Browning of white fat: does irisin play a role in humans?

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Abstract

The discovery of irisin as an exercise-regulated myokine inducing browning of WAT has gained interest as a potential new strategy to combat obesity and its associated disorders, such as type 2 diabetes. However, there are inconsistencies regarding the relevance of irisin in humans. The regulation of *FNDC5* mRNA expression by exercise and contraction could not be reproduced by a number of human studies using several exercise protocols and *in vitro* approaches. Furthermore, the nature of FNDC5 fragments and the presence of irisin in humans are questionable and probably contribute to conflicting data obtained with commercially available ELISA kits. Most importantly, the information regarding the concentration of circulating irisin in humans is not clear, as different studies using different kits measure irisin levels in a wide range. Data about the role of irisin in states of human obesity and metabolic diseases are conflicting and, in some cases, changes in irisin levels have been observed; they were only moderate in 10–20%. Independent of the presence and regulation of FNDC5/irisin in humans, the application of recombinant irisin could still represent a therapeutic strategy to fight obesity. However, the current data obtained from human cell models reveal that FNDC5/irisin has no effect on browning of the major WAT depots in humans and is likely to selectively target a small subpopulation of adipocytes, which are located in classical BAT regions, such as the supraclavicular adipose tissue. Thus, other candidates, such as BMP7 or CNPs, seem to be more prominent candidates as inducers of browning in humans.

Key Words
- adipocyte
- diabetes
- exercise
- obesity
- muscle

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Introduction

White adipose tissue (WAT) and brown adipose tissue (BAT) exert inverse functions in that WAT stores energy, whereas BAT, characterized by a large number of mitochondria containing uncoupling protein 1 (UCP1), mediates adaptive thermogenesis and contributes to the maintenance of body temperature. In 2009, five independent studies demonstrated the presence of BAT in adult humans (Cypess *et al.* 2009, van Marken Lichtenbelt *et al.* 2009, Saito *et al.* 2009, Virtanen *et al.* 2009, Zingaretti *et al.* 2009) and confirmed the existence of an inverse correlation between BMI and BAT activity. These observations have aroused considerable interest in the therapeutic potential of brown adipocytes for inducing weight loss, and recent data have confirmed that BAT oxidative metabolism contributes significantly to energy expenditure (Ouellet *et al.* 2012). The classical view of brown and white fat cells was modified in 2010 by a publication of the Cannon/Nedergaard group describing a third type of fat cells termed 'brite' (brown-in-white) adipocytes (Petrovic *et al.* 2010). These cells share biochemical features and the thermogenic potential with brown adipocytes, but are derived from different precursor cells (Seale *et al.* 2008).

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The possibility to differentiate adipose stem cells into brite adipocytes (browning of white fat) and to induce thermogenic activation and augment energy expenditure is currently considered as an important promising approach to combat obesity and obesity-related complications.

A novel hormone-like myokine termed irisin has recently been described to activate such a white-to-brown shift in adipocytes (Boström *et al*. 2012*a*). As this molecule was originally reported to be released after physical activity, it gained huge interest as a potential mediator of the health-promoting effects of physical exercise. Irisin is a 112 amino acid peptide cleaved from fibronectin type III domain containing protein 5 (FNDC5), a type I membrane protein which was claimed to be upregulated by exercise training in both mice and humans (Boström *et al*. 2012*a*). When writing this review, more than 100 papers dealing with irisin have been found in PubMed. However, the physiological role and the potential therapeutic value of irisin have remained highly controversial. In this review, we analyzed the current literature regarding exercise regulation and the functional impact of irisin in humans. We conclude that: i) the nature and concentration of circulating FNDC5 fragments remain unclear and ii) irisin has no effect on major WAT depots in humans and may only target a small subpopulation of adipocytes.

Impact of exercise on browning of white adipose tissue

As noticed before, the exercise-regulated myokine irisin was identified and described as a link between exercise and the promotion of WAT browning (Boström *et al*. 2012*a*). It is well known that the transcriptional coregulator PGC1α is induced in muscle in response to exercise in rodents and humans (Goto *et al*. 2000, Pilegaard *et al*. 2003). Therefore, transgenic mice overexpressing *Pgc1a* (*Ppargc1a*) in skeletal muscle can be used as an exercise model (Boström *et al*. 2012*a*). Interestingly, these transgenics exhibited increased expression of the brown marker genes *Ucp1* and cell death-inducing DFFA-like effector A (*Cidea*) only in the inguinal depot, suggesting that muscle activity promotes remodeling of subcutaneous WAT. Indeed, UCP1 expression was also strongly upregulated in the subcutaneous inguinal depot of mice after 3 weeks of wheel running.

From a physiological point of view, the induction of brown-like fat in response to exercise appears to be surprising due to several factors. First, exercise itself is an energy-consuming process and skeletal muscle is supplied with energy sources from other organs, such as free fatty acids released from WAT during physical activity. In this context, Kelly (2012) raised the question as to why physical activity would induce a program that burns fat stores, which are needed for the exercising muscle. Secondly, it has been discussed by Cannon & Nedergaard (2004) that there should be a diminished demand for non-shivering thermogenesis during exercise, as heat is generated by skeletal muscle contraction. In accordance, several studies on rats have demonstrated that treadmill running has no effect on *Ucp1* mRNA levels in BAT (Scarpace *et al*. 1994, Segawa *et al*. 1998, De Matteis *et al*. 2013) as well as on BAT mass (Segawa *et al*. 1998). The interscapular BAT of the *Pgc1a* transgenic mice used by Boström *et al*. (2012*a*) was also not altered when compared with WT mice. Furthermore, even a trend toward a decrease in *Ucp1* mRNA expression in BAT has been observed in rats after 3 weeks of endurance exercise (Roca-Rivada *et al*. 2013). By contrast, only one study demonstrated that treadmill exercise training of mice leads to enhanced expression of certain brown adipocyte-specific genes in BAT (Xu *et al*. 2011). Thus, exercise intervention is likely to have no effect on classical BAT in rodents. Regarding the browning of WAT, an increase in the number of mitochondria and enhanced expression of brown-specific genes in the visceral epididymal WAT of mice have been demonstrated after exercise (Xu *et al*. 2011). Another study carried out on rats also observed induction of UCP1 expression in the visceral but not the subcutaneous WAT after 3 weeks of training (Roca-Rivada *et al*. 2013). Boström *et al*. (2012*a*) demonstrated the most prominent effect of exercise on the subcutaneous WAT and the data obtained were in contrast to the two above-mentioned studies.

In addition to these studies on rodents, a potential induction of WAT browning in response to exercise remains to be clarified in case of humans. A recent study performing a 12-week training intervention in humans could not detect any alterations in WAT. Thus, expression of several brown adipocyte-specific marker genes, such as *UCP1* and *PRDM16*, were not significantly changed in subcutaneous adipose tissue after the intervention (Norheim *et al*. 2014). Hence, studies assessing the impact of exercise on WAT browning in rodents are conflicting, especially with regard to the WAT depot that undergoes remodeling in response to exercise. Furthermore, the browning of WAT in response to exercise has not yet been demonstrated in humans and, hence, further studies in humans are necessary.

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Exercise regulation of *FNDC5* mRNA in skeletal muscle: *in vivo* studies

In the initial report by Boström *et al.* (2012*a*), irisin is proposed to be a novel PGC1α-dependent and exercise-responsive myokine. This conclusion was based on the finding that skeletal muscle *Fndc5* mRNA levels from mice after 3 weeks of free wheel running were enhanced compared with sedentary mice (about 2.8-fold). Moreover, enhanced *Fndc5* expression was accompanied by increased *Pgc1a* mRNA levels (about 2.5-fold). Additionally, the expression levels of *FNDC5* and *PGC1A* were examined in muscle biopsies from human subjects before and after 10 weeks of endurance training. Expression of both genes was enhanced after the training session (about twofold) (Boström *et al.* 2012*a*).

After this initial description of FNDC5 and irisin as exercise-regulated proteins in mice and humans, *FNDC5* mRNA expression in skeletal muscle was analyzed in several human exercise cohorts (Table 1). Intriguingly, most of these studies performed in humans failed to reproduce enhanced *FNDC5* mRNA levels after exercise. The upregulation of FNDC5 in skeletal muscle by exercise was only demonstrated in four out of 15 studies, including the study by Boström *et al.* In this context, they reported the most prominent increase in *FNDC5* mRNA expression after exercise (twofold) (Boström *et al.* 2012*a*), whereas three other studies reported only a moderate increase of 1.3- to 1.4-fold (Timmons *et al.* 2012, Pekkala *et al.* 2013, Norheim *et al.* 2014).

Timmons *et al.* (2012) were the first unable to reproduce a substantial training-induced increase in *FNDC5* expression, neither in young men after endurance training nor in a different cohort (20–80 years old subjects) after resistance training. However, it is possible that *FNDC5* mRNA expression can be induced by exercise in a subset of individuals, as older active subjects had a 30% higher *FNDC5* expression than sedentary control subjects, while no difference was observed in younger subjects (Timmons *et al.* 2012). As FNDC5 has been described to be PGC1α-dependent (Boström *et al.* 2012*a*), this early study by Timmons *et al.* (2012) has been criticized for including exercise intervention studies without reported induction of *PGC1A* expression in muscle (Boström *et al.* 2012*b*). Thus, a lack of PGC1α induction in some of the exercise cohorts may explain the conflicting results.

In line with this suggestion, exercise intervention studies reporting increased muscle *PGC1A* expression would potentially clarify the relation between *FNDC5* and *PGC1A* mRNA expression in skeletal muscle. Recently, Norheim *et al.* (2014) have compared the effects of acute and chronic exercises and assessed the correlation between *PGC1A* and *FNDC5* expression in these exercise types. Therefore, a cohort of 13 sedentary men, aged 40–65 years, underwent a 12-week intervention of combined endurance and strength training. Muscle biopsies were taken after an acute endurance workload, both before (acute) and after (chronic) the 12-week intervention period. Interestingly, after 12 weeks of exercise, PGC1α was slightly activated (1.2-fold) and *FNDC5* mRNA levels were additionally increased (1.4-fold) (Norheim *et al.* 2014), being in line with the initial report by Boström *et al.* However, acute exercise intervention performed by the same subjects gave different results. Regardless of the prominent increase in *PGC1A* mRNA levels (7.4-fold), *FNDC5* mRNA expression levels did not differ at all (Norheim *et al.* 2014). Prominent effects on *PGC1A* mRNA expression have also been described in other acute exercise cohorts (twofold and fourfold (Pekkala *et al.* 2013)), whereas chronic exercise had a moderate effect (1.2-fold (Norheim *et al.* 2014) and 1.5-fold (Boström *et al.* 2012*a*)).

In conclusion, the regulation of *FNDC5* expression by exercise could not be reproduced by the majority of studies performed in humans (Table 1). Furthermore, even a strong increase in gene expression of the transcriptional coactivator *PGC1A* occurring after acute exercise does not necessarily lead to an activation of *FNDC5* expression (Pekkala *et al.* 2013, Norheim *et al.* 2014).

Exercise regulation of *FNDC5* mRNA in skeletal muscle: *in vitro* models

To further investigate the effect of PGC1α on FNDC5 expression in human skeletal muscle cells, *in vitro* studies were performed on primary human myotubes (Besse-Patin *et al.* 2014, Raschke *et al.* 2013, Norheim *et al.* 2014).

Treatment of primary human myotubes with drugs mimicking the activation of exercise signaling pathways, namely caffeine, ionomycin, and forskolin, significantly increased *PGC1A* expression. However, this induction of *PGC1A* mRNA expression was not accompanied by enhanced *FNDC5* expression. Indeed, *FNDC5* mRNA was reduced after incubating the myotubes for 24 h with these exercise mimetics (Norheim *et al.* 2014). In line with this, a second study also demonstrated significantly decreased *FNDC5* expression levels in primary human myotubes challenged with ionomycin and forskolin (Besse-Patin *et al.* 2014).

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Table 1 Human exercise cohorts analyzed for *FNDC5* mRNA expression in skeletal muscle after different modes of exercise

Study (reference)	**Patients/subjects**	***n***	**Exercise mode**	**Intervention**	**Main results**
Boström *et al.* (2012*a*)	Healthy adults	8	A	10 weeks of supervised endurance training	Twofold increase in *FNDC5* mRNA expression in muscle
Besse-Patin *et al.* (2014)	Obese, non-diabetic subjects	11	A	8 weeks of supervised endurance training	No change in *FNDC5* mRNA expression in muscle
Kurdiova *et al.* (2014)	Sedentary, obese individuals	16	A	12 weeks strength/endurance training	No change in *FNDC5* mRNA expression in muscle
	Sedentary vs trained	7 vs 8	B	1 h 75% of maximal capacity	No change in *FNDC5* mRNA expression in muscle
Norheim *et al.* (2014)	Normoglycemic, sedentary men	13	A	12 weeks combined endurance and strength training	1.4-fold increase in *FNDC5* mRNA expression in muscle
	Normoglycemic, sedentary men	13	B	45 min cycling at 70% $VO_{2\ max}$	No change in *FNDC5* mRNA expression in muscle
Pekkala *et al.* (2013)	Healthy, untrained male	17	B	1 h acute low-intensity aerobic exercise	No change in *FNDC5* mRNA expression in muscle
	Healthy, young male	10	B	Single resistance exercise bout	1.4-fold increase in *FNDC5* mRNA expression in muscle
	Healthy, old male	10	B	Single resistance exercise bout	No change in *FNDC5* mRNA expression in muscle
	Healthy, untrained middle-aged male	9	A	21 weeks combined endurance and resistance exercise	No change in *FNDC5* mRNA expression in muscle
Raschke *et al.* (2013)	Young, sedentary males	6	A	10 weeks of aerobic interval training	No change in *FNDC5* mRNA expression in muscle
	Young, sedentary males	7	A	11 weeks of strength training	No change in *FNDC5* mRNA expression in muscle
Timmons *et al.* (2012)	Young sedentary males)	24	A	6 weeks intense endurance cycling	No change in *FNDC5* mRNA expression in muscle
	20–80 years old subjects	43	A	20 weeks supervised resistance exercise study	No change in *FNDC5* mRNA expression in muscle
	Young vs older sedentary and age-matched endurance trained	10 vs 10	–	–	1.3-fold increase in *FNDC5* mRNA expression in muscle of older trained compared with older sedentary subjects

A, long-term exercise intervention; B, acute exercise.

To circumvent exercise mimetics as an *in vitro* exercise model, electrical pulse stimulation (EPS) has been performed to induce muscle contraction *in vitro*. This model is well established and primary human myotubes subjected to EPS are characterized by enhanced *PGC1A* mRNA expression, enhanced mitochondrial biogenesis as well as enhanced secretion of the well-known myokines IL6 and VEGFA (Lambernd *et al*. 2012). Moreover, this EPS model reflects a training model rather than acute exercise as shown by the enhanced *MYH1* mRNA level and enhanced mitochondrial content (Lambernd *et al*. 2012, Raschke *et al*. 2013). Similar to the results obtained with exercise mimetics, *FNDC5* mRNA expression was not significantly enhanced in primary human myotubes after EPS, although *PGC1A* expression was significantly increased (Raschke *et al*. 2013).

To sum up, all of these *in vitro* studies performed with primary human skeletal muscle cells failed to demonstrate an increase in *FNDC5* expression using several approaches to mimic exercise *in vitro*. The lack of PGC1α induction as a potential reason for the absence of augmented *FNDC5* expression can be excluded, as *PGC1A* gene expression was significantly enhanced in all models.

Taken all described human *in vivo* and *in vitro* studies together, discussing all these discrepancies between *PGC1A* activation and *FNDC5* mRNA expression, Norheim *et al*. (2014) speculated that *FNDC5* is not a direct PGC1α target gene but is rather upregulated in skeletal muscle *in vivo* via secondary mechanisms.

The initial characterization of a candidate myokine is frequently limited to the detection of mRNA expression in skeletal muscle tissue, as it has also been done for *FNDC5*. Moreover, determination of gene expression or protein level in skeletal muscle biopsies is critical, as besides skeletal muscle fibers, skeletal muscle contains extended layers of connective tissues, capillaries, and nerve cells among others. Thus, gene expression studies must be followed by the detection of the encoded protein specifically in skeletal muscle fibers, e.g., by additional immunostaining of the skeletal muscle tissue sections. Finally, for full validation of a protein as a myokine, secretion from skeletal muscle cells has to be demonstrated.

Exercise regulation of circulating irisin in humans

Key points in the study by Boström *et al*. (2012*a*) were that the irisin fragment was present in the plasma of mouse and humans and that circulating levels were enhanced following exercise, as observed for skeletal muscle *FNDC5* expression in this study. The presence of irisin protein in plasma was based on western blots using an antibody which detects the transmembrane segment of FNDC5 and thus would fail to detect the C-terminally cleaved, secreted irisin fragment (Erickson 2013, Raschke *et al*. 2013). However, later on, several ELISA assays to detect circulating irisin became commercially available and were used to quantify irisin concentrations in human exercise studies (Table 2).

Using these ELISA kits, some of these human studies reported moderately increased serum irisin levels after exercise intervention. Thus, Kraemer *et al*. (2014) reported transiently elevated circulating irisin levels in response to moderate aerobic exercise during the first hour after exercise (20% increase) (Kraemer *et al*. 2014). In line with this finding, Huh *et al*. (2012) observed a moderate increase in circulating irisin level 30 min after a sprint running session (18% increase) (Huh *et al*. 2012) and Norheim *et al*. (2014) demonstrated slightly increased irisin levels after 45 min cycling (20% increase) (Norheim *et al*. 2014). In contrast to *FNDC5* mRNA data, enhanced circulating irisin levels were found in acute exercise studies rather than in long-term training studies.

Despite these three reports, 12 out of 15 studies failed to demonstrate that exercise affects circulating irisin levels in humans, neither after an acute bout of exercise nor after chronic exercise training (Huh *et al*. 2012, Aydin *et al*. 2013, Hecksteden *et al*. 2013, Moraes *et al*. 2013, Pekkala *et al*. 2013, Kurdiova *et al*. 2014, Norheim *et al*. 2014). Recently, Hecksteden *et al*. (2013) have published a randomized control trial and focused on the serum irisin concentrations. Subjects performed two guideline-conforming training interventions, either endurance or strength endurance training for 26 weeks. Once again, a training-induced increase in circulating irisin levels could not be confirmed (Hecksteden *et al*. 2013). Interestingly, although Norheim *et al*. (2014) observed an increased expression of *PGC1A* and *FNDC5* after 12 weeks of exercise, this was not translated into chronically increased levels of irisin in plasma and training for 12 weeks even tended to reduce irisin levels.

Summarizing the above-mentioned ELISA results, the current data provide equivocal results for regulation of circulating irisin levels after exercise. Only some studies reported slightly increased irisin levels after acute exercise, while the majority of studies failed to reproduce the results obtained by Boström *et al*. (summarized in Table 2).

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Table 2 Human exercise cohorts analyzed for circulating irisin levels after different modes of exercise

Study (reference)	**Patients/subjects**	***n***	**Exercise mode**	**Intervention**	**Main result**
Boström *et al*. (2012*a*)	Healthy adults	8	A	10 weeks of supervised endurance training	WB with Abnova antibody – does not detect irisin
Hecksteden *et al*. (2013)	Healthy adults: Control Aerobic training Strength training	 39 23 40	A	26 weeks of supervised aerobic endurance or strength endurance training	No change in circulating irisin levels between groups
Huh *et al*. (2012)	Young, moderately trained, healthy males	15	B	1 week of exercise (2–3 sets of two 80-m sprints)	Circulating irisin levels were significantly induced (18%) 30 min after the exercise
	Young, moderately trained, healthy males	15	A	8 weeks of exercise (three times a week, 2–3 sets of two 80-m sprints)	No change in circulating irisin levels
Kraemer *et al*. (2014)	Healthy, young male	7	B	90 min treadmill exercise	Circulating irisin levels were significantly induced (20%) by 54 min exercise
Kurdiova *et al*. (2014)	Sedentary vs trained	7 vs 8	B	1 h 75% of maximal capacity	No change in circulating irisin levels
Moraes *et al*. (2013)	Hemodialysis patients	26	A	6 months supervised resistance exercise	No change in circulating irisin levels
Norheim *et al*. (2014)	Normoglycemic, sedentary men	13	A	12 weeks combined endurance and strength training	No change in circulating irisin levels
	Normoglycemic, sedentary men	13	B	45 min cycling at 70% $VO_{2\ max}$	1.2-fold increase in circulating irisin levels directly after exercise
Pekkala *et al*. (2013)	Healthy, untrained male	17	B	1 h acute low-intensity aerobic exercise	No change in circulating irisin levels
	Healthy, young male	14	B	Single resistance exercise bout	No change in circulating irisin levels
	Healthy, young male	10	B	Single resistance exercise bout	No change in circulating irisin levels
	Healthy, untrained middle-aged male	9	A	21 weeks high-intensity endurance exercise	No change in circulating irisin levels
	Healthy, untrained middle-aged male	9	A	21 weeks combined endurance and resistance exercise	No change in circulating irisin levels
Aydin *et al*. (2013)	Obese males vs healthy males	7 vs 7	B	45 min of moderate outdoor running (5.5 km/45 min)	No change in circulating irisin levels

A, long-term exercise intervention; B, acute exercise.

Impact of irisin on WAT browning

The idea of irisin as an exercise-regulated myokine in humans and the physiological role of exercise-mediated browning of WAT have been discussed in the previous section. Independent of the potential regulation of circulating irisin and skeletal muscle FNDC5 expression in response to acute or chronic exercise, the functionality of irisin in humans remains to be elucidated. Boström *et al*. (2012*a*) initially demonstrated that recombinant irisin and FNDC5 induce browning of WAT-derived murine preadipocytes *in vitro*. In the context of irisin as a therapeutic approach in the fight against obesity and its associated metabolic diseases, it is crucial to prove that irisin has an effect on white-to-brown transition in human cell models. Therefore, functional studies investigating the potency of irisin as an inducer of browning, performed in rodents and humans, will be discussed in this section.

After selection of secreted PGC1α- and exercise-regulated proteins/myokines in mice, Boström *et al*. (2012*a*) assessed their potential as inducers of browning and identified FNDC5 as a promising candidate. To study browning of WAT *in vitro*, murine preadipocytes were isolated from the inguinal fat depot and treated with commercially available recombinant FNDC5 (Abnova,

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Taiwan, China) during adipogenic differentiation. The inguinal depot is regarded as a subcutaneous and generally white fat depot, but has a high ability to undergo browning in response to a cold environment (Walden *et al*. 2012) and hormonal stimuli such as BMP7 (Schulz *et al*. 2011). Treatment of primary murine subcutaneous preadipocytes with 20 nM recombinant FNDC5 increased the expression of the BAT marker genes *Ucp1*, *Cidea*, and *Pgc1a* as well as oxygen consumption as a functional readout (Boström *et al*. 2012*a*). By contrast, FNDC5 failed to enhance brown marker genes in classical brown adipocytes isolated from the interscapular depot, suggesting tissue- and/or lineage-specific effects for FNDC5. The beneficial effect of irisin has also been demonstrated *in vivo*, as high-fat diet-induced obesity was reduced by adenoviral-mediated overexpression of FNDC5 in mice (Boström *et al*. 2012*a*).

The idea of a tissue-dependent impact of FNDC5 on browning was further elucidated by a second study from the same group. They proposed that brite or beige adipocytes display a distinct subpopulation within white adipose tissue and are highly responsive to β-adrenergic stimulation similar to classical brown adipocytes (Wu *et al*. 2012). In this context, *CD137* (*TNFRSF9*) and *TMEM26* were identified as selective cell surface marker genes highly expressed in brite adipocytes, but with a low expression in classical brown and white adipocytes. Interestingly, CD137-sorted cells from the stromal vascular fraction isolated from the inguinal depot of mice display a strong browning response toward 20 nM FNDC5 (Abnova) or 100 nM of the fusion protein irisin-Fc. On the other hand, cells expressing CD137 to a lesser extent did not show any significant response toward FNDC5 or irisin-Fc, indicating that only a subpopulation of preadipocytes isolated from the subcutaneous depot, which highly express CD137, is responsible for the browning effect of irisin (Wu *et al*. 2012).

Another study using murine 3T3-L1 adipocytes and primary rat adipocytes also observed a white-to-brown shift after exposure to 20 nM recombinant irisin, which was produced in yeast using the human irisin cDNA sequence (Zhang *et al*. 2014). Notably, the irisin-mediated increase in *Ucp1* mRNA expression was higher in 3T3-L1 adipocytes compared with primary rat adipocytes (sevenfold vs fourfold), indicating potential differences in the action of irisin between species. Unfortunately, *CD137* expression was not assessed in this study. In line with these results in murine and rat cell systems, a third study demonstrated the impact of irisin on browning of murine WAT. Treatment of primary murine preadipocytes isolated from the inguinal depot with 20 nM of a commercially available FNDC5 peptide (Abcam, ab117436) enhanced the expression of *Ucp1* and other brown marker genes (Shan *et al*. 2013). However, the synthetic peptide from Abcam used for this study corresponds to the C-terminal region of human FNDC5 and comprises the amino acids 149–178. This peptide is a part of the transmembrane domain and does not share any sequences with the irisin fragment, which comprises amino acids 32–143 (UniProt entry Q8NAU1). Therefore, the results observed by Shan *et al*. (2013) with this peptide are surprising and not related to the irisin fragment.

Summarizing the current data assessing the role of irisin in rodents, there is evidence for an impact of irisin on the browning of WAT. However, the function of irisin has not yet been validated in a human cell system and remains unclear. In addition to the previously described lineage-selective effect of FNDC5, potential differences in the molecular signature of adipose tissue depots between mice and humans have been proposed. Thus, Wu *et al*. (2012) observed that human adipose tissue from the supraclavicular region, and proven to be BAT positive by [^{18}F]FDG-PET/CT, shares the molecular signature of murine beige adipose tissue rather than murine classical BAT with a myogenic origin (Wu *et al*. 2012). However, three recent studies have provided evidence for the existence of classical brown adipocytes in humans, suggesting that the human neck adipose tissue depot is composed of both classical and brite adipocytes (Cypess *et al*. 2013, Jespersen *et al*. 2013, Lidell *et al*. 2013). In conclusion, these studies raise the question of whether results about browning obtained in murine models can be extrapolated to the human situation.

Relevance of irisin as an inducer of browning in humans

Studies investigating the functionality of FNDC5 and/or irisin in humans are rare and criticism has been raised regarding the relevance of irisin in humans. In this context, primary human preadipocytes isolated from the subcutaneous depot of different donors did not exhibit browning in response to irisin or FNDC5 (Raschke *et al*. 2013). The effects of three different recombinant proteins obtained from several sources (Phoenix, Abnova, and Caymen Chemicals) were compared in this study to exclude any potential differences between the suppliers. However, none of the proteins were able to induce browning, even when applied at high concentrations (1000 ng/ml for FNDC5 and 600 ng/ml for irisin).

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Moreover, preadipocytes isolated from donors highly expressing *CD137* strongly induced the browning program in response to BMP7, while no response toward irisin or FNDC5 was observed (Raschke *et al*. 2013).

Until recently, this was the sole study assessing the direct effect of FNDC5/irisin on browning in primary human adipocytes. More recently, a second study was published investigating the potency of FNDC5 to induce browning in primary human adipocytes from different depots. Lee *et al*. (2014) demonstrated a strong induction of several brown marker genes in primary human adipocytes isolated from neck biopsies after FNDC5 treatment for 6 days. Moreover, FNDC5 treatment of neck adipocytes increased basal and uncoupled oxygen consumption rates and FNDC5-treated cells were able to respond to β-adrenergic stimulation, a crucial feature of brown adipocyte functionality. As the myogenic marker *ZIC1* was not detectable, these neck adipocytes represent brite adipocytes rather than the classical brown ones. Interestingly, the effect of FNDC5 was only marginal or completely absent in primary adipocytes isolated from the subcutaneous and omental depots (Lee *et al*. 2014). In line with the study by Raschke *et al*. (2013), adipocytes from the subcutaneous depot do not appear to be able to undergo browning in response to FNDC5/irisin. One explanation could be the low expression of the brite-specific marker genes *TMEM26* and *CD137* in the subcutaneous depot compared with deep neck adipose tissue depots (Cypess *et al*. 2013, Lee *et al*. 2014). However, high *CD137* expression in human subcutaneous adipose tissue was not associated with a browning effect of FNDC5/irisin (Raschke *et al*. 2013).

The current data dealing with the effect of FNDC5/irisin on browning in humans lead to the suggestion that the sensitivity toward FNDC5/irisin is potentially dependent on the adipocyte lineage and in consequence the adipose tissue depot and the species (Fig. 1). Thus, FNDC5/irisin does not activate classical brown adipocytes (Boström *et al*. 2012*a*) and has no effect on pure white adipocytes (Wu *et al*. 2012, Raschke *et al*. 2013, Lee *et al*. 2014). It is likely that only a small subpopulation of adipocytes, which are highly expressing brite-specific markers, is responsible for the irisin effect (Wu *et al*. 2012). In this case, the relevance of irisin in humans and its potency as a pharmacological agent to treat obesity is questionable, as the major human adipose tissue depots (subcutaneous and omental) are pure white depots with a low expression of brite-specific markers and do not undergo browning upon FNDC5/irisin treatment. Probably, a subpopulation of cells present in deep neck adipose tissue from humans, which is suggested to be composed of classical brown and brite adipocytes, responds to FNDC5/irisin. However, the contribution of these cells to whole-body energy expenditure is not clearly determined. Moreover, other candidates seem to be more promising and have been shown to induce browning in human subcutaneous adipocytes, such as CNPs (Bordicchia *et al*. 2012) or BMP7 (Schulz *et al*. 2011, Elsen *et al*. 2014).

Role of irisin in metabolic diseases

In addition to the discovery of irisin as an exercise-regulated myokine, which induced browning of WAT, Boström *et al*. (2012*a*) demonstrated the beneficial effects of irisin on whole-body metabolism. Thus, adenoviral-mediated FNDC5 overexpression led to browning of WAT in lean mice as well as diet-induced obese mice. In addition, a moderate increase in circulating irisin levels by threefold augmented energy expenditure, reduced the body weight gain under high-fat diet, and improved diet-induced insulin resistance (Boström *et al*. 2012*a*). These results suggested a potential protective role of irisin in the development of type 2 diabetes, one of the major obesity-associated metabolic diseases. In order to investigate the relevance of irisin in humans, many clinical studies have focused on the relation between circulating irisin levels and metabolic parameters and diseases. The following section will give a comprehensive overview about the correlation of circulating irisin with metabolic parameters in humans and highlight existing discrepancies (Table 3).

As irisin has initially been described to protect against diet-induced weight gain, mediated by browning of WAT and thus increased energy expenditure, many studies have investigated the correlation of circulating irisin with obesity in humans. In line with the suggested protective role of the myokine irisin in the development of obesity, negative correlations of circulating irisin levels with the BMI have been reported in humans (Aydin *et al*. 2013, Choi *et al*. 2013, Moreno-Navarrete *et al*. 2013, Polyzos *et al*. 2014). However, controversy exists regarding the relation between irisin levels and the BMI. Several studies reported a positive correlation of serum irisin levels with BMI (Liu *et al*. 2013, Stengel *et al*. 2013, Crujeiras *et al*. 2014*a*, Liu *et al*. 2014, Park *et al*. 2014) or could not detect a change in circulating irisin in obesity (Huh *et al*. 2012, Gouni-Berthold *et al*. 2013, Kurdiova *et al*. 2014). This could be related to different populations analyzed in the different studies, as some include obese subjects without

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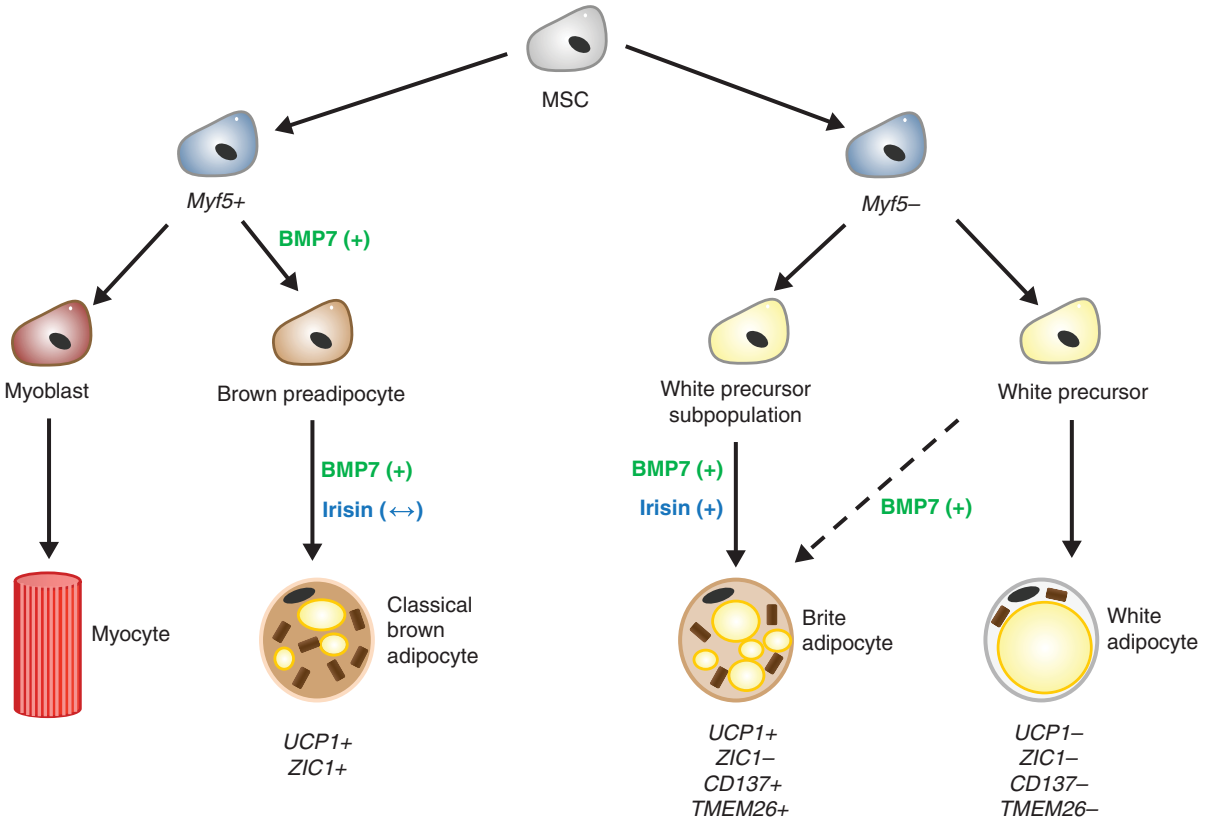

Figure 1
Classical brown adipocytes share a developmental origin with skeletal muscle cells and are derived from *Myf5*+ precursor cells. BMP7 is involved in lineage determination as well as differentiation of classical brown adipocytes which express *UCP1* and myogenic markers like *ZIC1*, while irisin does not promote classical brown adipocyte differentiation. White adipocytes develop from *Myf5*− precursor and give also rise to beige adipocytes in response to certain stimuli, such as BMP7. Only a subpopulation of white precursor cells, potentially characterized by high expression of beige markers such as *CD137* and *TMEM26*, is likely to undergo browning in response to irisin, as proposed by Wu *et al.* (2012). (+), positive effect; (↔), no effect.

metabolic disorders whereas others enclose obese patients with metabolic diseases such as type 2 diabetes.

However, recent intervention studies provide evidence for a positive correlation between BMI and circulating irisin levels, which is in conflict with the proposed anti-obesity effect of irisin. The earliest intervention study by Huh *et al.* (2012) assessed morbidly obese subjects undergoing gastric banding or gastric bypass and blood samples were collected at baseline and 6 months after surgery. Bariatric surgery led to a significant weight loss after 6 months, which was accompanied by decreased circulating irisin levels. These lower irisin levels were attributed to a lower fat-free mass and decreased *FNDC5* mRNA expression in skeletal muscle (Huh *et al.* 2012). However, the reduction of fat mass was not analyzed as a factor causing lower circulating irisin levels. The recent observation that irisin is not only a myokine but also an adipokine (Roca-Rivada *et al.* 2013) raises the question if circulating irisin can be solely attributed to skeletal muscle. Moreover, secretion of irisin is higher from WAT of diet-induced obese rats compared with lean controls (Roca-Rivada *et al.* 2013), suggesting that adipose tissue, especially in states of obesity, represents an important source of irisin besides skeletal muscle. Two other interventional studies, both with 8 weeks of hypocaloric diet, observed decreased irisin levels after weight loss (de la Iglesia *et al.* 2013, Crujeiras *et al.* 2014*b*). In addition to a positive association between irisin and BMI, irisin has been described to be positively associated with waist circumference and fat mass. (Crujeiras *et al.* 2014*a*). In summary, there is stronger evidence for a positive correlation of circulating irisin with adiposity markers. Nevertheless, data are still conflicting and it is not clear which organ is the major source of irisin.

Besides the association of irisin with obesity, many studies assessed the regulation of irisin in type 2 diabetes

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Table 3 Correlation of circulating irisin with metabolic parameters

Metabolic parameters	Correlation with circulating irisin	References
BMI	Positive correlation (T2DM cohort)	Liu *et al*. (2014)
	Positive correlation (non-diabetics)	Liu *et al*. (2013)
	Positive correlation (cross-sectional)	Park *et al*. (2014)
	Positive correlation	Stengel *et al*. (2013)
	Positive correlation	Crujeiras *et al*. (2014*a*)
	↑ (before vs after weight loss)	de la Iglesia *et al*. (2013)
	Negative correlation (non-diabetics)	Moreno-Navarrete *et al*. (2013)
	Negative correlation	Choi *et al*. (2013)
	↓ (obese vs lean)	Polyzos *et al*. (2014)
	↓ (obese vs lean)	Aydin *et al*. (2013)
	No correlation	Huh *et al*. (2012)
	No correlation	Gouni-Berthold *et al*. (2013)
T2DM	↓ (T2DM vs lean)	Kurdiova *et al*. (2014)
	↓ (T2DM vs lean)	Choi *et al*. (2013)
	↓ (T2DM vs lean)	Liu *et al*. (2013)
	↓ (T2DM with renal insufficiency vs T2DM)	Liu *et al*. (2014)
GDM	↑ (prior GDM vs no GDM)	Ebert *et al*. (2014*b*)
MetS	↑ (MetS vs lean)	Park *et al*. (2013)
HOMA-IR	Positive correlation	Park *et al*. (2013)
	Positive correlation	Ebert *et al*. (2014*a*)
	Negative correlation (girls)	Al-Daghri *et al*. (2013)
NAFLD	No difference (NAFLD obese vs obese)	Polyzos *et al*. (2014)
	↓ (NAFLD obese vs obese)	Zhang *et al*. (2013)
CKD	↓ (CKD vs control)	Wen *et al*. (2013)
	↓ (CKD stage 5 vs CKD stage 1)	Ebert *et al*. (2014*a*)

T2DM, type 2 diabetes; GDM, gestational diabetes mellitus; MetS, metabolic syndrome; HOMA-IR, homeostasis model assessment-estimated insulin resistance; NAFLD, non-alcoholic fatty liver disease; CKD, chronic kidney disease.

and other metabolic diseases. Similar to the conflicting observations regarding BMI, which have been discussed above, controversy exists. While some studies reported decreased irisin levels in type 2 diabetic subjects compared with lean subjects (Choi *et al*. 2013, Liu *et al*. 2013, Kurdiova *et al*. 2014) and a negative correlation of HOMA-IR with circulating irisin levels in girls (Al-Daghri *et al*. 2013), other studies demonstrated a positive correlation of circulating irisin with HOMA-IR (Park *et al*. 2013, Ebert *et al*. 2014*a*). In line, increased irisin levels were found in subjects with metabolic syndrome (Park *et al*. 2013) and higher circulating irisin concentrations have been reported in mothers with prior gestational diabetes (GDM) compared with those without prior GDM (Ebert *et al*. 2014*b*). Besides, reduced circulating irisin levels have been demonstrated in patients with chronic kidney disease (CKD) (Zhang *et al*. 2013) and non-alcoholic fatty liver disease (NAFLD) (Wen *et al*. 2013, Ebert *et al*. 2014*a*).

In addition to the different study populations, the different assays used to detect irisin may be a reason for these controversial results. Criticism regarding the reliability of commercially available irisin/FNDC5 antibodies and non-validated ELISA kits has already been raised (Erickson 2013). In this context, we summarized the cohort, the irisin concentrations measured, and the assay used in each study to gain an overview about the range of detected irisin concentrations (Supplementary Table 1, see section on supplementary data given at the end of this article). Interestingly, there is a wide range of irisin concentrations measured, from very low concentrations of 38.86 ± 2.48 pg/ml (Choi *et al*. 2013) to relatively high concentrations of 2157.9 ± 600.7 ng/ml (Moreno-Navarrete *et al*. 2013) in lean individuals. The concentrations measured in these different studies differ strongly, with about 50 000 times higher concentrations measured in the study by Moreno-Navarette *et al*. compared with those values originating from Choi *et al*. This tremendous difference between the circulating irisin concentrations indicates that at least some of the available ELISAs are unspecific and may have a high cross-reactivity to other proteins present in serum and plasma. Moreover, striking differences are observed even when using the same kit (Supplementary Table 1).

Is the irisin fragment present in humans?

Based on the huge variation in the current studies assessing circulating irisin concentrations in humans,

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the physiological circulating irisin levels in humans remain unclear. Moreover, the term irisin is often incorrectly and indistinctly used with FNDC5. The secretion mechanism of irisin by extracellular cleavage of FNDC5 proposed by Boström *et al.* (2012*a*) and the presence of a 12.6 kDa irisin fragment (theoretical molecular weight of the irisin chain amino acids 32–142 according to UniProt entry Q8NAU1) in the circulation has not been proven. As mentioned previously, for the initial description of irisin. an antibody raised against a peptide corresponding to amino acids 149–178 and thus mainly located in the transmembrane domain was used (Erickson 2013, Raschke *et al.* 2013). This antibody (ab117436, Abcam) is not predicted to detect irisin and is no longer available at Abcam. Nevertheless, a band of 22 kDa was designated to irisin, which was detected in western blot of deglycosylated cell media from HEK293 cells transfected with *FNDC5* (Boström *et al.* 2012*a*). This molecular weight of 22 kDa is more likely to reflect full-length FNDC5 (23.7 kDa, according to Q8NAU1) than irisin (12.6 kDa, see above), which would not be in conflict with the used antibody. A later study by Roca-Rivada *et al.* (2013) used two different antibodies, one solely against full-length FNDC5 (Abcam, raised against amino acids 149–178) and the other also detecting the irisin fragment (Phoenix Pharmaceuticals, raised against amino acids 42–142), to discriminate between these two proteins. With both antibodies, a band of 25 kDa was observed in conditioned media from rat skeletal muscle corresponding to full-length FNDC5. Interestingly, no additional band at the predicted lower molecular weight of irisin was observed with the anti-irisin antibody (Roca-Rivada *et al.* 2013), questioning the release of the initially described irisin fragment. Recently, a mass spectrometry approach has been used to clarify the identity of FNDC5-immunoreactive bands in human serum samples, which underwent deglycosylation. Several bands were observed with an anti-FNDC5 antibody from Abcam (ab131390) detecting both FNDC5 and the irisin fragment; but exclusively in a band of 24 kDa, a unique peptide located in the irisin sequence was identified by mass spectrometry, confirming that this band represents FNDC5 or fragments of FNDC5 (Lee *et al.* 2014). Unfortunately, this band was also designated as the irisin fragment, although the molecular weight is 24 kDa and the band could also represent other forms of FNDC5. Summarizing these studies, it remains unclear which fragments of FNDC5 are present in the circulation of humans.

Additionally, sequence differences between species have been reported. FNDC5 has already been described in 2011 to contain a non-canonical ATG start codon in humans (Ivanov *et al.* 2011). In line, multi-species alignment of the *FNDC5* exon 1 revealed that the canonical ATG start site is conserved in mouse, rat, gorilla, and chimp, but displays a mutation in the human sequence to ATA (Raschke *et al.* 2013). *In vitro* studies demonstrated that the translational efficiency of full-length *FNDC5* is strikingly reduced in this case and in consequence also the release of FNDC5 fragments. Usage of the next in-frame ATG as an alternative start site would lead to a truncated form of FNDC5 lacking the first 76 amino acids (Raschke *et al.* 2013). Interestingly, the specific fragment in the FNDC5-immunoreactive band with a molecular weight of 24 kDa identified by mass spectrometry (Lee *et al.* 2014) is a part of this truncated form (represented by NP_715637) described by Raschke *et al.* Taken together, FNDC5 fragments are present in humans, but the nature and the secretion mode have to be clarified in future studies and the presence of the initially described irisin peptide in humans is unclear.

Summary and conclusion

The discovery of irisin as an exercise-regulated myokine inducing browning of WAT has gained interest as a potential new strategy to combat obesity and its associated disorders, such as type 2 diabetes. However, there are inconsistencies regarding the relevance of irisin in humans. The regulation of *FNDC5* mRNA expression by exercise and contraction could not be reproduced by a number of human studies using several exercise protocols and *in vitro* approaches. Furthermore, the nature of FNDC5 fragments and the presence of irisin in humans are questionable and probably contribute to conflicting data obtained with commercially available ELISA kits. Most importantly, the information regarding the concentration of circulating irisin in humans is not clear, as different studies using different kits measure irisin levels in a wide range. Data about the role of irisin in states of human obesity and metabolic diseases are conflicting and, in some cases, changes in irisin levels have been observed; they were only moderate with 10–20%. Independent of the presence and regulation of FNDC5/irisin in humans, the application of recombinant irisin could still represent a therapeutic strategy to fight obesity. However, the current data obtained from human cell models reveal that FNDC5/irisin has no effect on browning of the major WAT depots in humans and is likely to selectively target a small subpopulation of adipocytes, located in classical BAT

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regions, such as the supraclavicular adipose tissue. Thus, other candidates, such as BMP7 or CNPs, seem to be more prominent candidates as inducers of browning in humans.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-14-0189.

Declaration of interest

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

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