'Exercise hormone' irisin may be a myth

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Credit: Peter Griffin/Public Domain

The discovery of the "exercise hormone" irisin three years ago and more than 170 related papers about it since have been called into question by recent research showing they were based on flawed testing kits.

Previous studies suggested that the hormone irisin—named for the Greek messenger goddess Iris—travels from muscle to fat tissue after exercise to tell fat cells to start burning energy instead of storing it. The finding ignited hope and press coverage that irisin could hold the key to

fighting diabetes and obesity, perhaps one day taking the form of a pill that could melt away the pounds without the hassle of a workout.

But new research from an international team of scientists has found that the antibodies used to measure levels of irisin in blood were poorly vetted and nonspecific. These researchers argue that the irisin levels reported by commercial kits were actually due to unknown blood proteins, misconstruing the role of the hormone in human metabolism.

The study, appearing March 9 in the journal *Scientific Reports*, directly tested the antibodies used in previous analyses and showed that they cross-reacted with proteins other than irisin, yielding a false positive result. Furthermore, none of the proteins detected by these test kits in any human or animal blood samples were the correct size to be irisin.

"From the start, the study of irisin has been complicated by unvalidated reagents and contradictory data that have raised flags about the existence of irisin and its role in humans and other species," said Harold P. Erickson, Ph.D., an author of the study and professor of cell biology and biochemistry at Duke University School of Medicine. "We provide compelling evidence that the signals reported by previous studies were due to non-specific blood proteins, not irisin. Hopefully, our findings will finally convince other researchers to stop chasing a myth."

Irisin's reputation as a fat-burning molecule can be traced back to a 2012 paper in the journal Nature. The researchers reported that in response to exercise, the tail end of a muscle protein called FNDC5 was lopped off and sent through the bloodstream to fat tissue, where it turned white fat into brown fat. Brown fat burns calories, and is what hibernating animals—and even human babies—use to keep warm.

A slew of studies quickly followed looking into how the levels of this protein fragment—which the original authors named irisin—were

affected by exercise, diabetes, obesity, even cancer. To measure irisin levels in blood, most researchers took a common shortcut and relied on commercial antibody kits called ELISA, which were marketed by several biotech companies.

The biotech companies and the scientists who purchased the kits largely ignored the possibility that their antibodies might be cross-reacting with other proteins and thus overestimating levels of irisin, Erickson said.

Erickson had raised this concern in a commentary article in the journal Adipocyte in 2013. The following year, he was excited to find two papers on the topic from the laboratory of German researcher Steffen Maak. The Maak studies had not relied on the ELISA, but used a more specific and time-consuming method known as a Western blot.

In an ELISA, all the proteins in the sample to be analyzed reside in a single dish, like alphabet soup. In this case, an antibody designed to stick to all the "p's" in the soup will give a signal for each "p" it binds, but scientists can't know for sure if it also bound some "q's" or "d's" in the process. In contrast, in a Western blot all of the proteins in a sample are run out singly, from "a" to "z." If an antibody binds other letters, the scientists know because they see a signal at the wrong spot in the alphabet. Likewise, if the antibody doesn't recognize the right letter, there won't be a signal where there should be.

Erickson emailed Maak and they initiated a collaboration, which was joined by the laboratories of Christian Devon in Norway and Vincenz Gerber in Switzerland. They ordered the antibodies that had been used in more than 80 studies to measure irisin levels, and they analyzed them using Western blots. The researchers turned up two critical issues. First, the antibodies failed to detect a protein of the correct size for irisin in any blood sample—even in horses that were training for long distance running. Second, each antibody reacted strongly with several blood

proteins that were the wrong size for irisin.

As a control, Erickson's laboratory synthesized two different versions of irisin, one dotted with sugars like the naturally derived protein, the other one sugar-free. The researchers then added various amounts of these proteins to their samples and repeated the Western blot. They showed the antibodies could detect even tiny amounts of both forms of irisin, and that they were the expected sizes of 20 and 13 kilodaltons.

The researchers believe that their findings dispute all previous data obtained with commercial ELISA kits for irisin and make it unlikely that the hormone plays a physiological role in humans.

"Our conclusions make sense, especially in light of the work of other researchers who have shown that the human version of the FDNC5 gene has a deleterious mutation at the beginning," Erickson said. "As a result, humans can produce less than one percent of the irisin present in other species. Humans are essentially a gene knock-out—they can't produce FNDC5, and therefore they can't produce irisin."

More information: "Irisin - a myth rather than an exercise-inducible myokine," Elke Albrecht, Frode Norheim, Bernd Thiede, Torgeir Holen, Tomoo Ohashi, Lisa Schering, Sindre Lee, Julia Brenmoehl, Selina Thomas, Christina A. Drevon, Harold P. Erickson, and Steffen Maak. *Scientific Reports*, March 9, 2015. DOI: 10.1038/srep08889

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