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SPOTLIGHT

Temporal and spatial metabolite dynamics impart control in adipogenesis

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The process of adipogenesis is critical for forming new, healthy adipocytes that are capable of storing lipids. In this issue, Sánchez-Ramírez and Ung et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202111137) reveal a novel role for the metabolite nicotinamide adenine dinucleotide in controlling differentiation of mesenchymal stromal cells into adipocytes.

Adipose tissue is a large endocrine organ with tremendous plasticity to expand and contract, a process that is driven via the coordination of a wide variety of cells within the adipose tissue (1). Adipose tissue is comprised of multiple cell types. Mature adipocytes make up the majority of fat mass, having lipid storing capabilities. However, the stromal vascular cell fraction of adipose tissue consists of endothelial cells, immune cells, preadipocytes, pericytes, and so-called "mesenchymal stromal cells," which have the capacity to proliferate and undergo differentiation, taking on the fate of an osteoblast, chrondrocyte, myocyte, or adipocyte (2). The capacity to generate new, healthy adipocytes enables efficient storing of circulating lipids, preventing excessive hypertrophy of existing adipocytes that can ultimately lyse, spilling their contents into the bloodstream to be taken up by other non-fat storing organs (3). Dysfunction of adipose tissue commonly occurs in the context of obesity, ultimately driving insulin resistance by the ectopic deposition of lipids in non-lipid storing organs such as the liver or muscle. However, white adipose tissue expansion that occurs in the context of obesity involves not only hypertrophy, but also excess proliferation of adipocyte progenitor cells, a process known as hyperplasia. Thus, the process of controlled adipogenesis is critical for maintaining healthy fat, and ultimately can protect an organism from insulin resistance and its comorbidities.

Though transcriptional reprogramming driven by transcription factors, including CCAAT/enhancer binding protein α and peroxisome proliferator-activated receptor γ (PPARγ), are required for terminal adipocyte differentiation, the adipogenic transition from the mesenchymal state involves a metabolic transition from glycolysis to oxidative phosphorylation. How these transcriptional and metabolic events coordinate temporally during the process of adipogenesis is poorly understood. The study by Sánchez-Ramírez and Ung et al. (4) utilizes innovative experimental approaches to reveal novel mechanisms involved in differentiation of mesenchymal stromal cells (MSCs) into adipocytes. Specifically, the authors use two-photon fluorescence lifetime microscopy (2P-FLIM) to show that the metabolite nicotinamide adenine dinucleotide (NAD^+), a metabolite tightly tied to the energy state of the cell (5), plays an inhibitory role in early stage adipogenesis, preventing commitment of MSCs to preadipocytes. Interestingly, although the sirtuin protein SIRT1 is activated by the metabolite NAD^+ and is also necessary for terminal differentiation, the authors found that suppression of NAD^+ synthesis by the blocking the so-called "salvage pathway" increased differentiation, suggesting that activation of SIRT1 in terminal differentiation does not depend on generation of NAD^+ by this salvage pathway.

To make these observations, the authors initially used human MSCs and subjected them to a protocol that induces adipogenesis in the presence or absence of NAD^+. Surprisingly, depletion of NAD^+ led to an increase in lipid accumulation at late stages of the differentiation paradigm, while NAD^+ supplementation had the opposite effect. Using label-free FLIM of lipid-associated fluorophores in live cells, the authors were able to quantify lipid accumulation over time, as lipid droplet–associated fluorophores had a longer lifetime with respect to the cell cytoplasm. In addition to identifying lipid accumulation over time in the presence of absence of NAD^+, the study also reveals that NAD^+ affects which PPARγ isoform is expressed at specific stages of differentiation. Using RNA sequencing, the authors identified a large change in the transcriptome of NAD^+-treated cells. Interestingly, most of the genes upregulated in differentiating MSCs treated with NAD^+ were associated with apoptosis or response to stress, while those downregulated included genes involved in cell differentiation and motility. Ribosomal proteins and pathways were also downregulated by NAD^+ supplementation, as were *STAT5A* and *STAT5B*, both known to regulate expression of PPARγ.

One of the most interesting findings of this study was that while NAD^+ plays a clear

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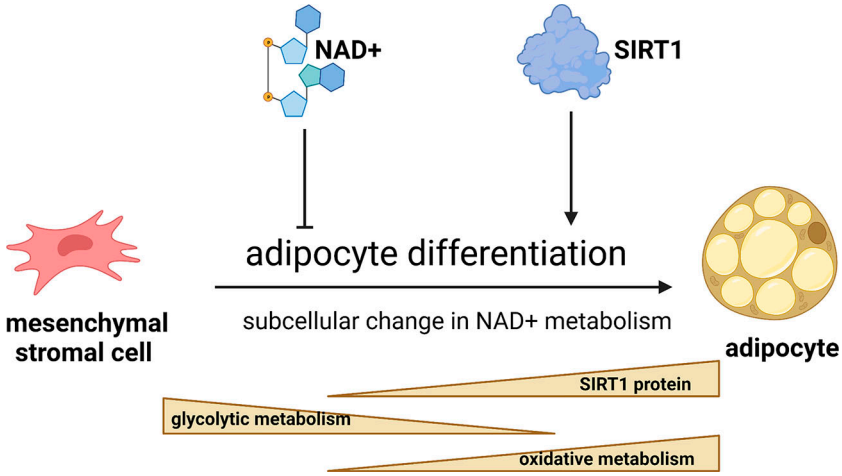

Figure 1. **NAD⁺ metabolism imparts temporal regulation in the process of adipogenic differentiation.** NAD⁺ plays an inhibitory role in the process of differentiation of mesenchymal stromal cells to lipid-storing adipocytes at early stages of adipogenesis, though the NAD⁺-regulated enzyme SIRT1 plays a minimal effect at this stage. NAD⁺ both induces a proapoptotic gene signature at early-stage differentiation and inhibits the shift from glycolytic to oxidative metabolism.

role in early stages of lineage commitment, gene expression analysis after SIRT1 inhibition at this stage revealed that SIRT1 plays a limited role during early stages of differentiation, but profoundly alters gene expression patterns at terminal stages of differentiation (Fig. 1). Thus, NAD⁺ metabolism plays temporally distinct roles during the process of adipogenesis. To further understand the temporal dynamics of NAD⁺ metabolism on adipogenesis, the authors used their 2P-FLIM approach to develop a novel method by which to quantitatively analyze bound NADH during adipogenesis in specific cellular compartments. The incentive to study compartmentalization came in part from the surprising result that MSCs treated with NAD⁺ at the induction of adipogenesis actually showed a lower intracellular concentration of NAD⁺ and NADH. Thus, to identify potential changes in the cellular distribution of NAD⁺ and NADH, as well as to determine the potential for cofactor binding, investigators analyzed bound NADH levels in single cells with micrometer pixel resolution. This analysis revealed that the fraction of bound NADH increased during adipogenesis, concomitant with the shift from glycolytic metabolism to oxidative metabolism. The increase in bound NADH was negated by the addition of NAD⁺, with levels of mitochondrial and cytoplasm/nuclear content both lowered compared to control treatment. However, while control-treated differentiating cells showed higher levels of bound NADH in the cytoplasm/nucleus compared to the mitochondria, the opposite was observed for NAD⁺-treated cells, a pattern that was abolished by inhibition of SIRT1 activity.

The results of the study by Sánchez-Ramírez and Ung et al. are the first to demonstrate that while NAD⁺ inhibits adipogenesis at early stages of differentiation, it appears to be independent of SIRT1 activity, as only blockade of SIRT1 activity at late stages of adipogenesis blocks terminal differentiation. In addition, it provides a context for subcellular compartmentalization of NAD⁺ metabolism and the extent to which adipogenesis might be gated by changes in metabolism at the subcellular level.

Targeting metabolism has been an attractive means to combat aging. NAD⁺ metabolism in particular has been at the center of active study, as NAD⁺ levels decline in cells and tissue throughout aging (6). NAD⁺ is a highly dynamic molecule, regulated by both the circadian clock and tightly linked to cycles of fasting and feeding (7, 8). Recent studies in preclinical models have revealed that supplementation with the NAD⁺ precursor, nicotinamide riboside, can reverse age-associated decline in mitochondrial respiration and transcriptional rhythms (9). Though less is understood regarding this metabolic pathway in healthy or pathogenic fat expansion, the work by Sánchez-Ramírez and Ung and colleagues provides new insight into its important role in adipocyte differentiation. This study has important implications. Though inhibiting adipogenesis by enhancing NAD⁺ may suppress adipose tissue expansion, it may be that the highly dynamic nature of NAD⁺ metabolism is required for optimal adipogenesis during healthy aging or under conditions of energy excess, when the need for healthy new adipocytes to accommodate extra lipid loads is present. Nevertheless, understanding the spatial and temporal dynamics in differentiating fat cells is an important step forward in understanding how we can exploit NAD⁺ metabolism to promote healthy fat during aging and under nutrient stress.

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