Browning fat

By Joanne Kotz, Senior Editor

Dana-Farber Cancer Institute researchers have identified a new hormone dubbed irisin that is induced by exercise and triggers the conversion of white fat to brown-like fat in mice, leading to increased energy expenditure.¹ **Ember Therapeutics Inc.** has licensed the findings and is generating stabilized versions of irisin in preparation for clinical trials in obesity and type 2 diabetes.

In a separate study, **Harvard University** researchers have developed a method for converting human pluripotent stem cells into white and brown adipocytes.² The Harvard team is collaborating with **Roche** to use the cell lines for multiple screens, including looking for molecules that promote brown-like phenotypes in white adipocytes.

There are two types of fat—white and brown. White adipose tissue (WAT) stores energy as triglycerides, whereas brown adipose tissue (BAT) metabolizes triglycerides to generate heat. The mobilization of triglycerides by BAT helps control weight and overall metabolic status.

BAT was long known to exist in rodents and human infants, but it was not until 2009 that a trio of papers in *The New England Journal of Medicine* identified BAT in human adults.^{3–5}

The hope is that molecules that activate brown fat or induce the conversion of white fat to brown-like fat could help treat metabolic diseases such as obesity and diabetes.

Muscling in

A team led by Bruce Spiegelman, professor in the Department of Cell Biology at Dana-Farber, was intrigued by the previous observation that transgenic mice with increased levels of peroxisome proliferation-activated receptor- γ coactivator 1 α (Ppargc1a; Pgc-1 α) in muscle were

resistant to age-related obesity and diabetes.⁶ PGC-1 α is induced in muscle by exercise.

Spiegelman's team hypothesized that exercise-induced PGC-1 α might be stimulating the secretion of factors from muscle that have beneficial effects on tissues involved in regulating metabolism.

The team first looked for a protein that was upregulated in response to PGC-1 α expression and identified the membrane protein fibronectin type III domain containing 5 (FNDC5) as a potential transcriptional target of PGC-1 α .

The next question was whether FNDC5 could be secreted. When FNDC5 was expressed in cells, it was proteolytically cleaved to release a shorter, previously uncharacterized variant that Spiegelman's team named irisin. Strikingly, the amino acid sequence of irisin is identical in mice and humans.

The team then looked to see if irisin levels were regulated by exercise. Indeed, exercise increased irisin levels in the blood of both mice and humans.

In mice with adenoviral vector-mediated overexpression of Fndc5, levels of irisin in the blood were threefold higher than those in mice with normal expression of Fndc5. These animals also had greater amounts of brown-like fat in their subcutaneous white fat pad than controls.

Figure 1. Exercise messenger. Dana-Farber Cancer Institute researchers have identified a new hormone, irisin, which mediates some of the benefits exercise has on metabolism.

Exercise induces increased expression of peroxisome proliferation-activated receptor- γ coactivator 1 α (PPARGC1A; PGC-1 α) [a], which boosts expression of the membrane protein fibronectin type III domain containing 5 (FNDC5) [b]. FNDC5 is proteolytically cleaved, resulting in the release of irisin [c], which is carried in the blood to white adipose tissue [d], where it stimulates the browning of white fat [e].

In mice fed a high-fat diet, greater expression of irisin decreased weight gain and increased glucose tolerance compared with normal irisin expression.

Together, the results suggest a pathway in which exercise induces irisin secretion, leading to the browning of white fat (see **Figure 1**, “Exercise messenger”).

Finally, the team asked whether irisin had therapeutic benefits.

When fed a high fat diet, mice overexpressing *Fndc5* had less weight gain, better glucose tolerance and lower fasting insulin levels than mice with normal *Fndc5* expression.

Results were published in *Nature*.

“Irisin certainly has appeal as a factor that is secreted in response to exercise and appears to be capable of inducing changes in fat metabolism and glycemic control in mice,” said Thomas Hughes, president and CEO of **Zafgen Inc.**

Zafgen’s ZGN-433 has completed Phase Ib testing in obesity. The compound inhibits methionine aminopeptidase 2 (MetAP2) and modulates the metabolic regulation of fat storage and breakdown.

According to Hughes, irisin’s therapeutic utility in humans is an open question awaiting clinical testing. “The authors speculate that the protein may exert its effects through induction of PPAR α . PPAR α stimulation has been seen to reduce body weight in laboratory animals but so far has not translated into body weight changes or improvements in glycemic control in humans,” he noted.

Spiegelman responded that “irisin does many things, only one of which is to regulate PPAR α in adipose cells.” He added that, in contrast to irisin, activators of peroxisome proliferation-activated receptor- α (PPAR α ; PPAR α) have never been shown to induce the browning of white fat.

Ember gets stoked

Ember, which was cofounded by Spiegelman and holds exclusive rights to the irisin technology from Dana-Farber, plans to put a variant of the hormone into the clinic in 2014 for obesity and type 2 diabetes.

Lou Tartaglia, Ember’s president and interim CEO, said the company has produced stabilized versions of irisin. He added that in mice, the variants produced browning of white fat and metabolic improvements.

The company also is pursuing alternative targets for inducing the browning of white fat or for activating brown fat. Tartaglia said Ember is running target-based *in vitro* screens and pathway-oriented cellular screens on a number of targets that have been identified by the company’s founders and scientific advisory board members, including bone morphogenetic protein 7 (BMP7; OP-1) and PR domain containing 16 (PRDM16).

Spiegelman’s laboratory is now focused on identifying irisin’s receptor.

Dana-Farber has filed a patent application covering the results reported in *Nature*.

Fat models

One promising outcome of Spiegelman’s study was that the hormone characterized first in mice, irisin, was subsequently shown to be activated by exercise in people. Historically, not all studies of metabolism in mice and in mouse adipocytes have directly translated to humans.

“Mice don’t get heart attacks. Many mice are hard to make fat. They don’t have the same metabolic problems as humans,” said Chad Cowan, an assistant professor in the Department of Stem Cell and Regenerative Biology at Harvard and a principal faculty member at the **Harvard Stem Cell Institute**.

On the flip side, human cell culture work has required immortalized cell lines because human WAT is not easily cultured. Cowan added that “there has been very limited access to human brown fat.”

To develop a better model system, a team led by Cowan turned to human pluripotent stem cells. The group first converted human induced pluripotent stem (iPS) cell lines and human embryonic stem cell (hESC) lines into mesenchymal progenitor cells. Transducing these progenitor cells with *PPARG* (*PPAR γ*) led to the formation of white adipocytes. Transduction with *PPAR γ* in combination with *CCAAT enhancer binding protein- β* (*CEBPB*; *CRP2*) and *PRDM16* generated brown adipocytes.

The response of the white adipocytes to insulin was modulated with known physiological regulators, suggesting the cells can model insulin sensitivity and resistance. The brown adipocytes had the expected increased level of mitochondrial activity, suggesting they are a functional model for brown fat.

Subcutaneous implantation of the adipocytes in mice led to the formation of BAT and WAT. Results were published in *Nature Cell Biology*.

Cowan’s team is collaborating with Roche to use the human adipocyte cell lines for drug discovery. Under a 2010 deal between Roche, Harvard University and **Massachusetts General Hospital**, the pharma is using stem cell lines developed by academics for drug screening in cardiovascular and metabolic diseases.

“The possibility to culture human brown and white adipose cells allows us to assess the efficacy of novel drug candidates in a highly relevant human cell system,” said Kurt Amrein, scientific expert in metabolism at Roche.

The pharma is using Cowan’s adipocyte cell lines for three types of screens.

First, Roche is “establishing human white adipocytes from iPS cells isolated from subjects with metabolic disorders. Cells with a clear deficit will be used in phenotypic screens aimed at normalizing function,” said Amrein.

The company is also setting up two screens focusing on brown fat. “We have a keen interest in identifying either small molecules that are able to convert white into brown-like adipocytes or identifying molecules that promote increased brown adipocyte formation from precursor cells,” he said.

Hughes said the feasibility and therapeutic outcome of activating brown fat in humans are unknown. “Brown fat serves a primary purpose of heating the blood as an adaptive response to cold exposure. Obese individuals may or may not tolerate increased heat production for long periods of time,” he said. “Secondly, brown fat function may need to be stimulated under physiological conditions—this stimulation is normally driven by sympathetic nerves—through adrenaline—and thyroid hormone. This stimulus may be needed to yield the benefits

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—Thomas Hughes, Zafgen Inc.

of brown fat induction. If multiple stimuli are needed, or true cold exposure is necessary, these limitations might impact the usefulness of a therapy.”

Amrein acknowledged that “the exact role BAT plays in obesity and the metabolic syndrome is still a matter of debate. However, many recent reports indicate that it may play a major role and may offer a completely new therapeutic target for metabolic diseases.”

Tartaglia, who also is a partner at **Third Rock Ventures**, told *SciBX* that Ember has studied the mice generated by Spiegelman in which transgenic expression of *Prmd16* leads to browning of white fat.⁷ “We’ve looked for things that were potentially concerning like increases in body temperature and haven’t found anything. However, until we do very thorough toxicology—especially in nonhuman primates and people—we really won’t know for sure,” he said.

Massachusetts General Hospital, where Cowan initiated the research, has filed a patent application covering the methods reported in *Nature Cell Biology*. Roche has a nonexclusive license, and the technology is available for additional licensing from **Partners HealthCare** Research Ventures & Licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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