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ORIGINAL ARTICLE

Impact of adipokines and myokines on fat browning

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Abstract

Since the discovery of leptin in 1994, the adipose tissue (AT) is not just considered a passive fat storage organ but also an extremely active secretory and endocrine organ that secretes a large variety of hormones, called adipokines, involved in energy metabolism. Adipokines may not only contribute to AT dysfunction and obesity, but also in fat browning, a process that induces a phenotypic switch from energy-storing white adipocytes to thermogenic brown fat–like cells. The fat browning process and, consequently, thermogenesis can also be stimulated by physical exercise. Contracting skeletal muscle is a metabolically active tissue that participates in several endocrine functions through the production of bioactive factors, collectively termed myokines, proposed as the mediators of physical activity–induced health benefits. Myokines affect muscle mass, have profound effects on glucose and lipid metabolism, and promote browning and thermogenesis of white AT in an endocrine and/or paracrine manner. The present review focuses on the role of different myokines and adipokines in the regulation of fat browning, as well as in the potential cross-talk between AT and skeletal muscle, in order to control body weight, energy expenditure and thermogenesis.

Keywords Leptin · Fat browning · Adipokine · Myokine

Abbreviations

AgRP	Agouti gene–related protein
ARC	Arcuate nucleus
AT	Adipose tissue
BAT	Brown adipose tissue
CART	Cocaine-amphetamine-regulated-transcript
CK	Cytokine
CNS	Central nervous system
MSC	Mesenchymal stem cells
MSTN	Myostatin
NPY	Neuropeptide Y
PDGFR-α	Platelet-derived growth factor receptor α
Pgc-1α	Peroxisome proliferator–activated receptor γ coactivator-1 α
PKA	Protein kinase A
POMC	Proopiomelanocortin
Ppar-γ	Peroxisome proliferator–activated receptor-γ
PRDM16	PR domain-containing 16

Key points
- Myokines are secreted and regulate physiological processes in an endocrine manner.
- The adipose tissue releases adipokines involved in the energy homeostasis.
- Myokines regulate fat browning with their activity being modulated by adipokines.

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PVN	Paraventricular nucleus
Tnf-α	Tumour necrosis factor-α
Ucp-1	Uncoupling protein 1
VTA	Ventral tegmental area
WAT	White adipose tissue

Introduction

Obesity spreads across the globe and has become an international public health issue, affecting the quality of life and being associated with many chronic diseases and early mortality [47, 136]. This complex chronic disease has a multifactorial aetiology, including genetic, epigenetic, physiological, behavioural, sociocultural and environmental factors, leading to an imbalance between energy intake and expenditure during an extended period [49, 91].

The metabolic health benefits of physical activity and exercise are well-described [131, 148]. A sedentary lifestyle or periods of low-intensity physical activity result in excess abdominal and visceral adiposity, which are associated with a higher risk of type 2 diabetes, impaired lipid metabolism and loss of muscle mass. By contrast, greater physical activity is associated with a lower risk of developing several chronic diseases and promotes multiple beneficial health effects [33]. The mechanisms underlying exercise-induced changes are complex to analyse since exercise is an intricate process, simultaneously involving integrative and adaptive responses in multiple tissues and organs at both cellular and systemic levels. Nevertheless, the energy expended in physical activity is often insufficient to counterbalance the excessive caloric consumption. Exercise results in multiple changes in several organs, including effects on the cardiovascular system, skeletal muscle, adipose tissue (AT) and bone.

The skeletal muscle is a large, highly plastic and adaptive organ that is critical to maintaining whole-body insulin sensitivity and metabolic homeostasis. It exhibits notable metabolic adaptations to exercise, including mitochondrial biogenesis, angiogenesis and improved substrate metabolism, but the mechanisms are still unclear [54]. During the last decade, it has been suggested that metabolic benefits of physical activity could be mediated by cytokines or cell-signalling proteins secreted in response to exercise that can regulate in an autocrine, paracrine and endocrine manner the function of muscle and other organs [103]. In this line, the skeletal muscle has emerged as an extremely active endocrine organ that secretes a huge variety of cytokines, chemokines, growth factors, hormones and vasoactive factors, collectively termed myokines, proposed as the mediators of physical activity–induced health benefits. Myostatin (MSTN, also known as growth differentiation factor 8) was the first secreted factor to accomplish the criteria of a myokine, being recognised as an endogenous inhibitor of muscle growth. *Mstn*-null mice exhibit a hypertrophic and hyperplastic skeletal muscle together with a suppression of body fat accumulation, suggesting an important crosstalk between the skeletal muscle and fat depots [12, 90]. In this regard, several studies demonstrate that endurance training, in addition to prevent insulin resistance in humans, can affect both muscle and AT mass in normal and obese animals and humans [126].

Different proteomics studies focused on the secretome of the skeletal muscle have identified numerous myokines with pleiotropic effects exerting their actions within the muscle itself or through systemic effects, including the liver, the AT and the immune system [99, 106]. In this sense, many myokines are known to be important endocrine mediators in the field of metabolic homeostasis through actions on AT.

Since the identification of leptin in 1994, the AT is not just considered a passive organ with functions including energy storage, heat insulation and mechanical protection. To date, AT is considered a highly dynamic endocrine organ that produces and releases bioactive factors, collectively known as adipokines, involved in the regulation of many physiological functions, including energy metabolism [19, 57, 58].

Fat browning

AT is considered an active participant in controlling the physiological and pathological processes associated to obesity [58]. AT has been traditionally subclassified into white AT (WAT) and brown AT (BAT), with clearly differentiated functions: WAT stores extra energy in the form of triglycerides, whereas BAT plays an important role in thermoregulatory heat production (nonshivering thermogenesis) and in diet-induced thermogenesis through the activation of the mitochondrial uncoupling protein UCP-1, which uncouples ATP production dissipating energy as heat [46]. The adipokines produced by WAT are involved in the regulation of many physiological functions, including fat browning, a process that induces a phenotypic switch from energy-storing white adipocytes to thermogenic brown fat–like cells. Both ATs present significant transcriptional, secretory, morphological and metabolic differences [1, 40, 41, 81]. The existence of brown fat–like cells that emerge within white fat pads, being designated as brite (corresponding to the contraction of "brown in white") or beige cells, has been reported [149].

WAT is predominantly composed of white adipocytes (35–70%), although other cell types in the stroma-vascular fraction including immune, endothelial and smooth muscle cells among others are found [31]. Triglycerides, the major cellular constituent of the adipocytes, are stored in a large unilocular lipid droplet, occupying 80–90% of the cell volume and

conferring them an ivory or yellow colour [48]. The nucleus and the thin cytoplasmic rim are displaced to the periphery of the adipocyte near the plasma membrane. White adipocytes are found in subcutaneous, abdominal, retroperitoneal, inguinal and gonadal fat depots [115, 133]. Brown fat cells, as mentioned above, also store energy in the form of triglyceride (TG), but in multiple small multilocular lipid droplets. The cytoplasm contains a central nucleus as well as large spherical mitochondria packed with laminar cristae, conferring the characteristic brown colour to the tissue. BAT is characterised by a highly sympathetic innervation and an extensive vascularisation [26]. In this regard, the plasma membrane protein calsyntenin 3β (CLSTN3β), highly expressed in thermogenic adipocytes, has a key role in the functional sympathetic innervation [153]. For many years, BAT was considered physiologically important only in small animals and newborns, allowing the adaptation to a cold environment by adaptive thermogenesis. Nonetheless, studies with positron-emission tomography integrated with computed tomography (^{18}F-FDG PET/CT) "rediscovered" the presence of functional and metabolically active BAT in different anatomical locations of human adults, particularly in the neck and supraclavicular regions [97, 123, 138, 143]. The activity and prevalence of BAT are inversely correlated with body mass index and white fat mass, evidencing an inverse relationship between its activity and obesity [138, 140]. These findings have revitalised the research on brown fat and the attempts to use it as a potential therapy against obesity [46].

The brown-like adipocytes discovered in WAT resemble white fat cells in morphology and gene expression patterns during basal states, but assume an intermediate appearance upon prolonged stimuli such as cold exposure, β-adrenergic stimulation or peroxisome proliferator–activated receptor (PPAR)-γ agonist treatment [102]. Furthermore, the thigh AT (tAT) has been recently identified as a naturally-existing beige adipose depot that, unlike WATs, conserves beige fat morphology even at room temperature and expresses key genes that promote energy expenditure in a higher extent than inguinal WAT [22]. The clusters of beige adipocytes exhibit multilocular lipid droplets surrounding large ones, being transformed (in a process called "browning" or "britening") in multilocular adipocytes with high mitochondrial content and a comparative thermogenic potential to brown fat cells, including UCP1, cell death–inducing DNA fragmentation factor alpha-like effector A (CIDEA) or PPAR-γ coactivator 1-α (PGC-1α). Brown fat cells express high levels of UCP1 under basal (unstimulated) conditions, whereas beige adipocytes maintain their thermogenic programme only in response to a constant stimulation [104]. Despite sharing the functional feature to undergo thermogenesis, many differences between brown and beige adipocytes exist that have to be considered, including their cellular origin.

Cellular origin

Both white and brown fat cells are derived from mesenchymal stem cells (MSC), although it is supposed they originate from different precursor cells [112]. The myogenic regulatory factor Myf5 plays a key role in the differentiation since MSC can be committed to either a myogenic lineage (Myf5-positive cells) or an adipogenic lineage (Myf5-negative cells). Both brown adipocytes and myocytes arise from MSC of the paraxial mesoderm that expresses the myogenic transcription factor Myf5, exhibiting a muscle-like gene signature [2, 128]. The transcription factor PR domain-containing 16 (PRDM16), necessary to promote BAT adipogenesis, selectively controls the bidirectional cell fate switch between myoblasts and brown fat cells. In this context, PRDM16 deficiency in cultured brown adipocytes inhibits brown adipogenesis and increases the expression of muscle genes [3, 128].

Although beige adipocytes arising in WAT share many of the morphological and functional features of brown fat cells, they are ontogenically different from those of the classic interscapular BAT [104, 129]. The precise origin of the distinct cells found in WAT is still a topic of intense discussion. Himms-Hagen et al. showed that multilocular mitochondria-rich adipocytes appear in WAT without dividing [62]. Cinti et al. observed beige adipocytes appearing as clusters of multilocular cells in WAT in response to cold or β-adrenergic agonists, revealing that under certain conditions, white adipocytes can transdifferentiate into brown adipocytes, evidencing the plasticity of the adipose organ [124]. In fact, in vivo lineage-tracing studies in transgenic mice confirmed the concept of transdifferentiation, supporting the idea that the thermogenic profile of beige adipocytes is reversible: beige adipocytes may lose UCP1 expression and gain white-adipocyte-specific gene expression profiles after a period of warm adaptation [118]. Wang and Scherer, using fate-mapping analyses in mice, showed that most beige adipocytes arise from adipogenic precursor cells in white fat pads through de novo differentiation rather than from pre-existing adipocytes [146]. Furthermore, lineage-tracing experiments performed by Lee et al. provided evidence that the induced brown adipocytes in WAT are derived from a population of cells that express platelet-derived growth factor receptor α (PDGFRα). This precursor can differentiate into either beige or white adipocytes depending on the environmental demand with β-adrenergic activators stimulating beige adipocyte development whereas high-fat feeding causing differentiation into white adipocytes. These bipotential adipocyte progenitors are morphologically flexible and might transdifferentiate when challenged with whitening or browning stimuli [129]. PDGFRβ has also been reported to mark beige mouse adipocyte progenitors [144]. In this line, the progenitor pool with dominant PDGFRα expression

generates beige adipocytes, whereas white adipocytes raises from the progenitor pool with dominant PDGFRβ expression [52]. Noteworthy, recently, glycolytic beige fat cells have been identified by Kajumura et al. [25]. Thermal stress induces progenitor cell plasticity, and the new called glycolytic beige adipocytes are formed via a myogenic state through GA-binding protein α as a transcriptional regulator. The capacity of browning varies depending on the WAT depot, with subcutaneous and perirenal WAT being more prone for the formation of brite adipocytes than epididymal/perigonadal WAT [130]. Strikingly, at least a subset of beige cells arises from a smooth muscle–like origin [86].

Transcriptional regulation of browning

The general programme of white and brown fat cell differentiation shares molecular components involved in their development and/or function including the transcription factor PPARγ or members of the CCAAT/enhancer-binding protein (C/EBP) transcription family. However, numerous signalling pathways involved in the emergence of brown-like adipocytes in WAT exist. The master regulator of adipocyte differentiation PPARγ is intimately involved in the regulation of brown adipocyte–selective characteristics of adipocytes. The recruitment of the PGC-1α/PPARγ complex is implicated in brown-like-induced adipocytes and mitochondrial biogenesis in WAT through agonists of PPARγ and the NAD-dependent deacetylase sirtuin 1 (SIRT1) [100]. Deacetylation of PPARγ is required to stabilise and recruit the coactivator PRDM16, leading to induction of the brown fat transcriptional programme through interactions with the mediator subunit (MED)-1 [60, 65, 109]. Furthermore, PRDM16 is required to supress the expression of many white fat genes and in the β-adrenergic-induced browning of subcutaneous WAT [130]. Recent studies have identified Krüppel-like factor 11 (KLF11) as another important mediator for the activation and maintenance of the brite selective gene programme [85]. Tumour growth factor β (TGF-β) together with different members of the bone morphogenetic protein (BMP) family, part of the TGF-β superfamily, are also involved in the induction of a brown adipocyte–like cell structure and function, such as BMP4 [108], BMP7 [9, 101] and BMP8b [87, 147]. Nuclear retinoid receptors [72], forkhead box C2 (FOXC2) [20, 51] and C/EBP family of transcription factors [50, 69] are also implicated in reprogramming white adipocytes towards brown fat differentiation.

Besides the transcriptional regulation, the endocrine and locally secreted factors originated from different organs in the periphery are also important regulators of fat browning, including myokines and adipokines. This review summarises the potential positive effects of exercise-induced myokines, as well as adipokines, on fat browning.

Myokines and fat browning

Regular physical activity or exercise training is associated with health benefits, including the improvement of cardiovascular health, type 2 diabetes, obesity and metabolic diseases [116, 152]. The fat browning process and, consequently, thermogenesis can be stimulated by physical exercise, and the potential underlying mechanisms remain incompletely understood. Physical exercise may trigger the browning process through actions on the central nervous system (CNS) [5]. Nevertheless, physical activity might also regulate fat browning through myokines, important mediators of the health benefits of exercise. Myokines may counteract the harmful effects of pro-inflammatory adipokines secreted by AT during physical inactivity, related to the chronic low-grade inflammatory condition associated with obesity, and maintain the whole-body homeostasis [103]. In this context, it is necessary to emphasise that intense resistance exercise is also associated with a transient inflammatory response and the release of pro-inflammatory myokines such as IL-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) or tumour necrosis factor-α (TNF-α). This post-exercise inflammatory response following acute resistance exercise represents a protective response to a cellular disturbance or stress, enhancing processes in muscle regeneration [15, 139]. Furthermore, endurance training further stimulates the expression of PGC-1α, a coactivator of PPAR-γ that not only modulates the expression of UCP1 and thermogenesis in BAT but also controls mitochondrial biogenesis and oxidative metabolism in many cell types, promoting multiple beneficial effects on health [107, 131]. Recently, screening of muscle cells overexpressing PGC-1α has led to the identification of PGC-1α-dependent myokines involved in promoting beige fat thermogenesis, including irisin, β-aminoisobutyric acid (BAIBA), myostatin and FGF-21 [11, 35, 52, 144] (Fig. 1).

Irisin

Irisin is a PGC-1α-dependent myokine that is cleaved from its cellular form, fibronectin type III domain-containing protein 5 (FNDC5), a transmembrane precursor protein expressed in rodent and human skeletal muscle [11]. Irisin from mice and humans is 100% identical, suggesting a highly conserved function. Irisin was identified as a myokine secreted into the circulation following physical exercise, capable of stimulating adipocyte browning of WAT and thermogenesis in both culture and in vivo [11]. The stimulation of primary subcutaneous white adipocytes during differentiation with irisin increases oxygen consumption together with an important induction of *Ucp1* mRNA and other brown fat–related genes, activating a broad programme of brown fat–like development [11]. After irisin administration in vivo through adenoviral vectors expressing full-length FNDC5, an increase in *Ucp1* gene

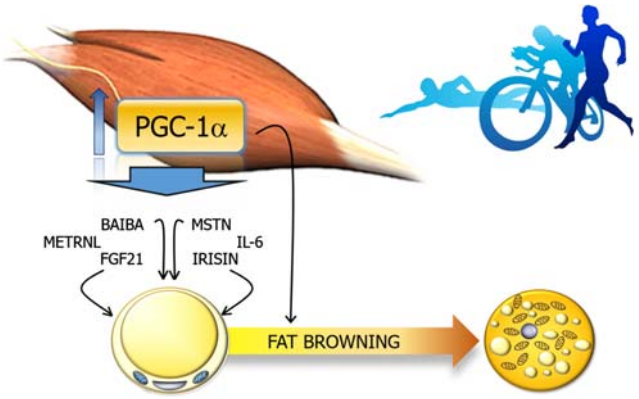

Fig. 1 Physical activity regulates fat browning through the expression of myokines, important mediators of the health benefits of exercise. The principal signalling pathway activated in a contracting muscle during physical exercise converges on PGC-1α by modulating *PGC1a* gene expression and/or post-translational modifications of the PGC-1α protein. As a consequence, PGC-1α coordinates the transcriptional network that stimulates mitochondrial biogenesis, angiogenesis and fibre type switch, and regulates different metabolic gene programmes [11]. In this line, PGC-1α stimulates the expression of several myokines involved in WAT browning, providing a new basis to understand the molecular mechanisms underlying the beneficial effects of exercise training. BAIBA, β-aminoisobutyric acid; FGF21, fibroblast growth factor 21; IL, interleukin; METRNL, meteorin-like; MSTN, myostatin

expression levels was detected, accompanied by an improvement in energy expenditure, obesity and diet-induced insulin resistance. The irisin-induced browning effect, characterised by enhanced expression of brown fat–specific genes (*Ucp1*, *Pgc1a*, *Tmem26*, *Ebf3*, *Elovl3*, *Cidea* and *Cox7a*), is mediated via the activation of p38 mitogen-activated protein kinase (p38 MAPK) and extracellular-regulated protein kinase (ERK) pathways [89]. In this regard, a positive correlation between basal levels of beige gene expression in subcutaneous WAT and the levels of UCP1 expression in response to irisin has been described [11, 111, 149]. The effects of irisin on the thermogenic programme on fat cells are directly mediated by a subset of integrins, especially those involving αV integrins [73]. Additionally, irisin inhibits adipogenesis during differentiation, reducing fat storage by suppressing formation of new adipocytes [111].

Boström et al. suggested that irisin is responsible for some of the beneficial effects of exercise and might constitute a potential therapeutic tool for the treatment of metabolic disorders. However, conflicting results emerged in human studies. Whereas some studies exhibit that exercise-induced irisin may stimulate WAT [11, 80], several well-conducted exercise intervention studies have shown that long-term exercise does not increase circulating irisin levels in humans [7, 14, 79]. It should be taken into account that irisin might be influenced by a number of phenotypic traits including increased adiposity, lean mass and fasting plasma glucose that may partially explain these conflicting results.

A direct action of irisin on skeletal muscle accretion has been reported by our group [145] and others [76] showing a direct effect of irisin on the myoblast proliferative response, upregulating myogenin and downregulating the myostatic

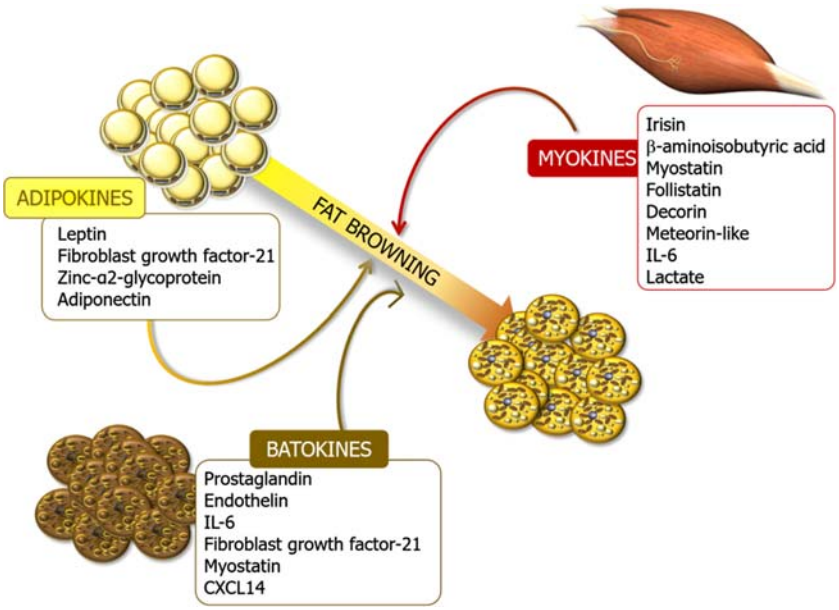

Fig. 2 A contracting skeletal muscle releases myokines, critical in exercise-induced metabolic adaptations. Specifically, muscular irisin, β-aminoisobutyric acid, myostatin, follistatin, decorin, meteorin-like, IL-6 and lactate lead to the induction of browning in WAT that can counteract obesity and its associated metabolic diseases. Several adipokines derived from adipose tissue, including leptin, fibroblast growth factor-21, zinc-α2-glycoprotein and adiponectin, are also involved in white fat browning and in the improvement of obesity-induced metabolic dysregulation. The brown fat is also able to secrete different factors called batokines such as prostaglandin, endothelin, IL-6, fibroblast growth factor-21, myostatin and CXCL14, also capable to contribute to fat browning

factors myostatin and dystrophin as well as the atrophy-related atrogin-1/MAFBx1 and MuRF1 [76, 145]. Furthermore, an increased metabolic rate in myocytes through irisin-mediated induction of mitochondrial biogenesis with the subsequent upregulation of mitochondrial genes (*Tfam*, *Nrf1* and *Ucp3*) [82] is also documented. The expression of the skeletal muscle FNDC5 is positively regulated by leptin [145], follistatin [24] and irisin itself [145], while being negatively regulated by myostatin [4], SMAD3 [90], glucose and palmitate [78].

Irisin is not only secreted by skeletal muscle but also, to a lesser extent, by AT, with an important autocrine and endocrine function [93, 113]. Short-term periods of exercise training induced FNDC5 secretion by WAT, being significantly higher in visceral than in subcutaneous AT. Interestingly, endurance training in rats upregulates FNDC5/irisin in parallel to the increased expression of brown and beige adipocyte genes (*Bmp7*, *Prdm16*, *Pgc1a*, *Cidea* and *Ucp1*) in eWAT, without affecting circulating irisin [114]. Myostatin and leptin negatively regulate the gene expression levels of *Fndc5* in the AT [4, 52, 145]. A discordant regulation of FNDC5/irisin in the skeletal muscle and WAT as well as a controversial response to short-term exercise training and fasting exists. However, no changes in FNDC5 expression or irisin secretion from subcutaneous and visceral WAT depots were observed by Tellerin et al. [137].

β-Aminoisobutyric acid

In line with these observations, Roberts et al. [144] sought to determine whether any additional mechanisms exist in the cross-talk between skeletal muscle and other metabolic organs. They analysed metabolites that were secreted from myocytes overexpressing PGC-1α and identified BAIBA, a metabolite derived from valine and thymine catabolism. This small myokine signals the positive effect of exercise from skeletal muscle to other tissues in an endocrine manner. In humans, BAIBA is released during muscle contraction in physical activity, being inversely associated with metabolic risk factors. BAIBA treatment in human-induced pluripotent stem cells led to a brown adipocyte–like gene expression signature as well as an increased mitochondrial activity. Diet-induced obese mice treated with BAIBA exhibited an increased BAT-specific gene expression (*Pgc1a*, *Ucp1*, *Cidea* and *Cytc*) together with a glucose tolerance improvement and decreased weight loss. Furthermore, an enhanced β-oxidation in hepatocytes, both in vivo and in vitro, is also observed after BAIBA treatment. Van Kuilenburg et al. [153] also demonstrated that the browning process as well as the β-oxidation in hepatocytes induced by BAIBA involved specific PPARα-dependent mechanisms.

The identification of BAIBA as an exercise-triggered signal and as a class of non-adrenergic activators of the thermogenic programme in WAT opens up new therapeutic possibilities for treating metabolic diseases.

Myostatin

MSTN, also termed growth/differentiation factor-8 (GDF-8), is a member of the TGF-β superfamily that is predominantly expressed and secreted by muscle fibres that negatively affects muscle growth and development [22]. Furthermore, MSTN also regulates the proliferation and differentiation of muscle stem cells and induces fibre-type switches [15, 25, 73, 137]. Overexpression of the *Mstn* gene is associated with lower muscle mass and decreased fibre size [92]. By contrast, loss of functional MSTN dramatically increased muscle mass in both mice [34] and humans [127] as a result from a combination of muscle fibre hypertrophy and hyperplasia. MSTN deficiency is also associated with suppression of body fat accumulation, involving MSTN in the control of energy balance beyond its effect on skeletal muscle [120]. *Mstn*-deficient mice exhibit remarkable changes in epididymal WAT, including elevated expression of genes involved in fatty acid oxidation, mitochondrial biogenesis, lipid transport together with the upregulation of key brown (*Pgc1a*, *Ucp1*, *Prdm16*, *Cidea* and *Dio2*)—and beige (*Tmem26* and *Cd137*)—AT-specific genes, confirming the existence of beige cells. In this regard, the observed browning phenotype in WAT of $Mstn^{-/-}$ mice is achieved by AMPK phosphorylation, necessary to induce the activation of PGC1α and FNDC5 [52, 120, 155]. MSTN post-transcriptionally suppresses, via a miR-34-dependent mechanism, FNDC5/irisin expression and secretion in white adipocytes. Loss of *Mstn* leads to decreased miR-34a expression, which subsequently promotes *Fndc5* expression, increasing thermogenic gene expression and browning in WAT [4].

Follistatin

Follistatin is a myostatin-binding glycoprotein that exerts an essential role in skeletal muscle development antagonising several members of the TGF-β superfamily including MSTN and activin A [10, 132]. Accordingly, targeted deletion of the gene encoding follistatin (*Fst*) causes neonatal death due to severe musculoskeletal defects [88]. Interestingly, mouse embryonic fibroblasts (MEFs) derived from *Fst*-deficient embryos display a lack of stimulation of key markers of energy metabolism and mitochondrial biogenesis (*Bmp7*, *Pparg*, *Pgc1a*, *Cidea*, *Cytc* or *Cd36*) [120] suggesting its potential role in the regulation of brown fat activity. In this regard, follistatin enhances the acquisition of brown adipocyte characteristics, by inducing the expression of key brown adipose-associated markers (*Pgc1a*, *Ucp1*, *Prdm16* and *Fabp4*) as well as increasing cellular oxygen consumption [13]. An important relationship between the muscle-derived

factor follistatin and irisin exists with the expression of skeletal muscle FNDC5 being positively regulated by follistatin and negatively by MSTN.

Decorin

Decorin is a crucial component of the extracellular matrix and a newly characterised contraction-induced myokine [68]. Decorin induces the upregulation of myogenic-associated factors such as MyoD and follistatin, and downregulates promoters of muscle atrophy including atrogin-1/MAFBx and MuRF-1. Mechanistically, secreted decorin binds and suppresses the activity of MSTN, suggesting a role in the muscle hypertrophic response and hence, in the regulation of fat browning [68].

Meteorin-like

Meteorin-like (METRNL) is a myokine induced in the skeletal muscle and WAT upon exercise and cold exposure, respectively. Contrary to the PGC-1α-dependent FNDC5, METRNL is primarily dependent on the PGC-1α isoform 4 (PGC-1α4) induced upon resistance exercise [110]. PGC-1α4 does not regulate most known PGC-1α targets including the regulation of the mitochondrial and oxidative metabolism programmes but induces muscle hypertrophy and strength [119]. METRNL is acutely induced after a single bout of high-intensity interval exercise in human skeletal muscle [30]. METRNL increases whole-body energy expenditure through the upregulation of genes involved in the brown/beige fat thermogenic and mitochondrial programme (*Ucp1*, *Pgc1a*, *Dio2* and *Erra*) as well as the anti-inflammatory genes *Il10* and *Tgfb* in AT. This activation of fat browning is not the consequence of a direct effect of METRNL on adipocytes but utilises an unconventional mechanism. METRNL indirectly activates the browning gene programme in WAT via stimulating the expression of the eosinophil-specific chemokines IL-4 and IL-13, promoting the activation of AT macrophages, essential to induce the production of catecholamines and to ultimately activate a pro-thermogenic programme [110].

IL-6

IL-6 synthesis and release by contracting skeletal muscle increases up to 100-fold after prolonged exercise, being considered the major myokine induced by exercise. Although IL-6 was originally classified as a pro-inflammatory cytokine, anti-inflammatory properties have also been described [77]. In this regard, IL-6 not only acts as a central mediator of inflammation but also serves as an endocrine modulator of metabolism, increasing glucose uptake and fatty acid oxidation in skeletal muscle while stimulating liver glucose output and fatty acid release from AT [55, 150]. *Ucp1* gene expression levels are increased in murine inguinal WAT after exercise training but not in *Il6*-deficient mice, suggesting that IL-6 plays a key role in the regulation of UCP1 expression. In this context, daily administration of IL-6 upregulates *Ucp1* expression in subcutaneous WAT [75]. Noteworthy, IL-6 is also released from differentiating human beige adipocytes facilitating the commitment of adipocyte precursors towards beiging, indicating its participation in fat browning [78].

Lactate

Lactate is the end product of anaerobic glycolysis and an important metabolite that mediates a large intercellular and inter-organ metabolic interplay. Lactate is considered a myokine, since it is a signalling molecule derived from muscle that communicates with other tissues, particularly the brain, the liver and the heart [83]. Lactate has also been reported to strongly increase thermogenic gene expression (*Ucp1*, *Cidea*, *Fgf21* and *Hoxc9*) in mouse and human WAT, being this effect dependent on the presence of active PPARγ signalling [17]. Interestingly, lactate induces the expression and secretion of FGF-21 from adipocytes [67] and myocytes [142], with this hormone being an important inductor of fat browning. Intense exercise causes a ~20-fold increase in circulating lactate, and further studies are needed to directly link an exercise-induced increase in lactate to the remodelling and browning of subcutaneous WAT.

Succinate

The mitochondrial tricarboxylic acid cycle intermediate succinate, produced by muscle shivering during cold exposure or exercise, is accumulated in a selective and substantial manner in both brown and beige ATs. The increased succinate levels are metabolised, triggering mitochondrial ROS production and UCP1-dependent thermogenesis activation [92].

Adipokines and fat browning

AT functions as both an energy storage and a secretory tissue producing a variety of bioactive substances, generally referred as adipokines, involved in regulating physiologic and pathologic processes. AT comprises two fractions, mature adipocytes and the stromovascular fraction that includes preadipocytes, fibroblasts, vascular endothelial cells and a variety of immune cells such as AT macrophages. AT also produces and secretes a wide spectrum of pro-inflammatory and anti-inflammatory factors implicated as active participants in energy homeostasis, including fat browning [58].

Two important adipokines, FGF21 and leptin, act in an autocrine/paracrine manner regulating the browning process induced by irisin.

Leptin

Leptin, the product of the *ob* gene, is a 16 kDa protein discovered in 1994 crucial for whole-body metabolism, thanks to the almost ubiquitous distribution of leptin receptors (LepR) in peripheral tissues [38, 154]. Leptin is mainly produced in adipocytes in relation to their triglyceride stores, representing a hormonal signal that circulates in proportion to body fat [37]. The lipolytic effect of leptin also helps to regulate adipocyte size and volume [42, 44]. Leptin predominantly influences energy balance via its effects on food intake and energy expenditure [43, 48]. Leptin crosses the blood-brain barrier affecting hypothalamic neural circuitry involved in the regulation of feeding behaviour and energy balance, including the arcuate nucleus (ARC), ventromedial hypothalamus (VMN) and dorsomedial hypothalamus (DMN) [61]. Leptin acts on appetite-suppressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) expressing neurons in order to suppress food intake and promote energy expenditure [96]. Central leptin administration activates WAT differentiation towards a BAT-like phenotype through the activation of the sympathetic nerve activity in BAT [105]. In this line, key markers of brown fat cell morphology and function are decreased in leptin-deficient *ob/ob* mice, suggesting a crucial role of leptin in brown adipogenesis and nonshivering thermogenesis. Furthermore, leptin and insulin act synergistically on distinct POMC neuronal subsets promoting WAT browning and energy expenditure as well as preventing the development of diet-induced obesity [28].

Another important target for leptin actions is the skeletal muscle, where it stimulates fatty acid oxidation via AMPK, increases both basal- and insulin-stimulated glucose uptake and oxidation and reduces inflammation and oxidative stress in muscle fibres. Furthermore, leptin enhances muscle cell proliferation and inhibits myofibrillar protein degradation by reducing the expression of negative regulators of muscle growth including myostatin, dystrophin or atrophy-related atrogin-1/MAFbx or MuRF1 [122]. Interestingly, leptin increases *Fndc5* gene expression levels in skeletal muscle, and stimulates irisin-induced muscle growth, as well as a proliferative response, suggesting that both molecules act synergistically on muscle accretion [145]. Contrary to what happens in the skeletal muscle, leptin downregulates the transcript levels of *Fndc5* in subcutaneous WAT [58]. Furthermore, leptin reduces irisin-stimulated expression of the brown adipocyte markers *Ucp1* and *Cidec*, as well as UCP1-positive cells, suggesting a negative regulation of subcutaneous fat browning induced by irisin.

Fibroblast growth factor-21

Fibroblast growth factor-21 (FGF21) is a member of the fibroblast growth factor super family, a large family of proteins involved in cell proliferation, growth and differentiation. FGF21 acts as a metabolic regulator, controlling glucose and lipid homeostasis [71], and ketogenesis, as well as energy expenditure and fat browning in both WAT and BAT [35, 63]. Although FGF21 is mainly expressed in liver, it is also secreted by the pancreas, the brain, the skeletal muscle, WAT and BAT. FGF21 signals to tissues primarily through the β-Klotho/FGFR1c receptor complex. FGFR1c has a broad tissue distribution, whereas β-Klotho is expressed in a limited number of metabolic tissues [8], suggesting that the tissue-specific effects of FGF21 are limited to those expressing β-Klotho. In this regard, both WAT and BAT express high levels of the critical coreceptor β-Klotho and are sensitive to exogenous FGF21 stimulation [35]. Adipocyte-derived FGF21 activates the thermogenic gene expression in BAT as well as browning in WAT depots. These processes are mediated by central (via sympathetic activation [29]) and local (via induction of the PGC-1α protein [35]) mechanisms. Notably, BAT is also an important source of FGF21, acting in an autocrine/paracrine manner and inducing the thermogenic programme of the brown adipocytes in response to cold exposure and β-adrenergic stimulation [23, 80]. Accordingly, *Fgf21*-knockout mice exhibit an impaired ability to adapt to chronic cold exposure, decreased thermogenic gene expression in BAT and impaired adaptive fat browning [35]. Interestingly, obesity is associated with increased circulating concentrations of FGF21, which is worsened by the presence of type 2 diabetes, [36, 59] together with a downregulation of β-Klotho in subcutaneous and visceral fat depots [36], suggesting a reduced responsiveness to FGF21 in these tissues.

Furthermore, FGF21 levels detected in skeletal muscle are essentially comparable with those in fasted liver, indicating that the skeletal muscle is also an important source of FGF21 production, with its expression being regulated by a PI3K/Akt signalling pathway. In this regard, stimulators of Akt1 signalling in skeletal muscle, such as resistance training exercise, may improve obesity-related metabolic disorders through production and secretion of FGF21, with endocrine effects leading to increased browning of WAT [66, 70].

FGF-21 is part of the potent bidirectional cross-talk between the skeletal muscle and AT. In this sense, the exercise-induced increase in irisin can stimulate the expression of FGF21 in brown fat, resulting in an increased browning of subcutaneous WAT in both human and mouse adipocytes [80].

Zinc-α2-glycoprotein

Zinc-α_2-glycoprotein (ZAG) is a 43-kDa adipokine identified as a lipid-mobilising factor that stimulates lipolysis and

inhibits lipogenesis in adipocytes. In this line, the expression and circulating levels of ZAG are inversely correlated to adiposity [6] being its expression decreased or lost in AT of obese mice and patients [94]. Intraperitoneal injection of a ZAG expression plasmid decreases body weight and adiposity and stimulates fat browning in the subcutaneous fat depot [84].

ZAG stimulates lipolysis in adipocytes via activation of β_3-adrenoceptors (AR) and the cAMP pathway which, in turn, activates PKA, increasing the adipocyte's lipolytic rate [121]. Intriguingly, activation of β_3-AR also stimulates thermogenesis in mature brown adipocytes, and chronic β_3-AR activation causes WAT browning [27]. Overexpression of ZAG in adipocytes enhances the expression of brown fat–specific markers (*Ucp1*, *Prdm16* and *Cidea*), mitochondrial biogenesis genes (*Pgc1a*, *Nrf1/2* and *Tfa*) and key lipid metabolism lipases [151]. In line with this observation, *ZAG* mRNA expression is positively correlated with the expression of several genes involved in fat browning in the subcutaneous WAT of overweight/obese patients [84].

Adiponectin

Adiponectin is one of the most abundant adipokines secreted from adipocytes, and it is expressed almost exclusively in WAT and BAT [125]. Adiponectin exerts anti-diabetic effects, protecting against insulin resistance, and its expression is downregulated during obesity [134]. Furthermore, it is well-established that adiponectin plays a crucial role in regulating immune responses such as inflammation [32]. In this regard, adiponectin regulates macrophage proliferation and polarisation, suppressing M1 macrophage activation and promoting M2 macrophage proliferation. The anti-inflammatory M2 macrophages have been proposed to be an important source of norepinephrine, a hormone involved in browning and thermogenesis in BAT [98]. The promotion of M2 macrophage proliferation by adiponectin provides a novel mechanism triggering a cold-induced browning effect in subcutaneous AT [64]. However, the relevance of M2 macrophages as source of norepinephrine is controversial [34, 120]. Fisher et al. suggest that alternatively M2 macrophages do not synthesise high amounts of catecholamines and hence are not likely to have a direct role in thermogenesis, whereas Lutz et al. conclude that the increased alternatively activated macrophages concomitant with enhanced sympathetic tone in AT promote the thermogenic programme [120]. Further research is needed to understand the role of M2 macrophages in thermogenesis.

Batokines

Brown and beige adipocytes also have a secretory role, which contribute, in an autocrine/paracrine manner, to the control of BAT expansion and activity as well as to the extent of browning of WAT [141]. Among these factors, prostaglandins are identified to directly induce browning in WAT [53]. Endothelin-1, which is released by brown adipocytes, is reported to repress the thermogenic activities of brown and beige AT [74]. The enhanced IL-6 expression in BAT may participate in the induction of browning of WAT in response to a cold environment [16], while BAT FGF21 expression is also capable of influencing systemic FGF21 levels, stimulating browning markers in WAT [23]. MSTN, believed to be produced mainly by muscle, is also expressed in BAT, contributing significantly to serum myostatin levels [120]. Overexpression of MSTN impairs BAT differentiation in vitro, with preadipocytes from *Mstn*-deficient mice exhibiting an increased propensity to differentiate into brown adipocytes. Furthermore, brown fat cells secrete CXCL14 (C-X-C motif chemokine ligand-14), leading to adaptive thermogenesis via M2 macrophage recruitment and enhancing BAT activation as well as the browning of WAT [21] (Fig. 2).

Beige adipocytes also secrete factors that can influence the function of fat cells or other organs. In this regard, the secreted factor Slit2, a member of the family of Slit homologue proteins, is released from beige adipose cells and promotes adipose thermogenesis, augmenting energy expenditure and contributing to browning of inguinal WAT [135].

Cross-talk between myokines and adipokines

Pedersen et al. [103] suggested that the well-established protective effects of exercise are regulated by the skeletal muscle via the secretion of myokines, preventing the adverse effects of pro-inflammatory adipokines and generating an important metabolic dialogue between the skeletal muscle and AT. In this line, evidence clearly demonstrates that skeletal muscle and AT function as endocrine organs producing a variety of factors, myokines and adipokines, respectively, involved in the complex network of interorgan communication required for energy homeostasis in a complex organism. This connection allows local autocrine/paracrine interactions, regulating different physiological processes such as myogenesis, adipogenesis, protein turnover and lipogenesis/lipolysis [45]. The high conservation of leptin among species points to the physiological relevance of this adipokine [95]. Noteworthy, the plausible role of other factors like caveolin-1, aquaporins or serum amyloid A, among others, which are involved in the development of the metabolic derangements should not be disregarded [18, 39, 56]. In this regard, fat browning is mediated by myokines, with their activity being modulated by adipokines, confirming the potential cross-talk between AT and skeletal muscle in order to control body weight, energy expenditure and thermogenesis [117].

Conclusion

Since the discovery of leptin in 1994, there has been an intense focus on the autocrine properties of AT. It has been demonstrated that AT plays a key role in the regulation of energy homeostasis, and alterations in the expression or activity of adipokines have a fundamental role in the pathogenesis of metabolic disorders. Nevertheless, contracting skeletal muscle has emerged as an endocrine organ secreting numerous myokines involved in the regulation of metabolism. Indeed, some molecules expressed in the skeletal muscle have recently been shown to modulate adipose metabolism, especially browning of WAT, confirming the metabolic interrelationships and cross-talk of signals derived from both organs. Understanding the complexity of the cross-talk between skeletal muscle cells and adipocytes will allow to delineate the molecular mechanisms involved in WAT browning as well as to identify novel pharmacological agents, holding great promise for protection against obesity and its related metabolic diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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