DIABETES

Insulin resistance grows in the dark

Riddle, M.R., *et al. Nature* **555**, 647–651 (2018)

They may not look it, but cave-dwelling Mexican tetra fish and river-dwelling Mexican tetra fish are the same species, *Astyanax mexicanus*. The cave dwellers are eyeless and pinkish, while the river fish are a lovely silver. It's just that about a million years ago, several populations of river-dwellers became trapped in limestone caves, where they evolved to survive in lightless, extremely resource-limited conditions.

In fact, nutrients only enter the cave with bat droppings or rare surges stemming from seasonal floods. The cavefish as a result evolved the means to survive long periods with no food.

"We're interested in how animals survive in environments with limited nutrients," said Misty Riddle, a post-doctoral researcher in the genetics department at Harvard Medical School, and a co-author of a recent paper in *Nature* that describes new insights into how the cavefish cope with those deficiencies.

Researchers already knew that the cavefish have some metabolic adaptations to their extreme environment: in the lab, they have increased appetites and a greater propensity for storing fat compared to their river-dwelling relatives. Riddle and her colleagues investigated further and found that an hour after a meal, the cavefish have much higher blood glucose levels than river fish. However, a 21-day fast caused blood glucose levels in the cavefish to crash while the river fish maintained normal levels.

On the other hand, glucose injections spiked blood glucose levels in both fish, but the return to baseline levels was delayed in cavefish compared to river fish. The two lines of evidence "led us to think that maybe they have some sort of defect in their ability to clear glucose from their blood," said Riddle.

In short, cavefish resemble human patients with type 2 diabetes, right down to a similar fatty liver. Insulin signaling plays a key role in blood glucose regulation. After a meal, blood glucose levels naturally rise, and the pancreas responds by releasing insulin, which binds to our cells and instructs them to absorb glucose from the blood. "Something was happening in the cavefish to prevent that from going smoothly," said Riddle.

The Mexican tetra varies by habitat: from rivers (top) to caves (bottom). Adapted with permission from *Nature* **555**, 647–651 (2018).

A genetic comparison between three populations from three separate caves as well as a river population revealed a potential explanation. Two of the three cave populations bore mutations in the insulin receptor gene (*insra*). The same mutation in humans causes a severe form of insulin resistance that leads to low weight and premature death.

To determine the functional impact of the change in the insulin receptor gene, the researchers introduced the sequences from both the cavefish and the river fish into cultured human cells, and found that the cavefish receptor had a much lower binding affinity for insulin. The insulin sequence from the third cavefish population was normal. "But they also have dysregulated glucose homeostasis. It seems like it's evolved through some other mechanism that we're not sure about yet," said Riddle.

Despite a mutation that leaves blood glucose at elevated levels, the cavefish don't appear to suffer any of the negative consequences that humans with types 2 diabetes experience, suggesting that the fish have developed a compensatory mechanism to avoid harm.

Next, the researchers exploited the fact that the river fish and cavefish, despite their differences, can still interbreed and produce fertile offspring. Male hybrids that carried one or two copies of the mutant insulin receptor genotype from their cavefish parent weighed 27% more on average. "It could be that the increased weight may be an adaption to increase their nutrient storage capacity during times when food is pretty rare," said Riddle.

Strikingly, the altered insulin receptor had a similar effect in a completely unrelated fish. When the researchers used CRISPR/Cas9 to introduce it into the zebrafish, the animals gained more weight and were insulin resistant. They had reduced scale size, which suggests that the mutation caused ill effects in the zebrafish. The researchers observed no such effects in the cavefish.

The researchers hope to identify hybrid tetra fish that bear the altered insulin receptor but not the protective mechanism. "We can then see if the health of those fish is affected, and potentially what genes those fish lack to make them sick when they have the insulin receptor mutation," said Riddle.

Despite the insulin resistance, the cavefish appear quite healthy in the lab. The oldest are at least 14 years old, and they seem to be aging better than the river fish. They haven't yet developed the signs of senescence, such as sunken fins and an arched back, that have appeared in their river cousins.

The cavefish also don't accumulate advanced glycation end-products—the altered proteins that appear in humans with diabetes and are linked to pathology of diabetes, cardiovascular disease, and aging. "Figuring out how they do that could potentially have implications for the treatment of diabetes or prevention of the pathologies associated with chronically elevated blood sugar levels," said Riddle.

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