Chemical tracers reveal oxygen-dependent switch in cellular pathway to fat

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Using tracer compounds, scientists have been able to track the cellular production of NADPH, a key coenzyme for making fat, through a pathway that has never been measured directly before.

By tracking this pathway, known as malic enzyme metabolism, which is one of a few recognized routes to make NADPH, researchers from Rabinowitz lab discovered a novel switch in the way fat cells make NADPH depending on the presence of oxygen. The findings were published in *Nature Chemical Biology*.

"No one had ever shown an environmental dependent switch in any NADPH production pathway," said Joshua Rabinowitz, Professor of Chemistry and the Lewis-Sigler Institute for Integrative Genomics at Princeton and principal investigator of the work. "No one had the tools to look," he said.

NADPH is critical to not only fat synthesis, but also protein and DNA synthesis, and antioxidant defense, implicating it in many diseases such as cancer and diabetes. By understanding and monitoring the pathways through which NADPH is made, scientists can work towards influencing these processes using therapeutic compounds.

The Rabinowitz lab first applied their tracer method in 2014 to study the most well known NADPH production pathway, the oxidative pentose phosphate pathway (oxPPP). The method relied on compounds labeled with deuterium atoms, hydrogen's heavier cousin, which can be deployed

in the cell and measured by a technique called mass spectrometry.

In this work, the researchers extended their method to probe the lesser-known malic enzyme pathway by developing two new, orthogonal tracer compounds specific to this pathway. One tracer, a deuterated succinate compound, enters the cycle more directly but is somewhat challenging for the cell to uptake, while the other, a deuterated glucose molecule, is taken up by the cell readily but takes an extra step to enter the pathway.

The research team investigated the malic enzyme pathway under various concentrations of oxygen. Low oxygen environments, which are found in fat cells in obesity, are of particular clinical interest. They found that in a low oxygen environment, the oxidative pentose phosphate pathway produced more NADPH than the malic enzyme pathway, but in a higher oxygen environment, the pathway contributions completely flipped.

"It's like the cells are quite clever. They choose the pathway depending on what they want to make, and what nutrients they can access," said Ling Liu, a graduate student in the Rabinowitz lab and lead author on the work.

One advantage of this method is that it tracks NADPH made specifically in the cytosolic compartment of the cell, whereas the previous leading technique, which relied on tracer compounds with carbon-13 atoms, is unable to differentiate between malic enzyme activity in the cytosol and mitochondria.

NADPH involvement in essential cellular processes has a direct impact on diseases such as diabetes, obesity and cancer. "All of these central biomedical questions depend on an understanding of NADPH pathways, and if you can't quantify how a metabolite is made and used, you can't understand what's going on," Rabinowitz said. "Ultimately, we're trying to understand the fundamental chemistry that's leading to these

important biological outcomes," he said.

More information: Ling Liu et al. Malic enzyme tracers reveal hypoxia-induced switch in adipocyte NADPH pathway usage, *Nature Chemical Biology* (2016). DOI: 10.1038/nchembio.2047

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