



# MicroRNAs Emerge as Modulators of NAD<sup>+</sup>-Dependent Energy Metabolism in Skeletal Muscle



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Obesity, type 2 diabetes, and related metabolic disorders are associated with reduced mitochondrial function in skeletal muscle and other organs (1). However, causality of aberrant mitochondrial activity in the etiology of these diseases and the exact mechanism(s) linking metabolic pathologies to mitochondrial dysfunction are still under debate. Therefore, a better molecular understanding of this observation is required in order to design novel therapeutic strategies aimed at modulation of mitochondrial function in these disease contexts. The silent mating type information regulation 2 homolog 1 (SIRT-1) and the peroxisome proliferator-activated receptor  $\gamma$  coactivators 1 (PGC-1) are important regulators of mitochondrial function in skeletal muscle (1). In response to deacetylation by SIRT-1, PGC-1 $\alpha$  is activated and subsequently promotes an oxidative muscle fiber phenotype. In turn, SIRT-1 activity is regulated by the substrate NAD<sup>+</sup>, and thus acts as an intracellular rheostat by increasing mitochondrial biogenesis to match the energy needs of the cell (2).

Interestingly, recent studies have exploited this mechanism by using the NAD<sup>+</sup> precursors nicotinamide riboside (3) and nicotinamide mononucleotide (4) to increase SIRT-1 activity and thereby the oxidative capacity of skeletal muscle. Alternatively, intracellular NAD<sup>+</sup> can be elevated through inhibition of other enzymes that use NAD<sup>+</sup>. In particular, the poly(ADP-ribose) polymerases (PARPs), which metabolize NAD<sup>+</sup> to form polymers of ADP-ribose (PAR) on other proteins, are major consumers of NAD<sup>+</sup> in various cell types. PARPs are involved in regulation of several distinct cellular processes, such as DNA repair, inflammation, and differentiation (5), but they have also recently been recognized as having important roles in metabolism (6). In fact, germ-line deletion of either PARP-1 or PARP-2, or pharmacological inhibition of PARP activity by PJ-34, results in elevated intracellular NAD<sup>+</sup> levels in skeletal

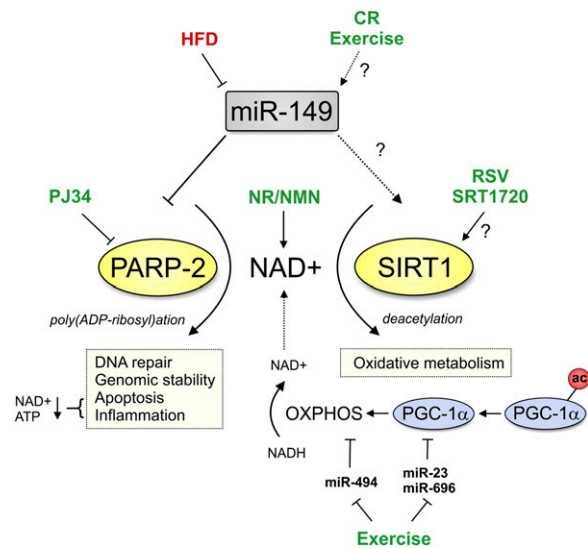
muscle with the expected outcome of increased SIRT-1 activity and mitochondrial oxidative function (7,8). In most tissues, PARP-1 accounts for the vast majority (>85%) of cellular PARP activity, and is therefore also the major NAD<sup>+</sup> consumer (6). Thus, PARP-1 deletion presumably affects SIRT-1 primarily due to increased NAD<sup>+</sup> levels (8). In contrast, even though PARP-2 null mice also show increased NAD<sup>+</sup> levels (7), PARP-2 also acts as a transcriptional repressor of SIRT-1 (7).

Modulation of PARP activity provides a novel therapeutic strategy to increase SIRT-1 expression and activity in skeletal muscle, and represents an alternative to pharmacological activation of SIRT-1 using resveratrol or SRT1720 (9,10). Interestingly, several studies indicate that PARP activity in murine skeletal muscle is dysregulated during high-fat diet (HFD) feeding (8) and aging (11), implying a direct involvement of PARPs in development of metabolic dysfunction in muscle. This idea is now further supported in the study by Mohamed et al. (12) published in this issue, in which the authors demonstrate that skeletal muscle PARylation was increased in HFD-fed mice concomitant with elevated expression of PARP-2. Surprisingly, in contrast to other studies that have reported elevated PARP-1 in HFD-fed mice (8), the authors detected no differences in PARP-1 expression with HFD feeding. As expected however, elevated PARP-2 expression was associated with lower NAD<sup>+</sup> levels and reduced SIRT-1 expression, together with a reduction in mitochondrial oxidative function. Depletion of intracellular NAD<sup>+</sup> through increased PARP-2 activity was therefore postulated as a major mechanism leading to mitochondrial dysfunction in these mice.

Although simple and attractive, the findings do not exclude the idea that PARPs, apart from acting as a transcriptional repressor of SIRT-1 (7), also interact with

other transcriptional regulators such as forkhead box class O1 (FoxO1) (13) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) (14). Further, direct PARylation of mitochondrial proteins has also been demonstrated to reduce oxidative function (15), suggesting that the connection between PARP-2 and mitochondrial dysfunction in skeletal muscle is multifaceted. In a series of elegant experiments, Mohamed et al. (12) investigated the long-elusive mechanism behind increased PARP-2 expression in HFD-fed mice by performing a microRNA (miRNA) screen in skeletal muscle. Several differentially regulated miRNAs were discovered, of which two (miR-149 and miR-712-3p) were significantly downregulated with HFD feeding, and therefore considered potential candidates for the observed regulation of PARP-2. However, only miR-149 was able to reduce PARP-2 levels in vitro, and it bound to a conserved seed region in the 3'UTR of PARP-2. In a well-designed in vitro setup, the authors could then demonstrate that overexpression of miR-149 in cultured myotubes led to a reduction in PARP-2, elevated NAD<sup>+</sup> levels, increased SIRT-1 activity, and concomitantly promoted mitochondrial biogenesis. These in vitro data are in many aspects opposite to the muscle phenotype observed in HFD-fed mice, and it will therefore be interesting to see whether overexpression of miR-149 in skeletal muscle in vivo will have a protective effect against obesity-induced metabolic dysfunction. Skeletal muscle-specific inhibition of PARP-2 activity would also circumvent the dichotomy present in global PARP-2 null mice, which simultaneously exhibit increased peripheral insulin sensitivity as well as  $\beta$ -cell dysfunction and insulinopenia (7). Furthermore, it will be important to elucidate whether the miR-149/PARP-2/SIRT-1 axis postulated here in a mouse model is also dysregulated in human obesity, and thus a valid therapeutic target in patients.

Mohamed et al. have identified a novel miRNA that modulates oxidative function in skeletal muscle by affecting the intracellular levels of the metabolite NAD<sup>+</sup>. Besides miR-149, other miRNAs, such as miR-494 (16), miR-23 (17), and miR-696 (18), analogously affect mitochondrial biogenesis and function in skeletal muscle, however by inhibiting different targets (Fig. 1). Interestingly, all of these miRNAs are regulated by exercise (16–18). Since both exercise and caloric restriction are linked to increased SIRT-1 activity and improved mitochondrial function, it will be interesting to investigate whether miR-149, and by extension also PARP-2, is differentially regulated in these metabolically beneficial states. In conclusion, Mohamed et al. (12) provides novel evidence of dysregulated PARP-2 during obesity, and proposes an important role of miR-149 in the control of intracellular NAD<sup>+</sup> levels, thus providing another example of a miRNA that acts as a major regulator of cellular metabolism. The relevance of this article is underscored by the emerging role of PARPs in metabolic regulation (6), and thus provides a framework for future studies on the connection between PARP activity and mitochondrial function. Moreover, these



**Figure 1**—MiRNA-controlled regulation of metabolic reprogramming of skeletal muscle cells by the metabolite NAD<sup>+</sup>. Expression of miR-149 is regulated by HFD feeding and potentially by caloric restriction (CR) as well as exercise. PARP-2 is inhibited by miR-149 while directly or indirectly, this miRNA increases the activity of SIRT1. Thus, by shifting the balance between two major NAD<sup>+</sup> consumers, miR-149 determines the metabolic fate of muscle cells and promotes SIRT-1-dependent deacetylation of the transcriptional coactivator PGC-1 $\alpha$ . As a consequence, mitochondrial function and oxidative metabolism in muscle cells are boosted. This system has recently been shown to likewise be under exercise-controlled regulation via different miRNAs. Inversely, HFD-mediated repression of miR-149 results in increased activity of PARP-2, which, in turn, not only promotes DNA damage repair, but also apoptosis and inflammation in a cellular context of depleted NAD<sup>+</sup> and ATP. This dichotomy in the regulation of cellular metabolism in muscle can furthermore be modulated by pharmacological interventions aimed at PARP-2 and SIRT-1 activities, as well as elevation of cellular NAD<sup>+</sup> levels using PJ34, resveratrol (RSV), SRT1720, nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN), respectively. OXPHOS, oxidative phosphorylation.

findings put an emphasis on the clinical relevance of PARP inhibitors, not only in the field of oncology (19), but also as an incipient therapeutic strategy for treatment of obesity and obesity-related metabolic disorders.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

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