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Loss of Enzyme Produces Diabetes-Like Symptoms

Researchers have identified a protein that appears to play an important role in signaling muscle cells to take up glucose from the bloodstream.

In genetic studies in mice, researchers knocked out the gene that produces the enzyme Akt2 and noted that the mice developed insulin resistance and symptoms that resembled type 2 diabetes. Insulin resistance is caused by environmental factors and genetic mutations that result in cells becoming resistant to insulin. Normally, cells respond to insulin by taking up glucose from the bloodstream. Type 2 diabetes mellitus ensues when the insulin-producing cells of the pancreas fail to compensate for abnormalities in insulin action. Diabetes causes high blood sugar levels, which can lead to cardiovascular disease, blindness and kidney malfunction.

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— **Morris J. Birnbaum**

In an article published in the June 1, 2001, issue of the journal *Science*, researchers led by Howard Hughes Medical Institute investigator Morris J. Birnbaum at the University of Pennsylvania School of Medicine reported that they have pinned down a function for the somewhat enigmatic protein, Akt2.

"Dozens of papers have been published arguing both for and against Akt2 as having a role in insulin-stimulated signaling of glucose transport," he said. "Most people agree that insulin's action on the insulin receptor activates the enzyme PI 3-kinase which initiates a signaling cascade that somehow leads to the movement of glucose transporters to the cell membrane.

"But PI 3-kinase was the most downstream member of the signaling pathway for which there was a consensus," he said. "The Holy Grail in this field is to link the signal transduction cascade to the movement of glucose transporters."

Akt2 was not necessarily a favorite candidate for being that downstream enzyme, said Birnbaum. In fact, some researchers had begun to discount Akt2, arguing that other similar protein kinases might play a more important role in insulin signaling. But in earlier experiments, Birnbaum and his colleagues had shown that overactivation of Akt2 mimicked insulin action in mice.

"While the data in those experiments were very clear, it was an artificial system that only showed that Akt could be sufficient under certain conditions. But the experiments did not address whether Akt2 was necessary for insulin-stimulated glucose transport," he said. Other scientists had reduced Akt2 activity in tissue culture cells, but their experiments proved inconclusive because they had not managed to eliminate Akt activity completely in their experimental systems.

Birnbaum's colleague and lead author on the *Science* paper, Han Cho, with the help of HHMI investigator Marisa S. Bartolomei, successfully created a strain of mice in which all Akt2 activity was eliminated. With Akt2 function abolished, the scientists found that the mice grew normally, but had mildly elevated blood sugar a telling symptom of diabetes. The researchers found that the elevated blood sugar was not caused by inadequate insulin production in the pancreas. Studies of the pancreas revealed that its insulin-producing cells had greatly increased, most likely to compensate for an insulin resistance characteristic of type 2 diabetes, said Birnbaum.

When Birnbaum and his colleagues studied the animals' muscle tissue, they discovered a partial defect in insulin-stimulated glucose uptake. Co-authors Jason Kim and HHMI investigator Gerald I. Shulman at Yale University School of Medicine performed glucose clamp studies to measure the liver production of glucose in response to insulin. The researchers infused precise amounts of glucose and insulin through a small catheter inserted into a mouse's vein and measured the blood glucose levels. These experiments as well as studies using a radioactive glucose tracer revealed that the livers of the Akt2 knockout mice did not respond to insulin by lowering glucose production. Furthermore, the animals' tissues were consuming less glucose in response to insulin.

"These findings are important because they represent the first evidence in an intact animal of a signaling pathway by which insulin shuts off hepatic glucose production," said Birnbaum. "And taken together, finding effects in both liver and muscle are especially significant because dual abnormalities are exactly what occurs in type 2 diabetes," he said.

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Birnbaum and his colleagues plan to knock out Akt2 in specific tissues such as muscle, liver and fat, and study the effects on insulin signaling in those tissues. They also plan to explore the signaling pathway downstream of Akt2, in an effort to determine whether genetic defects in the Akt2 pathway could

contribute to diabetes.