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From white to brown fat through the PGC-1α-dependent myokine irisin: implications for diabetes and obesity

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Summary and comment on a recent *Nature* paper entitled 'A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis' (Boström et al., 2012).

The benefits of good diet and exercise have been extensively documented, and are the cornerstones for non-pharmacological treatment of cardiovascular and metabolic diseases (Dunstan, 2011). However, the molecular mechanisms by which exercise exerts its positive effects remain largely unexplained. An ongoing challenge in the field is to identify these mechanisms, enabling the development of exercise-mimetic drugs (Narkar et al., 2008).

A well-described effect of exercise is 'browning' of white adipose tissue (WAT) – that is, exercise increases the relative amount of brown adipose tissue (BAT). In contrast to the primarily fat-storing function of WAT, BAT has non-shivering thermogenic properties owing to expression of uncoupling protein-1 (UCP1) and increased mitochondrial content (Handschin and Spiegelman, 2008; Lidell and Enerbäck, 2010; Ravussin and Galgani, 2011). In addition, higher BAT levels are associated with resistance to metabolic diseases. Induction of a browning programme in rodents reduces body weight and improves glucose homeostasis (Zhou et al., 2003). Although it was previously thought that, in humans, BAT regresses with age to an almost non-existent brown depot in adults, recent evidence has proven otherwise (Virtanen et al., 2009; van Marken Lichtenbelt et al., 2009; Zingaretti et al., 2009). Importantly, however, van Marken Lichtenbelt et al. showed that the amount of BAT is significantly lower in overweight or obese subjects, and that there is a negative relationship between BAT and both body mass index and percentage of body fat (van Marken Lichtenbelt et al., 2009). Therefore, it is clear that BAT is both present and functional in adult humans, and that lower amounts of BAT are associated with obesity. An exciting new study from the Spiegelman laboratory has now uncovered a molecular mechanism by which exercise-induced browning can occur, involving a newly identified PGC-1α-induced myokine (Boström et al., 2012) (Fig. 1).

PGC-1α is a transcriptional coactivator that regulates genes in response to nutritional and physiological cues (Finck and Kelly, 2006). Exercise (particularly chronic) is accompanied by increased muscle expression of PGC-1α, whereas type 2 diabetes or a sedentary lifestyle are associated with reduced expression [see Handschin and Spiegelman, and references therein (Handschin and Spiegelman, 2008)]. Increasing the muscle expression of PGC-1α in mice protects against weight gain, inflammation, oxidative stress, muscle wasting and bone loss. Moreover, increasing PGC-1α expression improves metabolic parameters such as insulin sensitivity and insulin signalling (Wenz et al., 2009). However, it is still largely unknown how a single transcriptional coactivator can mediate such broad systemic effects and, particularly, how muscle-specific expression can influence whole-body metabolism. Boström et al. shed light on this issue by identifying a muscle-derived secreted factor that mediates systemic effects (Boström et al., 2012).

First, Boström and colleagues identified that muscle-specific overexpression of PGC-1α induced a brown-like adipose tissue gene programme. Mice overexpressing PGC-1α showed markedly increased levels of BAT-associated transcripts, including *Ucp1* and *Cidea*, in the subcutaneous inguinal fat layer, correlating with increased UCP1 expression in WAT (Boström et al., 2012). A similar genetic profile was induced by 3 weeks of wheel running, indicating that muscle-specific PGC-1α expression and exercise both triggered a 'browning' programme in WAT (Boström et al., 2012).

To determine the mechanism behind this effect, Boström et al. treated subcutaneous adipocytes with either control medium or medium from cells ectopically expressing PGC-1α (Boström et al., 2012). The latter induced a brown-like genetic programme, suggesting that a secreted molecule from the muscle cells was responsible for inducing browning of WAT.

To identify the secreted molecule, the authors combined Affymetrix gene expression arrays and an algorithm to predict secreted peptides. This led to the identification of five candidates: IL-15, FNDC5, VEGF-β, LRG1 and TIMP4. Importantly, these factors were also upregulated in the muscles of mice and humans after endurance exercise. Additional experiments implicated FNDC5 as the most likely candidate mediating the browning effect; its action was stronger than the known inducer, BMP7. In vitro, FNDC5-treated undifferentiated white adipocytes became UCP1-positive, and showed increased mitochondrial density and mitochondrial gene expression. FNDC5 treatment also augmented oxygen consumption and energy expenditure, with the majority of respiration being uncoupled.

Next, Boström et al. addressed how muscle FNDC5, a type I transmembrane protein, gets into the adipose tissue, and determined that FNDC5 is C-terminally cleaved and secreted into the media (Boström et al., 2012). They named this previously undescribed portion of FNDC5 irisin, after the Greek messenger goddess Iris.

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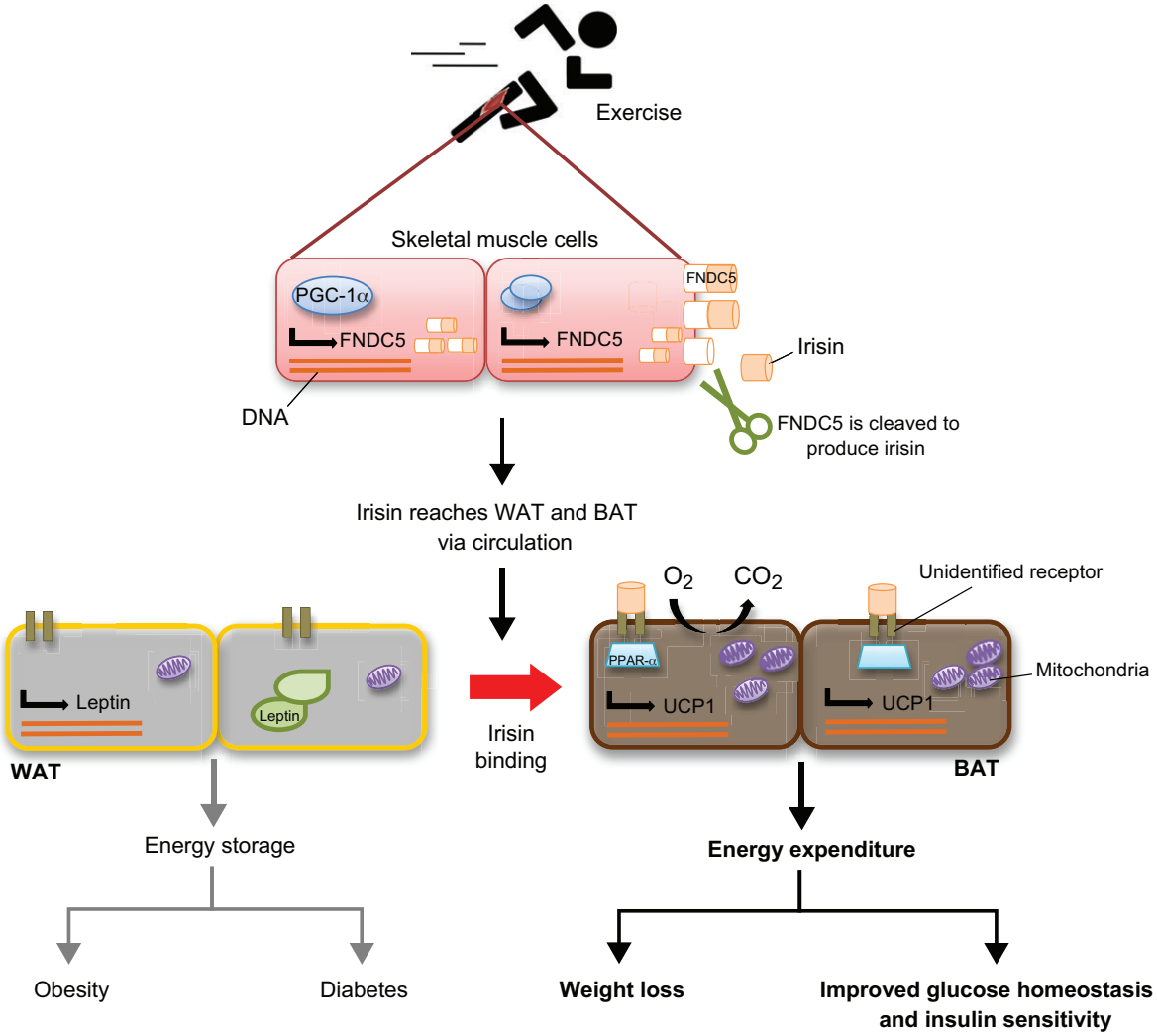

Fig. 1. Exercise-induced adipose tissue browning through PGC-1α and irisin. Exercise increases the expression levels of PGC-1α in the muscle. This, in turn, upregulates the expression of FNDC5, a type I membrane protein, which is C-terminally cleaved and secreted as irisin into the circulation. Binding of irisin to an unknown receptor on the surface of adipocytes in WAT changes their genetic profile. In particular, irisin induces the expression of PPAR-α, which is thought to be an intermediate downstream effector that increases the expression of UCP1 (highly expressed in BAT and a marker of browning). The browning of WAT is associated with augmented mitochondrial density and oxygen consumption. Browning is accompanied by an increase in the energy expenditure profile, leading to favourable effects on metabolism.

To determine whether irisin was involved in mediating the PGC-1α-induced browning of WAT, they treated medium from PGC-1α-expressing muscle cells with anti-FNDC5 before supplementing it to adipocytes. This treatment completely blocked the induction of the browning programme.

Boström and colleagues found irisin in the plasma of wild-type mice, and showed that muscle-specific knockout of PGC-1α decreased irisin levels by 72%. Notably, they also identified a band of similar electrophoretic mobility in the plasma of healthy human subjects. After 3 weeks of free wheel running, plasma irisin levels had increased by 65% in mice and, correspondingly, plasma irisin levels were found to double in healthy humans after 10 weeks of endurance exercise.

The authors also investigated at the molecular level how irisin induces browning; in particular, they analysed the upregulation of UCP1 expression. Gene arrays indicated that one possible mechanism might be increased expression of PPAR-α. PPAR-α is a member of the PPAR family of ligand-activated receptors, which have become relevant as therapeutic targets owing to their roles in lipid and glucose metabolism and vascular biology (Hiukka et al., 2010). FNDC5 induced a threefold increase in *Ppara* mRNA levels in white adipocytes differentiated from stromal vascular cells. Moreover, pharmacological inhibition with a PPAR-α-selective antagonist limited the induction of the browning programme by FNDC5, indicating that the increased expression of UCP1 and browning of WAT by FNDC5 are at least partially mediated via PPAR-α.

Boström et al. then evaluated whether FNDC5 was functional in vivo, and whether it could improve disease in a mouse model of diet-induced obesity and insulin resistance (Boström et al., 2012). They first established in control mice that delivery of FNDC5 by adenoviral vectors increased *Fndc5* mRNA by 15-fold and plasma levels of irisin by three- to fourfold. At 10 days post-injection, a 13-fold increase in *Ucp1* mRNA levels was

detected, accompanied by significantly higher expression of *Cidea*. Next, they showed that delivery of FNDC5-expressing adenovirus to high-fat-fed C57BL/6 mice showed similar browning patterns. Importantly, this modest increase of irisin (threefold) was accompanied by increased oxygen consumption, a slight but significant reduction in body weight, improved glucose tolerance and reduced fasting insulin levels.

Finally, Boström and colleagues tested whether irisin is necessary for the exercise-induced gene-browning programme by injecting anti-FNDC5 antibody into mice before swimming training and showed that this caused a complete blockade of the response, as measured by *Ucp1* and *Cidea* mRNA levels. Hence, in addition to inducing a browning genotype and phenotype that improves the metabolic parameters in a disease model, irisin is also required for the exercise-induced browning of WAT.

In summary, Boström et al. showed that increased levels of PGC-1α in muscle cells induced the expression of the type I membrane protein FNDC5, which is cleaved and secreted into the circulation (Boström et al., 2012). The secreted portion of FNDC5, a newly identified myokine known as irisin, binds to undetermined receptors on the surface of WAT. By an incompletely understood mechanism, irisin induced the expression of Ucp1 and other BAT-associated genes, partly via increased PPAR-α expression. Thus, irisin functions as a muscle-derived energy-expenditure signal that directly communicates with adipose tissue and induces browning. This effect improved the tissue metabolic profile and increased whole-body energy expenditure, making irisin a potential new target for the treatment of metabolic diseases (Fig. 1).

An important next step will be to identify the specific molecular pathways that underlie browning. Boström and colleagues identified PPAR-α as a key factor for the expression of UCP1, but there are probably other proteins involved. For example, another member of the PPAR family has been implicated as a candidate for browning. Recently, Ohno et al. showed that PPAR-γ ligands, coupled with PRDM16 (a determinant for the development of BAT), could switch subcutaneous white adipose cells to a BAT programme (Ohno et al., 2012). Another important observation was recently made by Ortega-Molina et al., who determined that inhibition of PI3K signalling by overexpression of its natural repressor, PTEN, can cause browning of WAT (Ortega-Molina et al., 2012). It will be thought provoking to address whether and how different browning programmes can work together. Moreover, other molecules such as AMPK and PPAR-δ have been put forward as exercise mimetics (Narkar et al., 2008), so it will be interesting to explore these in the context of irisin.

The implications of these findings for metabolic diseases are broad and exciting, particularly because the authors showed that irisin is present in humans and increases in response to endurance exercise. Although browning of WAT has not been described in humans, the existence of functional BAT depots in adults suggests that the conversion observed in rodents might be conserved. Reflecting the animal studies presented by Boström et al., could FNDC5 (or irisin) be delivered in humans in a similar way to increase irisin plasma levels? However, it will be crucial to investigate other potential effects of circulating irisin, beyond those described by Boström et al., on other metabolic tissues such as hepatocytes, and smooth or cardiac muscle.

In summary, this study will undoubtedly stimulate more PGC-1α–FNDC5–irisin research, and it will be stimulating to see whether this axis is part of the next generation of therapeutics for insulin-related diseases and obesity. Furthermore, PGC-1α is known to benefit tissues that do not have a primary metabolic function, such as the brain (Lin et al., 2004; Cui et al., 2006; Castillo-Quan, 2011). Thus, might FNDC5 and irisin be relevant for neurodegenerative diseases? The benefits of exercise on the brain are increasingly recognised, so it would be interesting to determine whether the muscle-derived FNDC5 can signal to the brain in an endocrine manner, perhaps improving brain function.

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