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REVIEW

Crosstalk between adipokines and myokines in fat browning

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Abstract

Skeletal muscle is the largest organ determining whole-body insulin sensitivity and metabolic homoeostasis. Adaptive changes of skeletal muscle in response to physical activity include adjustments in the production and secretion of muscle-derived bioactive factors, known as myokines, such as myostatin, IL-4, IL-6, IL-7 and IL-15, myonectin, follistatin-like 1 or leukaemia inhibitory factor. These myokines not only act locally in the muscle in an autocrine/paracrine manner, but also are released to the bloodstream as endocrine factors to regulate physiological processes in other tissues. Irisin, derived from the cleavage of FNDC5 protein, constitutes a myokine that induces myogenesis and fat browning (switch of white adipocytes to brown fat-like cells) together with a concomitant increase in energy expenditure. Besides being a target for irisin actions, the adipose tissue also constitutes a production site of FNDC5. Interestingly, irisin secretion from subcutaneous and visceral fat depots is decreased by long-term exercise training and fasting, suggesting a discordant regulation of FNDC5/irisin in skeletal muscle and adipose tissue. Accordingly, our group has recently reported that the adipokine leptin differentially regulates FNDC5/irisin expression in skeletal muscle and fat, confirming the crosstalk between both tissues. Moreover, irisin secretion and function are regulated by other myokines, such as follistatin or myostatin, as well as by other adipokines, including fibroblast growth factor 21 and leptin. Taken together, myokines have emerged as novel molecular mediators of fat browning and their activity can be modulated by adipokines, confirming the crosstalk between skeletal muscle and adipose tissue to regulate thermogenesis and energy expenditure.

Keywords adipokines, beige adipocytes, energy expenditure, myokines.

Fat browning

In addition to its traditional functions (energy storage, heat insulation and mechanical protection), adipose tissue is a highly dynamic endocrine organ that produces and releases a huge variety of bioactive factors known as adipokines, which regulate many physiological functions, including energy metabolism (Rodríguez *et al.* 2015b). Two types of adipose tissue can be distinguished by morphology, function and

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location: white (WAT) and brown (BAT) adipose tissues (Frühbeck *et al.* 2009a, Cinti 2012). On the one hand, white adipocytes are huge (25–200 μm), round cells with a large unilocular lipid droplet, few mitochondria in a thin cytoplasmic rim and a peripheral nucleus (Frühbeck 2008, Cinti 2012). The main functions of WAT are the storage of energy in the form of triacylglycerols, lipolysis and secretion of adipokines. WAT is located in the subcutaneous, abdominal, inguinal, retroperitoneal, gonadal and peri-cardial regions (Cinti 2012). On the other hand, brown adipocytes are smaller cells (15–60 μm) with polygonal shape, multi-locular lipid droplets, a central nucleus with large spherical mitochondria packed with laminar cristae (Fig. 1) (Cinti 2012). Mitochondria in brown adipocytes are marked by the expression of uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis, thereby resulting in heat production (Cannon & Nedergaard 2004). In this regard, BAT plays an important role in non-shivering and diet-induced thermogenesis through UCP1 activation and it is particularly abundant in hibernators and cold-acclimated rodents (Frühbeck *et al.* 2009a). In animals, BAT is located in interscapular, subscapular, axillary, peri-subclavian and peri-carotid regions (Giordano *et al.* 2014), although in some species, such as lambs and cattles, the perirenal adipose tissue represents the main depot in the newborns (Smith *et al.* 2004, Taga *et al.* 2012). In humans, BAT is mainly found in the interscapular, paravertebral and axillary regions in newborns, allowing their adaptation to a cold environment by adaptive thermogenesis (Frühbeck *et al.* 2009a). Metabolically active BAT is also detectable by positron-emission tomography integrated with computed tomography (^{18}F-FDG PET/CT) particularly in the neck and supraclavicular regions in adults (Nedergaard *et al.* 2007, van Marken Lichtenbelt *et al.* 2009, Saito *et al.* 2009, Virtanen *et al.* 2009, Vijgen *et al.* 2010). BAT activity can be induced in response to cold and sympathetic nervous system activation and is inversely correlated with BMI and adiposity, evidencing an inverse relationship with obesity (van Marken Lichtenbelt *et al.* 2009, Vijgen *et al.* 2010). Thus, BAT activation has been proposed as a potential therapy against obesity based on its energy-dissipating properties (Frühbeck *et al.* 2009a).

The existence of brown fat-like cells that emerge within white fat pads, designated as brown-in-white ('brite') or beige adipocytes, has been recently reported (Petrovic *et al.* 2010). Beige adipocytes resemble white fat cells in morphology and gene expression patterns during basal states, but acquire an intermediate brown-like appearance upon prolonged cold exposure, β-adrenergic stimulation or peroxisome proliferator-activated receptor (PPAR)-γ agonist treatment in a process called 'fat browning' (Fig. 1) (Petrovic *et al.* 2010, Wu *et al.* 2012). These clusters of active beige adipocytes exhibit multi-locular lipid droplets, high mitochondrial content and express thermogenic factors such as UCP1, PPAR-γ coactivator 1-α (PGC-1α), cell death-inducing DFFA-like effector a (CIDEA), deiodinase type II (DIO2) and β_3-adrenergic receptor (ADRB3) (Wu *et al.* 2012). Moreover, beige adipocytes also exhibit a unique gene signature characterized by the expression of beige-specific markers, such as TNF receptor superfamily member 9 (CD137), transmembrane protein 26 (TMEM26), T-box-associated transcription factor (TBX1), homeobox C8 and C9 (HOXC8 and HOXC9) or CITED1 (Petrovic *et al.* 2010, Walden *et al.* 2011, Sharp *et al.* 2012, Wu *et al.* 2012, Jespersen *et al.* 2013). Regarding the location of beige adipose tissue in humans, recent data demonstrate that human BAT might consist of both classical brown and recruitable brite adipocytes, important for future considerations on how to induce BAT activity (Sharp *et al.* 2012, Jespersen *et al.* 2013). Beige adipocytes can be induced by chronic cold exposure, physical activity and lactation as well as by obesity (Cinti 2012, Rodríguez *et al.* 2015b). It remains controversial whether beige adipocytes are formed *de novo* from precursor cells in the adipose tissue (Wang *et al.* 2014, Gustafson *et al.* 2015) or arise from white-to-brown adipocyte transdifferentiation (Cinti 2012) (Fig. 2). Fat browning might be of particular medical relevance, because animal data indicate that higher amounts of fat browning are positively associated with resistance to obesity and its comorbidities (Petrovic *et al.* 2010, Wu *et al.* 2012).

Transcriptional regulation of brown and beige adipogenesis

The developmental origin and transcriptional regulation of classic brown adipocytes and beige fat cells is different (Fig. 2), although both types of adipocytes are UCP1-expressing cells with high mitochondrial content and thermogenic capacity.

White and brown fat cells derive from the same mesenchymal stem cells in the embryonic mesoderm (Enerbäck 2009). These mesenchymal stem cells can be committed to either an adipogenic lineage (MYF5-negative cells) or a myogenic lineage (MYF5-positive cells), with MYF5 being a key myogenic regulatory factor (Pownall *et al.* 2002, Seale *et al.* 2009). Brown adipocytes and myocytes arise from MYF5-expressing precursors in the paraxial mesoderm, showing a muscle-like gene signature (Seale *et al.* 2007, 2008) (Fig. 2a). Several members of the bone morphogenetic

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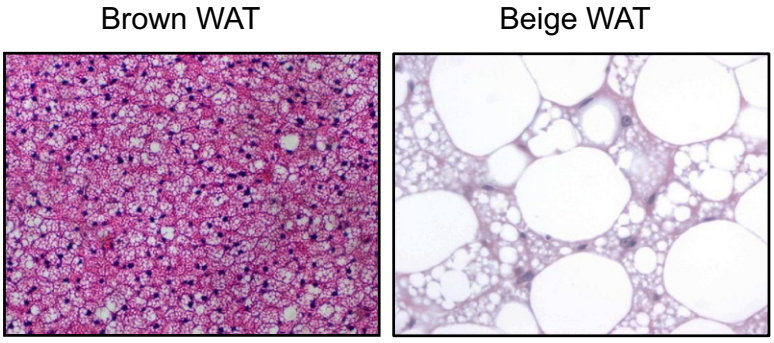
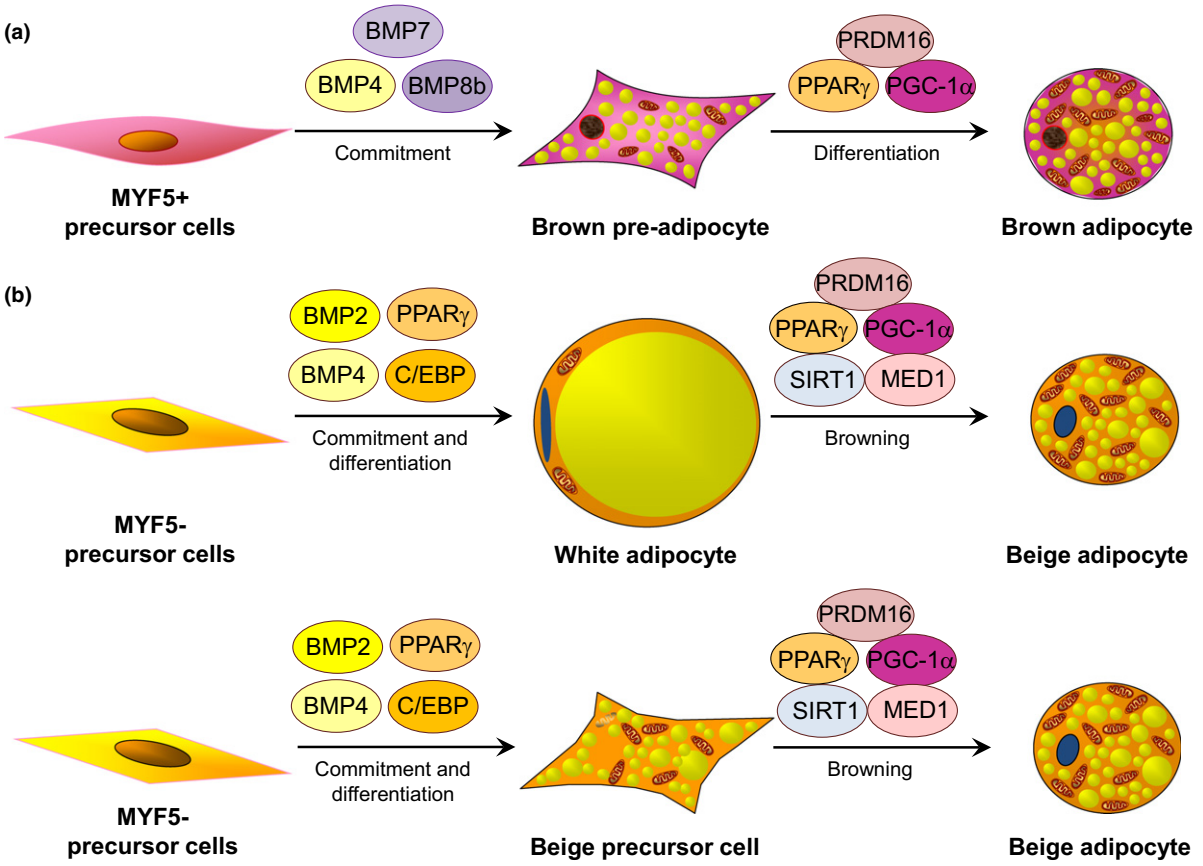


Figure 1 Representative histological sections of brown and beige adipose tissue obtained from rodents. Haematoxylin–eosin staining, magnification 100×.



Figre 2 Developmental origin and transcriptional regulation of brown and beige adipocytes. (a) During early embryonic development, brown adipocytes are derived from MYF5+ progenitor cells. Several members of bone morphogenetic proteins, such as BMP4, BMP7 and BMP8a, are involved in the commitment of MYF5+ cells towards brown pre-adipocytes. PRDM16 stimulates brown adipocyte differentiation through the binding to PPAR-γ and PGC-1α, activating the transcription of brown-selective genes (PGC-1α/β, UCP-1, ZIC1, ELOVL3, among others). (b) It remains controversial whether beige adipocytes derive from the transdifferentiation of white adipocytes towards a brown-like phenotype (*upper panel*) or they are formed *de novo* from precursor cells in the adipose tissue (*lower panel*). The deacetylation of PPAR-γ by SIRT1 is required to stabilize and recruit the coactivator PRDM16, which induces brown fat transcriptional programme through interactions with PGC-1α and the mediator subunit MED1 beige adipocytes display thermogenic properties inducible by cold exposure, β-adrenergic stimulation or exercise, and to show a unique gene signature (TMEM26, CD137 and TBX1). PPAR, proliferator-activated receptor.

protein (BMP) family, which belongs to the TGF-β superfamily, are involved in the commitment of MYF5-positive cells into brown adipocyte precursors, such as BMP4 (Qian *et al.* 2013), BMP7 (Tseng *et al.* 2008) and BMP8b (Whittle *et al.* 2012). The transcription factor PR domain containing 16 (PRDM16)

constitutes the molecular switch deciding the fate of the common progenitor cell to become either a skeletal myoblast or a brown adipocyte (Seale *et al.* 2007, Frühbeck *et al.* 2009b, Becerril *et al.* 2013). PRDM16 robustly induces the expression of UCP1 and other brown fat-selective genes through binding to and stimulating PGC-1α and PGC-1*β* (Seale *et al.* 2008, Frühbeck *et al.* 2009b, Becerril *et al.* 2013). In addition, different studies have identified the implication of microRNAs in brown adipogenesis. Both miR-196a (Mori *et al.* 2012) and miR-155 (Chen *et al.* 2013) stimulate brown lineage commitment targeting C/EBPβ, while miR-133 directly interacts with and reduces *Prdm16* transcripts decreasing both brown and beige adipocyte differentiation (Trajkovski *et al.* 2012).

On the other hand, beige adipocytes develop in WAT in response to several stimuli (Harms & Seale 2013). Cold exposure and β-adrenergic system activation are the best-known mediators of fat browning (Murano *et al.* 2009, Nguyen *et al.* 2011a), but recently, the existence of novel endocrine BAT activators beyond the sympathetic tone has been reported (Villarroya & Vidal-Puig 2013, Rodríguez *et al.* 2015b), including fibroblast growth factor 21 (FGF21) (Fisher *et al.* 2012), cardiac natriuretic peptides (Bordicchia *et al.* 2012), follistatin (Braga *et al.* 2014), bile acids, BMP8B (Whittle *et al.* 2012), the intermediate metabolites lactate and ketone body β-hidroxybutyrate (Carrière *et al.* 2014) or leptin (Rodríguez *et al.* 2015a). In addition, fat browning can be triggered by pharmacological agents (Bonet *et al.* 2013), such as agonists of PPAR-γ (Petrovic *et al.* 2010), β3-adrenergic receptor (Lee *et al.* 2012) or thyroid hormone receptor (Lin *et al.* 2015) as well as with synthetic inhibitors of histone deacetylases (Galmozzi *et al.* 2013), among others. The process of beige adipogenesis is regulated by several transcriptional factors and coregulators, such as PPAR-γ, PRDM16 or sirtuin 1 (SIRT1), generally functioning in a combinatorial manner (Fig. 2b). The activation of PPAR-γ, the absolute master regulator of adipocyte differentiation, increases UCP1 expression in different WAT depots, particularly in the inguinal depot (Petrovic *et al.* 2010). PGC-1α binds the heterodimer formed by PPAR-γ and retinoid X receptor alpha (RXR-α) and promotes the expression of thermogenic UCP1 (Rosen *et al.* 2000). The NAD^+-dependent type III deacetylase SIRT1 also activates PPAR-*γ*, PPAR-α and PGC-1α in adipocytes to contribute to fat browning (Qiang *et al.* 2012, Wang *et al.* 2013, Fu *et al.* 2014). Deacetylation of PPARγ is required to stabilize and recruit the coactivator PRDM16, which downregulates the expression of white-specific genes and induces the brown fat transcriptional programme through interactions with the mediator subunit MED1 (Becerril *et al.* 2012, Qiang *et al.* 2012, Harms *et al.* 2015, Iida *et al.* 2015), T-box 15 (TBX15) (Gburcik *et al.* 2012) or Zfp516 (Dempersmier *et al.* 2015), playing an essential role in differentiation and activation of the beige adipocytes.

In the present review, we will focus on the role of physical activity in fat browning as well as the crosstalk of adipokines and myokines in this process.

The skeletal muscle as an endocrine organ: impact of myokines on metabolic homoeostasis

The impact of physical activity and exercise on health is well known (Handschin & Spiegelman 2008, Neufer *et al.* 2015). A sedentary lifestyle and even short periods of physical inactivity are associated with a decrease in insulin sensitivity, impaired lipid metabolism, loss of muscle mass and accumulation of visceral fat. By contrast, exercise training results in adaptive structural and metabolic changes in skeletal muscle, including a change in the type of muscle fibres, mitochondrial biogenesis and angiogenesis. Moreover, regular exercise promotes multiple beneficial effects on health, which are mediated in part by the activation of the PGC-1α transcription factor (Handschin & Spiegelman 2008).

Physical activity protects against all causes of mortality (Blair *et al.* 1995), and the identification of the skeletal muscle as an endocrine organ has provided a mechanistic explanation for the beneficial effects of the regular practice of exercise on the prevention of metabolic diseases (Pedersen & Febbraio 2012). Skeletal muscle is the largest organ influencing whole-body insulin sensitivity and metabolic homoeostasis. Since the identification of myostatin in 1997 (McPherron *et al.* 1997) and interleukin-6 (IL-6) in 2000 (Steensberg *et al.* 2000) as muscle-secreted factors, 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, that have been proposed as the mediators of the beneficial actions of physical activity (Table 1) (Pedersen & Febbraio 2012). IL-6 is recognized as the prototype myokine exerting autocrine, paracrine and endocrine functions (Pedersen 2009), but during the last decade, several proteomics studies focusing on the secretome of skeletal muscle have revealed a large number of myokines with pleiotropic effects such as myostatin, IL-6, IL-7 and IL-15, FGF-21, myonectin, follistatin, leukaemia inhibitor factor or the more recently identified, musclin, irisin, β-aminoisobutyric acid (BAIBA) or meteorin-like (Norheim *et al.* 2011, Pedersen &

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Table 1 Proteins expressed and/or secreted by skeletal muscle with endocrine effects

Protein	Main metabolic effect	References
ANGPTL4	Inhibitor of the lipoprotein lipase enzyme increased by acute exercise	Catoire *et al.* (2014a)
Apelin	Peptide induced by endurance training that is involved in the control of blood pressure and cardiac contractility	Besse-Patin *et al.* (2014)
BAIBA	Myokine that constitutes the natural catabolite of thymine involved in hepatic FFA β-oxidation and fat browning	Roberts *et al.* (2014)
BDNF	Trophic factor for innervating motor neurones that also inhibits myogenic differentiation	Mousavi & Jasmin (2006)
Calprotectin	DAMP released from muscle during exercise involved in extravasation of leucocytes and with antimicrobial activity	Mortensen *et al.* (2008)
CX3CL1	Chemokine involved in leucocyte adhesion and in macrophage-directed rescuing of skeletal muscle cells from apoptosis	Catoire *et al.* (2014b)
Decorin	Myokine released by contracting myotubes that promotes muscle growth by inhibiting myostatin and atrophy markers as well as by increasing MyoD	Kanzleiter *et al.* (2014)
DPP4	Cell surface type II membrane glycoprotein that cleaves N-terminal dipeptides of post-prandial activated incretins GLP1 and GIP	Raschke *et al.* (2013a)
FGF21	Factor involved in the regulation of systemic glucose, lipid metabolism and browning	Izumiya *et al.* (2008), Chavez *et al.* (2009)
FNDC5/Irisin	Myokine with myogenic properties that stimulates browning of white adipose tissue	Boström *et al.* (2012), Huh *et al.* (2014), Rodríguez *et al.* (2015a)
FSTL1	Glycoprotein of the SPARC family that promotes endothelial cell function and revascularization in ischaemic tissues	Ouchi *et al.* (2008)
IGF-1	Growth factor involved in skeletal muscle hypertrophy and regeneration	Pedersen & Febbraio (2012)
IGF-BP5	Binding protein that inhibits myoblast differentiation by sequestering IGF-1	James *et al.* (1993)
IL-4	Interleukin that enhances muscle regeneration by stimulating the fusion of myoblasts with myotubes	Horsley *et al.* (2003)
IL-6	Prototype myokine that increases muscle hypertrophy and whole-body fat oxidation as well as promotes insulin resistance	Bartoccioni *et al.* (1994), van Hall *et al.* (2003), Febbraio *et al.* (2004)
IL-7	Interleukin involved in muscle hypertrophy that acts on satellite cells and is required for T-cell and B-cell development	Haugen *et al.* (2010)
IL-8	Interleukin acting as modulator of inflammation and proangiogenic factor	Pedersen & Febbraio (2012), Amir Levy *et al.* (2015)
IL-15	Interleukin that promotes muscle hypertrophy and decreases lipid deposition in pre-adipocytes and white adipose tissue mass	Carbo *et al.* (2001)
INSL6	Myokine markedly induced by muscle injury that promotes muscle progenitor cell proliferation and survival	Zeng *et al.* (2010)
LIF	Contraction-induced cytokine that induces satellite cell proliferation for proper muscle hypertrophy and regeneration	Broholm *et al.* (2008), Broholm *et al.* (2011)
MCP-1	Chemokine involved in attracting macrophages and other immune cells for repair and growth of skeletal muscle	Catoire *et al.* (2014b)
Meteorin-like	Myokine that activates eosinophils and macrophages and thermogenic programme in the adipose tissue	Rao *et al.* (2014)
Musclin	Vasoconstrictor myokine that also attenuates insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle	Nishizawa *et al.* (2004), Lin *et al.* (2014)
Myonectin	Nutrient-responsive myokine that enhances glucose uptake and stimulates fatty acid oxidation	Seldin *et al.* (2012)
Myostatin	Hormone involved in the inhibition of muscle hypertrophy, in the maintenance of metabolic homoeostasis and in modulation of adipose tissue function and mass	McPherron *et al.* (1997), Feldman *et al.* (2006)
PAI-1	Serin protease inhibitor (serpin) that acts as an antifibrinolytic factor	Norheim *et al.* (2011)
PEDF	Glycoprotein of the non-inhibitory serpin group with anti-angiogenic and neurotrophic properties	Steele *et al.* (1993), Raschke *et al.* (2013a)

(continued)

Table 1 (continued)

Protein	Main metabolic effect	References
Somatotropin	Pleiotropic peptide hormone with an important role in the regulation of metabolism via stimulation of lipid mobilization and oxidation promotes anabolic effects on skeletal muscle	Raschke *et al.* (2013a)
SPARC	Matricellular protein involved in differentiation, regeneration and proliferation	Jorgensen *et al.* (2009)
VEGF	Factor that is the potential mitogen of endothelial cells and is involved in angiogenesis in response to exercise.	Hoffner *et al.* (2003)

ANGPTL4, angiopoietin-like 4; BAIBA, β-aminoisobutyric acid; BDNF, brain-derived neurotrophic factor; BMP-7, bone morphogenetic protein; CX3CL1, chemokine (C-X3-C motif) ligand 1 (also referred to as fractalkine); DAMP, damage activated molecular pattern protein; DPP-4, dipeptidyl peptidase 4; FGF-21, fibroblast growth factor-21; FSTL1, follistatin-like protein 1; IGF-1, insulin growth factor 1; IGF-BP5, insulin-like growth factor-binding protein-5; IL, interleukin; INSL6, insulin-like 6; LIF, leukaemic inhibitory factor; MCP-1, monocyte chemoattractant protein 1; PAI-1, plasminogen activator inhibitor-1; PEDF, pigment epithelium-derived factor; SPARC, secreted protein acidic and rich in cysteine; VEGF, vascular endothelial growth factor; FFA, free fatty acid.

Febbraio 2012). Besides the well-known interleukins such as IL-4, IL-6, IL-7 and IL-15, further interleukins are secreted by the skeletal mucle cells, such as IL-1α, IL-3, IL-16, IL-22, IL-28a, IL-29 and IL-31 (Raschke *et al.* 2013a).

Regarding the biological function of myokines (Table 1), skeletal muscle has inbuilt control mechanisms to prevent overgrowth as well as muscle atrophy with myokines acting as positive and negative inducers of skeletal muscle growth. In this regard, IL-4, IL-6, IL-7, IL-15 and leukaemia inhibitory factor (LIF) promote muscle hypertrophy, while myostatin inhibits muscle hypertrophy (McPherron *et al.* 1997). The contracting skeletal muscle secretes enhanced levels of myokines in response to exercise, which have beneficial endocrine effects, playing a crucial role in the dialogue between skeletal muscle and other metabolic tissues, such as adipose tissue, pancreas, intestine or liver (Raschke & Eckel 2013). Brain-derived neurotrophic factor (BDNF) and IL-6 are involved in AMPK-mediated free fatty acid (FFA) oxidation, and IL-6 also stimulates lipolysis in the visceral fat depot and increases insulin secretion by inducing the expression of glucagon-like peptide 1 (GLP-1) by intestinal L cells (Pedersen & Febbraio 2012). IGF-1, FGF-2 and TGF-β are involved in bone formation, and follistatin-related protein 1 improves endothelial function and revascularization of ischaemic blood vessels. Several myokines, such as irisin, BAIBA and meteorin-like, have a role in browning of WAT (Boström *et al.* 2012, Ruas *et al.* 2012, Roberts *et al.* 2014), which is extensively explained in the next section.

Impact of myokines on fat browning

Regular physical activity and exercise training induce profound adaptations in WAT, such as an increase in mitochondrial activity, decrease in adipocyte cell size and lipid content or regulation of adipokines, that mediate in part whole-body metabolic health (Stanford *et al.* 2015a). In rodent models, exercise training also increases the expression of *Ucp1, Prdm16* and other markers of beige adipocytes in both visceral and subcutaneous adipose tissue (Boström *et al.* 2012, Stanford *et al.* 2015b), although these effects are more pronounced in the subcutaneous fat depot. The underlying mechanisms whereby exercise promotes fat browning have been focus of several investigations. It has been proposed that the exercise-induced sympathoactivation contributes to fat browning (Ghorbani *et al.* 1997, Nedergaard & Cannon 2014). Nonetheless, the discovery of the contracting muscle as an endocrine organ has revealed that IL-6 as well as novel myokines also act on adipocytes as positive (IL-6, irisin, BAIBA and meteorin-like) and negative (myostatin) regulators of fat browning (Boström *et al.* 2012, Ruas *et al.* 2012, Shan *et al.* 2013, Knudsen *et al.* 2014, Roberts *et al.* 2014) (Fig. 3). The secretion of these myokines in response to exercise and their impact on fat browning provide a novel mechanism to explain the benefits of physical activity on weight loss and metabolic disease prevention. Interestingly, it has been recently reported that lactate, a metabolite released by skeletal muscle during and after exercise, induces a robust increase of the thermogenic gene expression (*Ucp1, Cidea, Fgf21* and *Hoxc9*) in mouse and human white adipocytes through PPAR-γ activation (Carrière *et al.* 2014). Thus, the release of several myokines and lactate during exercise could contribute to the browning remodelling of adipose tissue. Until now, human studies are scarce and whether physical activity *per se* recruits brown and beige adipocytes (Dinas *et al.*

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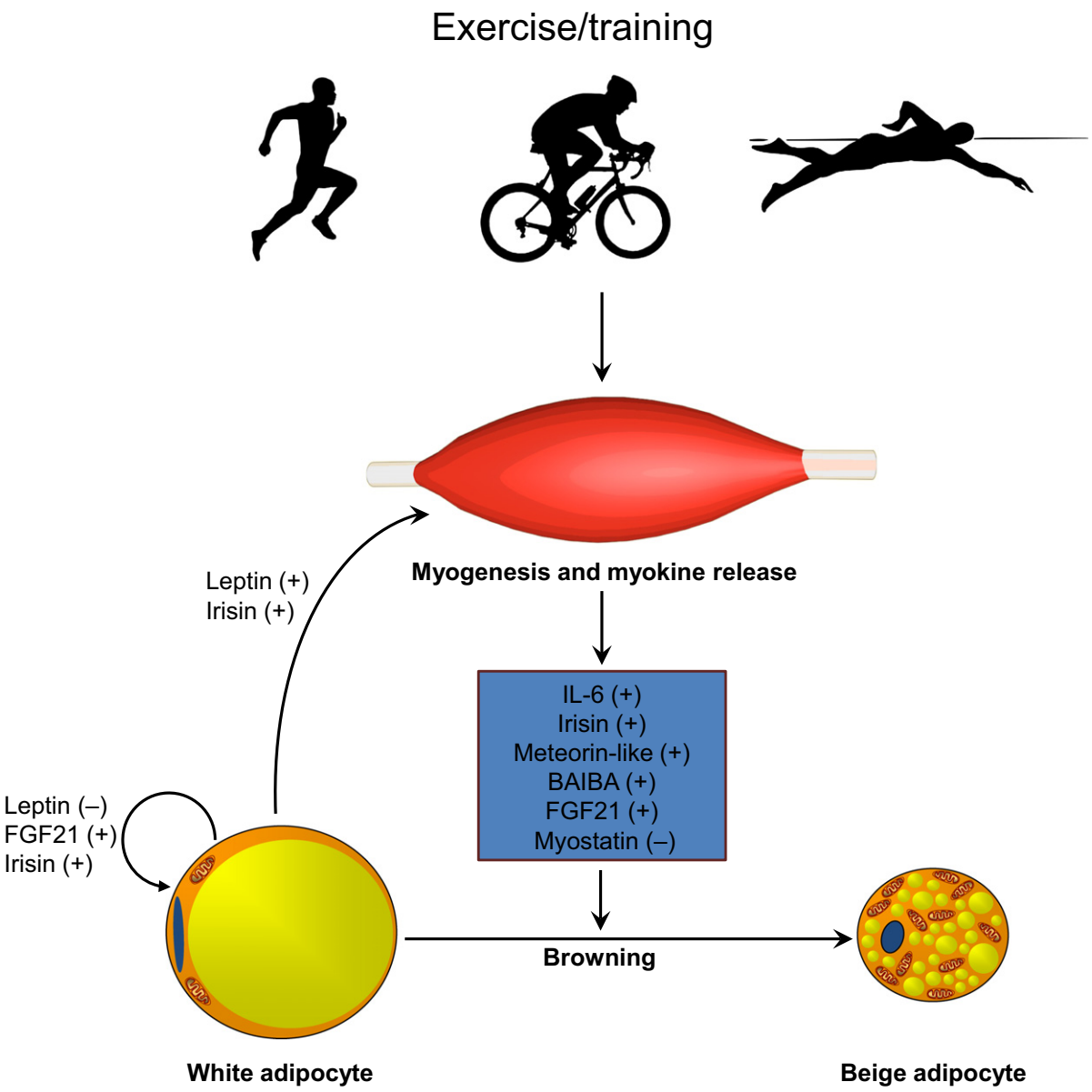

Figure 3 Crosstalk between adipokines and myokines in fat browning after exercise. Exercise training induces muscle growth as well as the production and secretion of myokines, which are in part responsible for the beneficial metabolic effects of physical activity on other organs, including the adipose tissue. Several myokines regulate the differentiation of energy-accumulating white adipocytes into energy-dissipating beige adipocytes, a process called fat browning. In this regard, the myokines IL-6, irisin, BAIBA and meteorin-like positively regulate fat browning, while the myostatic factor myostatin represses this biological process. On the other hand, the adipose tissue secretes the adipokine leptin and FGF-21 as feedback signal, closing the adipocyte–myocyte loop. Leptin stimulates myogenesis and induces the expression and release of irisin in skeletal muscle, but reduces its browning effect in subcutaneous adipocytes. FGF-21 acts in an autocrine/paracrine manner enhancing irisin-induced beige adipogenesis. Finally, irisin is not only a myokine, but also an adipokine with myogenic and browning effects that induces a positive self-regulation in both skeletal muscle and adipose tissue.

2015) or not (Vosselman *et al.* 2015) remains controversial. Therefore, abusive extrapolation of rodent data to humans should be avoided and it will be important to disentangle the true impact of exercise training on fat browning in humans to gain further insight into metabolic health.

IL-6

The prototype myokine IL-6 has been proposed as an important factor in the crosstalk between skeletal muscle and adipose tissue. IL-6 increases up to 100-fold in the circulation during exercise due to the

increased release of IL-6 by type I and type II contracting muscle fibres (Pedersen & Febbraio 2008). Circulating IL-6 acts as a potent regulator of fat metabolism in humans, increasing lipolysis and FFA oxidation in adipocytes (van Hall *et al.* 2003). Interestingly, IL-6 also regulates exercise training-induced UCP1 expression in murine inguinal WAT (Knudsen *et al.* 2014), suggesting its participation in fat browning. To our current knowledge, no studies have reported this effect in humans, so further studies are needed to analyse the IL-6-induced beige adipocyte differentiation and/or activation in response to exercise training.

Irisin

The fibronectin type III domain containing 5 (*FNDC5*) gene encodes a protein in the skeletal muscle that is proteolitically cleaved to form the active form, irisin (Boström *et al.* 2012). Exercise and/or PGC-1α induce FNDC5 expression and irisin secretion from skeletal muscle in rodents and humans (Boström *et al.* 2012, Huh *et al.* 2012, Gouni-Berthold *et al.* 2013, Hecksteden *et al.* 2013, Moreno-Navarrete *et al.* 2013, Roberts *et al.* 2013, Shan *et al.* 2013, Wrann *et al.* 2013, Kurdiova *et al.* 2014, Norheim *et al.* 2014). In this regard, a direct action of irisin on skeletal muscle accretion by increasing myogenic molecules, while decreasing myostatic factors as well as atrophy-related genes, has been recently proposed by our group (Rodríguez *et al.* 2015a) and others (Huh *et al.* 2014). The stimulation of murine C2C12 myocytes with irisin induces their proliferative response, upregulates myogenin, which is essential for the terminal differentiation of committed myoblast, and downregulates the myostatic factors myostatin and dystrophin as well as the atrophy-related atrogin-1/MAFBx1 and MuRF1 (Rodríguez *et al.* 2015a). Moreover, irisin treatment also promotes mitochondrial biogenesis with the subsequent upregulation of mitochondrial genes (*Tfam*, *Nrf1* and *Ucp3*) in C2C12 myocytes (Vaughan *et al.* 2014). The expression of skeletal muscle FNDC5 is positively regulated by leptin (Rodríguez *et al.* 2015a), follistatin (Vamvini *et al.* 2013) and irisin itself (Rodríguez *et al.* 2015a), while being negatively regulated by myostatin (Shan *et al.* 2013), TGF-β (Tiano *et al.* 2015) and palmitate (Kurdiova *et al.* 2014) (Fig. 4). Furthermore, several pharmacological treatments, such as lipid-lowering statins (Gouni-Berthold *et al.* 2013) or antidiabetic metformin (Li *et al.* 2015a), reportedly regulate the transcription of FNDC5.

Large controversy exists on the physiological role of irisin in humans with several studies showing that exercise and high-intensity training protocols are effective in raising circulating irisin in humans (Boström *et al.* 2012, Huh *et al.* 2012, Norheim *et al.* 2014, Jedrychowski *et al.* 2015), while others were not able to find any association (Timmons *et al.* 2012, Hecksteden *et al.* 2013, Hofmann *et al.* 2014, Kurdiova *et al.* 2014), which highlights the doubts on the robustness of the exercise data. Furthermore, recent reports even argued against the existence of circulating irisin (Erickson 2013, Raschke *et al.* 2013b, Albrecht *et al.* 2015), as the human *FNDC5* gene harbours a mutation in the conserved ATG codon to ATA that might represent a null mutation preventing irisin transcription (Raschke *et al.* 2013b). Most of the studies used for circulating irisin detection relied on commercial antibodies and ELISA assays that revealed prominent cross-reactivity with non-specific proteins in human and animal sera (Albrecht *et al.* 2015). In this regard, the detection and quantitation of circulating irisin by quantitative mass spectrometry with heavy stable isotopes as standards have so far settled the existence of human irisin in plasma and its regulation by exercise (Jedrychowski *et al.* 2015).

Exogenous administration of irisin induces the browning of subcutaneous fat and thermogenesis in mice, thereby promoting oxygen consumption (Boström *et al.* 2012). The expression of fat browning-specific genes (*Ucp1*, *Pgc1a*, *Tmem26*, *Ebf3*, *Elovl3*, *Cidea* and *Cox7a*) is mediated through the activation of p38 MAPK and ERK1/2 pathways (Zhang *et al.* 2014). In humans, a positive correlation of circulating irisin and energy expenditure has been also found (Swick *et al.* 2013, Lee *et al.* 2014). Apparently, it seems paradoxical that exercise increases the secretion of a thermogenic hormone that would burn the fat stores (Kelly 2012), but it has been hypothesized that this mechanism has evolved from shivering-related muscle contraction to increase thermogenesis through BAT expansion (Lee *et al.* 2014). In this regard, irisin secretion is induced in proportion to the shivering intensity after cold exposure, in a magnitude similar to exercise-stimulated secretion (Lee *et al.* 2014). Nonetheless, other authors have found similar circulating irisin levels between individuals with active BAT detected by ^{18}FDG-PET/CT and those without BAT (Choi *et al.* 2014, Norheim *et al.* 2014), so further studies evaluating the role of irisin on human fat browning are needed. The adipose tissue not only constitutes a target for irisin, but also expressed the *FNDC5* gene and secretes irisin, but to a lesser extent than the skeletal muscle (Moreno-Navarrete *et al.* 2013, Roca-Rivada *et al.* 2013). An increased irisin secretion from subcutaneous and visceral adipose tissues is observed after only 1 week of exercise in rats (Roca-Rivada *et al.* 2013). Moreover, the gene expression levels of *Fndc5* in the adipose tissue obtained from rodents is also positively regulated by irisin itself (Rodríguez *et al.* 2015a), while

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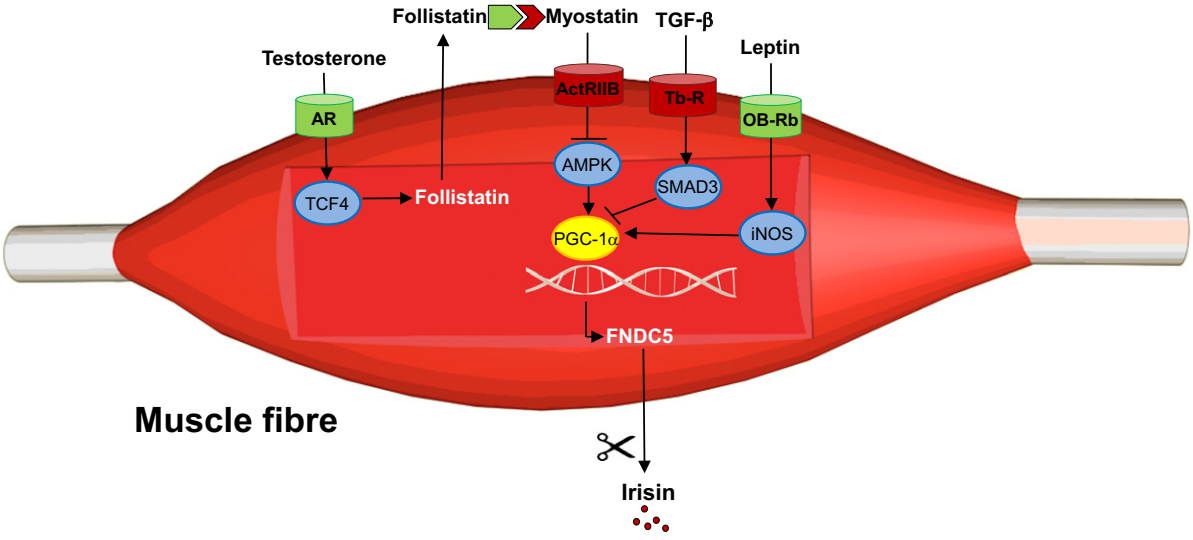

Figure 4 Factors involved in the myogenic action of irisin.

negatively regulated by myostatin (Shan *et al.* 2013) and leptin (Rodríguez *et al.* 2015a) (Fig. 3).

Myostatin

Myostatin [also known as growth differentiation factor 8 (GDF-8)] was the first secreted factor to fulfil the criteria of a myokine (McPherron *et al.* 1997). Myostatin is a member of the TGF-β superfamily that is predominantly expressed and secreted by muscle fibres (McPherron *et al.* 1997). On binding to its receptor, the transmembrane activin receptor type II B, myostatin inhibits muscle growth and the suppression of this pathway stimulates muscle growth (Lee & McPherron 2001, Schuelke *et al.* 2004, Relizani *et al.* 2014). Moreover, myostatin also inhibits the proliferation and differentiation of myoblast and satellite cells (Thomas *et al.* 2000, Joulia *et al.* 2003, McCroskery *et al.* 2003) and induces fibre-type switches (Mouisel *et al.* 2014). Myostatin gene deficiency results in an extensive skeletal muscle hypertrophy in mice (McPherron *et al.* 1997) and humans (Schuelke *et al.* 2004) as a result from a combination of muscle fibre hypertrophy and hyperplasia. Conversely, systemic overexpression of the myostatin gene (*MSTN*) leads to cachexia, which is characterized by extensive muscle loss (Zimmers *et al.* 2002). For this reason, myostatin blockade (e.g. antibodies, soluble decoy activin receptor type II B or propeptides) has been proposed as a therapeutic target for the treatment of muscular dystrophies, sarcopenia, cachexia and other muscle-wasting conditions (Lebrasseur 2012).

The loss of functional myostatin not only increases muscle mass, but also decreases body fat accumulation. Myostatin gene deficiency as well as its inactivation using soluble decoy activin receptor type II B protects against diet-induced obesity through the induction of genes involved in lipolysis and mitochondrial fatty acid oxidation in adipose tissue and liver (Zhang *et al.* 2012). Accordingly, the absence of myostatin in genetic models of obesity, such as leptin-deficient *ob/ob* or agouti lethal yellow (A^y/a) mice, partially suppresses both fat accumulation and the development of hyperglycaemia (McPherron & Lee 2002). In addition, mice carrying a targeted disruption of the myostatin gene ($Mstn^{-/-}$) drive fat browning through the upregulation of brown (*Pgc1a, Ucp1, Cidea* and *Dio2*)- and beige (*Tmem26* and *Cd137*)-specific genes in the white adipose tissue (Zhang *et al.* 2012, Shan *et al.* 2013). The fat browning induced in the absence of myostatin is non-cell autonomous, as it is triggered by the activation of the AMPK enzyme and the subsequent induction of PGC-1α and FNDC5 (Shan *et al.* 2013). These findings highlight the relevance of the inactivation of myostatin as potential anti-obesity drugs through the increase in fat browning-induced energy expenditure.

Follistatin binds and inhibits several TGF-β family members, including myostatin and activin A (Hill *et al.* 2002, Amthor *et al.* 2004, Boström & Fernández-Real 2014). Testosterone induces myogenic differentiation of multi-potent stem cells by the activation of follistatin through the interaction of the androgen receptor with T-cell factor-4 (TCF-4), resulting in the inhibition of the TGF-β signalling pathway (Fig. 4) (Singh *et al.* 2009). Interestingly, irisin levels are positively correlated with those of follistatin, which leads to muscle growth (Vamvini *et al.* 2013, Boström & Fernández-Real 2014). Accordingly, irisin directly reduces the mRNA expression of myostatin in C2C12 myocytes, suggesting a negative feedback of the inhibitory signals

in order to promote muscle accretion (Rodríguez *et al.* 2015a). Follistatin also acts on the adipose tissue-inducing genes involved in adipogenesis and fat browning (*Pgc1a, Ucp1, Prdm16* and *Fabp4*) (Braga *et al.* 2014). Taken together, myostatin activity can be antagonized by follistatin, which promotes myogenesis and fat browning.

β-Aminoisobutyric acid

Roberts and colleagues recently identified BAIBA, a natural catabolite of thymine, in the screening of metabolites that were released to the culture media of myocytes overexpressing the PGC-1α transcription factor (Roberts *et al.* 2014). BAIBA exerts an autocrine/paracrine action on skeletal muscle fibres by: (i) increasing mitochondrial FFA oxidation, (ii) attenuating the impairment of IRS-1/Akt-mediated insulin signalling and (iii) reducing the inflammation *in vivo* through AMPK-PPARδ-dependent mechanisms (Roberts *et al.* 2014, Jung *et al.* 2015). The endocrine effects of BAIBA involve the reduction fat accumulation in mice through the stimulation of mitochondrial FFA oxidation and reduction of hepatic *de novo* lipogenesis via the activation of PPAR-α in the liver (Maisonneuve *et al.* 2004, Begriche *et al.* 2008, Roberts *et al.* 2014). In this regard, the improvement of non-alcoholic fatty liver disease (NAFLD) in obese children after treatment with the probiotic VSL#3 is associated with a decrease in urinary BAIBA levels (Miccheli *et al.* 2015). Moreover, the peripheral actions of BAIBA also include fat browning as BAIBA treatment increases the expression of thermogenic genes (*Pgc1a, Ucp1, Cidea* and *CytC*) in murine WAT (Roberts *et al.* 2014). In humans, circulating BAIBA levels are increased during exercise training and are inversely correlated with cardiometabolic risk factors (Roberts *et al.* 2014). Together, the identification of BAIBA as an exercise-triggered signal provides further information for understanding the protective role of exercise against the development of metabolic diseases (Kammoun & Febbraio 2014, Roberts *et al.* 2014).

Meteorin-like

A splice form of the gene encoding PGC-1α, termed PGC-1α 4, is induced by resistance training and promotes muscle hypertrophy and strength in mice and humans (Ruas *et al.* 2012). The muscle-specific PGC-1α 4 overexpression in mice stimulates the expression and secretion of a hormone called meteorin-like (also known as subfatin) (Li *et al.* 2014, Rao *et al.* 2014). Rao and colleagues reported that meteorin-like is induced by exercise in the skeletal muscle with the increase in circulating meteorin-like inducing an upregulation of genes involved in brown/beige fat thermogenic and mitochondrial programme (*Pgc1a, Ucp1, Dio2* and *Erra*) as well as anti-inflammatory cytokines IL-10 and TGF-β in WAT (Rao *et al.* 2014). This activation of fat browning is not the consequence of a direct effect of meteorin-like on adipocytes. Meteorin-like activates the secretion of IL-4 and IL-13 from the eosinophils embedded in WAT and promotes the activation of adipose tissue macrophages as well as the thermogenic programme (Rao *et al.* 2014). Regarding the potential role of meteorin-like in the regulation of inflammation, Ushach and colleagues found that meteorin-like is produced by alternatively activated M2 macrophages and M-CSF cultured bone marrow macrophages (M2-like macrophages), with its expression being increased in skin disease such as psoriasis, actinic keratosis or atopic dermatitis as well as in rheumatoid arthritis (Ushach *et al.* 2015). However, other authors did not observe differences in the gene expression of anti-inflammatory factors (IL-4, IL-10 and IL-13), thermogenic genes (*Pgc1a, Ucp1, Dio2* and *Erra*) as well as eosinophils and anti-inflammatory M2 macrophages markers (*Siglec F, Ccr3, Mrc1, Clec10a* and *Retnla*) in both adipocyte-specific meteorin-like (*Mtrnl*)-knockout mice and transgenic mice with an adipocyte-specific overexpression of the *Metrnl* gene (Li *et al.* 2015b). Thus, further studies are required to elucidate the real contribution of meteorin-like on fat browning and immunity.

Meteorin-like is not only a myokine, but also an adipokine (Li *et al.* 2014, Rao *et al.* 2014). The expression of meteorin-like is downregulated in white adipose tissue during caloric restriction, while being dramatically upregulated in the adipose tissue during adipocyte differentiation and diet-induced obesity in rodents (Li *et al.* 2014). Meteorin-like induces adipocyte differentiation and improves insulin sensitivity in adipocytes through PPAR-γ-dependent mechanisms (Li *et al.* 2015b). Adipocyte-specific *Mtrnl* knockout exacerbates insulin resistance induced by high-fat diet, while adipocyte-specific transgenic overexpression of *Metrnl* prevents insulin resistance induced by diet-induced obesity or leptin deletion.

Crosstalk between adipokines and myokines in fat browning

Exercise increases PGC-1α in the skeletal muscle, which, in turn, activates the expression and secretion of irisin, BAIBA and meteorin-like in myocytes. These myokines are released to the bloodstream and induce fat browning and energy expenditure. The next question is whether the adipose tissue secretes factors acting as positive/negative feedback signals, closing the myocyte–adipocyte circle. Among the plentiful factors released by the adipose tissue (Rodríguez *et al.* 2015b),

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interestingly, two important adipokines, FGF21 and leptin, act in an autocrine/paracrine manner regulating the browning process induced by irisin. The crosstalk between the adipose tissue and skeletal muscle is of considerable interest, since a dysregulation in the secretion and production of adipokines and myokines might contribute to the development of excess adiposity, favouring the onset of whole-body insulin resistance.

Fibroblast growth factor-21

FGF21, an atypical member of the FGF superfamily, is involved in the control of glucose homoeostasis (Kharitonenkov *et al.* 2005), insulin sensitivity (Wente *et al.* 2006), ketogenesis (Badman *et al.* 2007) as well as thermogenesis and fat browning in BAT and WAT (Hondares *et al.* 2011, Fisher *et al.* 2012). The human *FGF21* gene encodes a 209 amino acid protein that contains a 28 amino acid signal sequence and a 181 amino acid secreted polypeptide (Nishimura *et al.* 2000). FGF21 is abundantly expressed in the liver, and to a lower extent in the skeletal muscle, adipose tissue, pancreas and thymus, among others (Nishimura *et al.* 2000, Izumiya *et al.* 2008, Muise *et al.* 2008, Hondares *et al.* 2011). The cellular response to FGF21 is activated on its binding to FGF receptor 1c (FGFR1c) with the coreceptor β-Klotho, forming the ternary complex FGF21–FGFR1c–β-Klotho (Adams *et al.* 2012, Gallego-Escuredo *et al.* 2015, Giralt *et al.* 2015). Both BAT and WAT express high levels of the critical coreceptor β-Klotho and are sensitive to exogenous FGF21 stimulation (Hondares *et al.* 2011, Fisher *et al.* 2012). In this sense, FGF21 stimulates the browning of WAT and BAT through central (Douris *et al.* 2015) and local (Fisher *et al.* 2012) mechanisms to provide a robust defence against hypothermia. Adipocyte-derived FGF21 acts in an autocrine/paracrine manner to increase the expression of UCP1 and other thermogenic genes in response to cold exposure and β-adrenergic stimulation in both fat depots (Fig. 2) (Chartoumpekis *et al.* 2011, Hondares *et al.* 2011, Fisher *et al.* 2012, Lee *et al.* 2014). Accordingly, *Fgf21*-knockout mice shows larger BAT depots containing larger lipid droplets and display an impaired ability to adapt to chronic cold exposure, which diminished browning of WAT (Fisher *et al.* 2012). FGF21 secretion is induced by cold exposure and stimulated both basal and irisin-induced expression of beige genes in human neck adipocytes (Lee *et al.* 2014), confirming its role as an endocrine activator of BAT function also in humans. Interestingly, human obesity is associated with increased circulating FGF21 levels and with an abnormal decrease in the expression of β-Klotho coreceptor in WAT, suggesting a reduced sensitivity to FGF21 in the obese state (Gallego-Escuredo *et al.* 2015).

Skeletal muscle is also a source of FGF21 with its expression being regulated in a PI3K/Akt signalling pathway-dependent manner (Izumiya *et al.* 2008). A recent study showed that FGF21 is also induced by the integrated stress response in UCP1 transgenic mice expressing this uncoupling protein in skeletal muscle (Keipert *et al.* 2014). Myocytic FGF21 is also the major insulin-responsive myokine with its expression being increased in young healthy men during a hyperinsulinaemic–euglycaemic clamp (Hojman *et al.* 2009, Kim *et al.* 2013). Interestingly, palmitate suppresses the skeletal muscle transcription of FGF21 and other myokines (CTRP15 and irisin), which might contribute to the palmitate-induced insulin resistance in myotubes (Yang *et al.* 2013). Plasma FGF21 levels are increased in insulin-resistant states and correlated with hepatic and muscle insulin resistance (Chavez *et al.* 2009), suggesting a role of this hepatokine/adipokine/myokine in the pathogenesis of type 2 diabetes.

Leptin

Leptin is a 16-kDa peptide hormone encoded by the *OB* gene, which was discovered in 1994 (Zhang *et al.* 1994, Friedman & Mantzoros 2015). Leptin constitutes a marker of the amount of energy stores in the body, as circulating leptin is proportional to the amount of body fat, the main production site of the hormone (Maffei *et al.* 1995). Leptin decreases body weight by reducing food intake and by increasing energy expenditure and lipolysis to maintain energy balance (Frühbeck *et al.* 2014). After crossing the blood–brain barrier, leptin activates several hypothalamic nuclei involved in the regulation of feeding behaviour and energy balance including the arcuate nucleus (ARC), ventromedial hypothalamus (VMN) and dorsomedial hypothalamus (DMN) (Harvey & Ashford 2003). On binding its hypothalamic receptors, leptin stimulates a population of neurones containing the anorexigenic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), thereby decreasing food intake and body weight (Harvey & Ashford 2003). Moreover, leptin increases energy expenditure through the stimulation of sympathetic nerve activity in BAT (Scarpace *et al.* 1997). In this sense, leptin plays a crucial role in brown adipogenesis and non-shivering thermogenesis, as leptin deficiency is associated with an impaired BAT morphology and function (Becerril *et al.* 2010, 2012). Leptin also exerts an autocrine/paracrine effect on white adipocytes through the stimulation of lipolysis counteracting the adenosine deaminase-induced tonic inhibition (Frühbeck *et al.* 1997, 1998, 2001).

The ubiquitous distribution of leptin receptors (OB-R), which show structural resemblance to the class I cytokine receptor family, underlies the pleiotropic

effects of leptin (Tartaglia *et al.* 1995). In this regard, the skeletal muscle also constitutes a target for the metabolic effects of leptin (Sáinz *et al.* 2009, 2010, 2012, Rodríguez *et al.* 2015a) (Fig. 2). Leptin promotes AMPK-induced FFA oxidation, enhances GLUT4-mediated glucose uptake and reduces inflammation and oxidative stress in muscle fibres (Muoio *et al.* 1997, Sáinz *et al.* 2010, 2012). Moreover, leptin increases muscle mass by increasing myocyte cell proliferation and by reducing the expression of negative regulators of muscle growth including myostatin, dystrophin or atrophy markers MAFbx or MuRF1 (Sáinz *et al.* 2009, Hamrick *et al.* 2010, Rodríguez *et al.* 2015a). Interestingly, leptin upregulates *Fndc5* expression in the skeletal muscle and enhances irisin-induced myocyte proliferation as well as the muscle growth enhancers myogenin and myonectin, suggesting a synergic effect of both molecules on muscle accretion (Rodríguez *et al.* 2015a) (Fig. 3). It seems plausible that these effects of leptin are mediated via OB-Rb, as leptin receptor-deficient *db/db* or POUND $Lepr^{db/lb}$ mice reportedly show an impaired muscle regeneration (Nguyen *et al.* 2011b, Arounleut *et al.* 2013). Despite the direct action of leptin on FNDC5/irisin expression and function on skeletal muscle, serum irisin levels are unaltered in leptin-deficient *ob/ob* mice before and after exogenous leptin administration (Quiñones *et al.* 2015, Rodríguez *et al.* 2015a). The lack of changes in circulating irisin in leptin deficiency and after leptin replacement might be related to the current debate on whether the antibodies used to detect plasma FNDC5/irisin are valid or not (Erickson 2013, Raschke *et al.* 2013b, Boström *et al.* 2014, Jedrychowski *et al.* 2015).

The crosstalk of leptin and irisin is also extended to the adipose tissue (Gutierrez-Repiso *et al.* 2014, Rodríguez *et al.* 2015a). Contrary to what is observed in the skeletal muscle, leptin downregulates *Fndc5* expression in the subcutaneous adipose tissue of wild-type and leptin-deficient *ob/ob* mice (Rodríguez *et al.* 2015a). Moreover, leptin reduces irisin-stimulated *Ucp1* and *Cidec* transcription as well as the generation of UCP1-positive cells, suggesting a negative regulation on the phenotypic transdifferentiation towards beige adipocytes (Rodríguez *et al.* 2015a). Interestingly, the incubation of human subcutaneous adipose tissue explants with leptin also downregulates *FNDC5* transcript levels (Gutierrez-Repiso *et al.* 2014). This inhibitory effect of leptin may explain at least in part the decreased serum irisin concentration found in morbid obese patients, which are characterized by hyperleptinaemia.

Conclusions

During the last three decades, the existence of diverse 'organokines' (adipokines, myokines, hepatokines and osteokines) has been identified, which encompass factors produced and released exclusively or mainly by specific organs and tissues with relevant metabolic activity (Gómez-Ambrosi *et al.* 2008, Pedersen & Febbraio 2012, Stefan & Häring 2013, Rodríguez *et al.* 2015b). Since the discovery of leptin in 1994, adipokines have focused extensive research on the metabolic impact of circulating factors (Rodríguez *et al.* 2015b). However, the discovery of myokines has also provided a new basis to understand the molecular mechanisms underlying the beneficial effects of physical activity on the reduction of morbidity and mortality rates, due to the action of myokines in metabolically active tissues, such as the adipose tissue, liver or brain. Both skeletal muscle and adipose tissue act as endocrine organs individually, but growing evidence points to a crosstalk of their metabolic mediators, namely myokines and adipokines, underlining a more complex scenario in the metabolic dialogue between organs. In the present review, we have focused on the crosstalk of adipokines and myokines in the switch of the phenotype of energy-storing white adipocytes into energy-dissipating beige adipocytes (fat browning) (Bartelt & Heeren 2014). Further studies are needed regarding the potential impact of the dysregulation of adipokine and myokine secretion and/or function due to a sedentary lifestyle and muscle atrophy on the development of obesity and its associated pathologies, such as insulin resistance and type 2 diabetes, among others.

Conflict of interest

The authors have nothing to disclose.

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