in response to noradrenergically mediated thermogenic stimulus (Whittle *et al.* 2012). Further studies have confirmed that *Bmp8b* is among the most intensely upregulated genes in murine brown adipocytes exposed to thermogenic stimuli (Verdeguer *et al.* 2015, Quesada-López *et al.* 2016). In contrast with other members of the Bmp family, the role of BMP8b in brown adipocyte biology is not associated with the early stages of adipogenic differentiation but with the secretory activity of fully differentiated brown adipocytes. Whittle and his collaborators (2012) found that BMP8b sensitises the BAT to sympathetically mediated thermogenic activation of BAT and WAT browning. *Bmp8b* gene expression in brown adipocytes is responsive not only to noradrenergic actions, but also to non-sympathetic activators of BAT activity such as G-protein-coupled receptor 120-mediated polyunsaturated fatty acid signalling (Quesada-López *et al.* 2016) or oestrogens (Grefhorst *et al.* 2015). Recent research has clarified some of the actions of the BMP8b secreted by brown adipocytes and its important autocrine and paracrine roles. Pellegrinelli *et al.* (2018) have found that BMP8b indirectly promotes sympathetic innervation and the vascularisation of brown and beige adipose tissues when the thermogenic capacity of the tissues has to be increased. BMP8b induces the endogenous production of NRG4, another known brown adipokine (Rosell *et al.* 2014, Wang *et al.* 2014), that promotes the outgrowth and branching of sympathetic nerve axons. Moreover, the release of BMP8b enhances the vascularisation of the brown fat depot by the induction of angiogenic factors. Therefore, BMP8b appears to be a brown adipocyte-secreted factor that promotes active brown and beige adipose tissue remodelling to increase thermogenic efficiency by directly and indirectly targeting innervation and vascularisation, in a manner reminiscent of that by which brown adipocyte-secreted CXCL14 targets immune cells. Moreover, in addition to paracrine actions, it has been shown that BMP8b acts centrally and, via AMPK inhibition in the ventromedial hypothalamus and the subsequent increase in orexin signalling, leads to the sympathetically mediated thermogenic activation of BAT and WAT (Whittle *et al.* 2012, Martins *et al.* 2016). Whether the BMP8b that exerts these central actions originates in the BAT or is locally produced is currently unknown.

The identification of an increasing number of brown adipokines targeting the sympathetic nerve endings at the BAT

The extent of innervation of sympathetic nerve endings is essential for the functionality and activity of BAT depots. The capacity of secreted factors from brown adipocytes to target nerve endings and promote their outgrowth as a part of the brown fat recruitment process are among the first recognised biological roles of the components of the brown adipocyte secretome. In 1994, M Nechad and her collaborators had reported that co-cultures of BAT explants with sympathetic ganglia induced neurite outgrowth by producing a neurotrophic factor (Néchad *et al.* 1994), which was identified to be nerve growth factor (NGF); this effect was found to be more intense in case of BAT explants obtained from thermogenically stimulated rodent models (newborn hamsters, cold-exposed rats). This role of NGF, as a batokine targeting sympathetic neurite outgrowth was further confirmed (Nisoli *et al.* 1996); however, in this report, an inverse correlation between the NGF release and the extent of thermogenic BAT activation was found.

Later, Rosell and her collaborators identified NRG4 as a neurite outgrowth-promoting brown adipokine, preferentially expressed in thermogenically stimulated brown adipocytes (Rosell *et al.* 2014). As mentioned earlier, NRG4 has recently been found to respond to the autocrine effects of brown adipocyte-secreted BMP8b. Notably, B M Spiegelman's team has recently identified a new brown adipocyte-secreted factor: the S100b protein, which promoted neurite outgrowth from sympathetic neurons at adipose depots (Zeng *et al.* 2019). S100b does not contain a signalling peptide corresponding to standard secretion pathway; this may have hampered its identification as a batokine in previous studies. Although Zeng *et al.* have not reported the effects of experimental thermogenic stimuli (cold, noradrenaline) on S100b secretion, examination of

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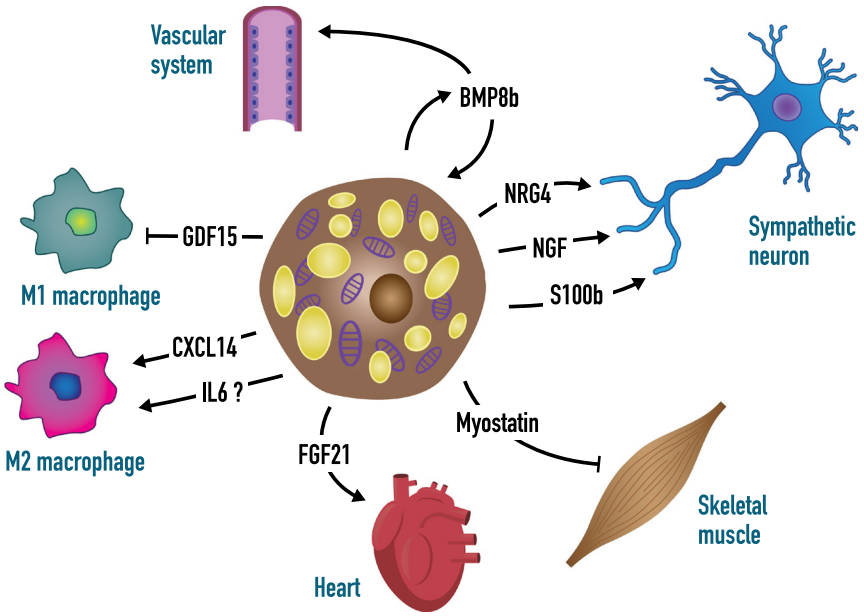

Figure 2
Representation of novel brown adipokines and their tissue targets. Bone morphogenetic protein-8b (BMP8b) targets vascular cells and, through its autocrine action on brown adipocytes, leads to the release of neuregulin-4 (NRG4) which promotes sympathetic neurite outgrowth. Nerve growth factor (NGF) and S100b protein are also secreted by brown adipocytes and target sympathetic nervous endings promoting innervation. C-X-C motif chemokine-14 (CXCL14) is a chemokine released by brown adipocytes; it promotes the recruitment of M2 macrophages, whereas brown adipocyte-secreted growth-and-differentiation factor-15 (GDF15) inhibits the pro-inflammatory activity of M1 macrophages. The BAT-released fibroblast growth factor-21 (FGF21) targets the heart, favouring cardioprotective effects, whereas the level of BAT-secreted myostatin controls the performance of skeletal muscles.

currently available transcriptomics databases (e.g. GEO GDS4850) indicate that acute cold exposure induces the expression of the S100b transcript in the BAT in mice; this is consistent with its role as a thermogenically induced batokine.

In summary, it appears that the secretome of thermogenically active brown adipocytes plays a key role in promoting the expansion of sympathetic nerve endings as a part of the recruitment of active BAT in response to environmental stimuli. NGF, NRG4, and S100b appear to be relevant components of the brown adipocyte secretome that mediate this action; however, we do not know whether there are other similar molecules, which are yet to be identified.

Conclusions

BAT is a secretory tissue producing brown adipokines, which exert autocrine, paracrine, and endocrine actions. In recent years, novel brown adipocyte-derived signalling molecules that have local actions and target vascular cells (e.g. BMP8b), macrophages (CXCL14, GDF15), and sympathetic nerve endings (NRG4, S100b), and contribute to the adaptive remodelling of BAT in response to thermogenic stimuli, have been identified. The capacity of BAT to serve as a source of endocrine factors has been recently reinforced by the recognition that BAT-released FGF21 targets the heart to exert cardioprotective effects. Moreover, BAT-secreted myostatin signals skeletal muscles to negatively regulate muscle performance (Fig. 2). Recent proteomics-based analyses have unveiled novel components of the brown adipocyte secretome, such as members of the complement system and ECM components.

The extent of BAT activity, even in humans in which the BAT size is small, is associated with a healthy systemic metabolic profile. In addition to its intrinsic glucose- and lipid-oxidising capacity, the secretory role of BAT and its modulation according to its thermogenic activity may be related to this association. Further research is warranted into this aspect to comprehensively understand the BAT secretome, and the identity of brown adipokines and their targets and actions. The identification of brown adipokines and the characterisation of their effects is expected to enhance our knowledge regarding potential tools and targets useful in formulating intervention strategies for complex metabolic diseases such as obesity, diabetes, and cardiovascular diseases.

Supplementary data
This is linked to the online version of the paper at https://doi.org/10.1530/JOE-19-0295.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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