Greasing insulin

By Benjamin Boettner, Senior Writer

An entirely new class of fatty acids has just been discovered that potentially is more potent than the ω -3 fatty acids found in fish oil. The new fatty acids—which work through G protein-coupled receptor 120, a target that had once been discarded by researchers—could be used to restore insulin sensitivity in patients with diabetes.¹

G protein-coupled receptor 120 (GPR120; O3FAR1) was tapped as a potential obesity and diabetes target following a 2005 paper in *Nature Medicine* that showed the receptor triggered the release of glucagon-like peptide-1 (GLP-1) from intestinal cells when stimulated by unsaturated long-chain free fatty acids.² But further studies showed that GPR120's direct effect on GLP-1 release would be minor or undetectable, and as a result companies abandoned the target in favor of related receptors that were able to stimulate insulin secretion.

In 2010, however, a team at the **University of California, San Diego** found that ω -3 fatty acids—such as **GlaxoSmithKline plc's** Lovaza fish oil extract—exert their anti-inflammatory and insulin-sensitizing effects through GPR120.³ The receptor was shown to be highly expressed in mature adipocytes, where it helped increase glucose uptake, and in proinflammatory macrophages, where it decreased inflammatory responses.

Two years later, an international team showed that dysfunction of GPR120 led to obesity and obesity-associated metabolic disorders such as glucose intolerance and fatty liver.⁴

Despite the new angles presented by the target, there was one key problem—exogenous ω -3 fatty acids are not very effective at activating GPR120. Now, a team at **Harvard Medical School** has identified fatty acids that operate through GPR120 and could be more potent correctors of glucose intolerance and insulin resistance than ω -3 fatty acids.

The team, led by Barbara Kahn, started out by performing a comparative lipidomics analysis in adipose cells from wild-type mice and mice that overexpressed solute carrier family 2 facilitated glucose transporter member 4 (Slc2a4; Glut4). Her group had previously shown that adipose-selective overexpression of Glut4 enhanced glucose tolerance in mice.^{5,6} Kahn is a professor of medicine at Harvard Medical School and the **Beth Israel Deaconess Medical Center**.

The comparative analysis led to the discovery of fatty acid-hydroxy fatty acids (FAHFAs), a class of lipids specifically synthesized by GLUT4-overexpressing adipocytes. The molecules consist of a hydroxyl fatty acid linked to a second fatty acid via ester bonds at variable positions. Among the FAHFAs, palmitic acid-hydroxy stearic acids (PAHSAs) were the most highly upregulated. PAHSAs also were synthesized by other tissues like the liver and were present in mouse chow and human foods.

The researchers next sought to understand whether PAHSA levels also varied with glucose tolerance and insulin sensitivity in humans. Indeed, levels of the lipids in serum and subcutaneous fat were lower in samples from insulin-resistant individuals than in those from insulin-sensitive individuals.

The team then asked whether two of the PAHSAs—9-PAHSA and 5-PAHSA—had therapeutic potential. They focused on these two molecules because 9-PAHSA was the most upregulated PAHSA in white adipose tissue of Glut4-overexpressing mice and 5-PAHSA was the most consistently downregulated PAHSA in white adipose tissue from insulin-resistant mice and humans.

In insulin-resistant mice fed a high-fat diet, each PAHSA given separately improved glucose tolerance and insulin sensitivity after glucose stimulation. Moreover, in adipose tissue from normal mice fed a high-fat diet, 9-PAHSA reduced adipose tissue inflammation, as measured by a reduction in inflammation-associated macrophages secreting tumor necrosis factor- α (Tnf- α) and Il-1 β . In normal mouse and human β cells stimulated with glucose, 5-PAHSA promoted insulin secretion.

Finally, the team showed that 5- and 9-PAHSAs directly bound to Gpr120 and depended on the lipid sensor for their effects.

Data were published in *Cell*.

“Prior to these findings, the existence of these endogenous ligands and their relevance to the regulation of GPR120 was not known,” said Jim Hauske, president and founder of **Sensor Pharmaceuticals Inc.** “Members of this new class of lipids likely are endogenous ligands not only to GPR120 but to a wider range of nutrient-sensing G protein-coupled receptors expressed in a range of human tissues.”

“These are very solid findings,” said Jerrold Olefsky, associate dean for scientific affairs and a professor of medicine at the **University of California, San Diego School of Medicine**. “The idea that the newly identified lipids work through GPR120 makes a lot of sense. They seem to engage all the biology that has been worked out for GPR120.”

In 2010, Olefsky's group identified GPR120 as a receptor for ω -3 fatty acids.³ This year, an Olefsky-led team that included researchers from **Merck & Co. Inc.** reported the development of a small molecule agonist of GPR120 that improved insulin resistance and adipose tissue inflammation in mice.⁷

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—Jerrold Olefsky,
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“Even with modest PAHSA elevations in serum, we see beneficial effects. Our mouse models show that 16- to 18-fold elevations chronically in adipose tissue are associated with no adverse effects, suggesting that the lipids may be well tolerated.”

—Barbara Kahn,
Harvard Medical School

Picturing PAHSAs

A crucial aspect of developing PAHSAs as therapeutic agents will be better defining their biology.

“We are carrying out chronic studies in diabetic mice to determine how effective the lipids are at reducing insulin resistance, improving glucose control and reversing adipose tissue inflammation,” Kahn said. “In addition we are studying whether we can also prevent type 1 diabetes in mice. To address the lipids’ efficacy in humans, we are planning a proof-of-concept study in a small number of diabetic people.”

Philippe Froguel said that measuring PAHSA concentrations in large human cohorts would be useful. Froguel is a professor at **Imperial College London**, and his group discovered associations between GPR120 levels and insulin sensitivity and tissue inflammation in both mice and humans.⁴

Steve Watkins, CTO at the metabolic biomarker company **Metabolon Inc.**, added, “Humans consume PAHSAs and their precursors in food. If these indeed are potent compounds, what impact does diet quality have? Nutritional studies in humans therefore would be interesting.”

Both Olefsky and Kahn expect the animal data on PAHSAs to translate to humans.

“It is encouraging that human tissues have the same properties as mouse tissues regarding the PAHSAs,” Olefsky said. “It will be important to find out whether the lipids are protected from lipases in the blood that could attack their ester bonds. This is why triglycerides don’t circulate freely. If PAHSAs would indeed be subject to cleavage, they would have to be further engineered for enhanced stability.”

“Even with modest PAHSA elevations in serum, we see beneficial effects,” said Kahn. “Our mouse models show that 16- to 18-fold elevations chronically in adipose tissue are associated with no adverse effects, suggesting that the lipids may be well tolerated. PAHSAs are also available orally with good absorption.”

Froguel thought that PAHSAs also could bind to and activate receptors other than GPR120. “I am convinced that GPR120 is the tip of the iceberg and that other signaling pathways exist for PAHSAs,” he said. “In the gut, for example, different GPCRs seem to sense lipids. It is very important to test this hypothesis.”

Kahn agreed. “We think there are most likely additional receptors besides GPR120 that mediate the beneficial effects of PAHSAs,” she said. “Knowing what other GPCRs are activated by PAHSAs is a priority for us.”

PAHSA comparisons

Hauske noted that small molecule GPR120 agonists could have greater intrinsic potencies than PAHSAs. However, he said, the

longer-term relative efficacy of such agonists, which will also depend on bioavailability, will have to be compared with PAHSAs in disease models.

He also wanted to see an analysis of the stereospecific requirements of the PAHSAs as he said it is possible that only one of the two enantiomers might be an active ligand. According to Hauske, “So far there has been no effort to synthesize enantiomers individually and to test their activities side by side and versus known GPR120 agonists. This is a very important piece of the mosaic.”

Sensor is developing a gut-restricted GPR120 agonist that is at lead discovery stage in preclinical development.

Merck, which holds rights to the GPR120 agonist it developed with UCSD, could not be reached for comments on its development strategy for the small molecule.

Harvard Medical School has filed for a patent covering the PAHSAs. The IP is available for licensing.

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