Year: 2014

MicroRNAs emerge as modulators of NAD+-dependent energy metabolism in skeletal muscle

Svensson, Kristoffer and Handschin, Christoph

Originally published as:

Svensson, Kristoffer and Handschin, Christoph. (2014) *MicroRNAs emerge as modulators of NAD+-dependent energy metabolism in skeletal muscle.* Diabetes, Vol. 63, H. 5. S. 1451-1453.

MicroRNAs emerge as modulators of NAD^+-dependent energy metabolism in skeletal muscle

Kristoffer Svensson and Christoph Handschin*

Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland

Published in Diabetes. 2014 May;63(5):1451-3. PMID: 24757196. doi: 10.2337/db14-0166

MicroRNAs emerge as modulators of NAD^+-dependent energy metabolism in skeletal muscle

Kristoffer Svensson and Christoph Handschin*

Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland

*Corresponding author: Christoph Handschin (christoph.handschin@unibas.ch)

Word count: 1054

Tables: 0

Figures: 1

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 mechanisms 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 (SIRT1) and the peroxisome proliferator-activated receptor γ co-activators 1 (PGC-1) are important regulators of mitochondrial function in skeletal muscle (1). In response to deacetylation by SIRT1, PGC-1α is activated and subsequently promotes an oxidative muscle fiber phenotype. In turn, SIRT1 activity is regulated by the substrate nicotinamide adenine dinucleotide (NAD^+), 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^+ precursors nicotinamide riboside (NR) (3) and nicotinamide mononucleotide (NMN) (4) to increase SIRT1 activity and thereby the oxidative capacity of skeletal muscle. Alternatively, intracellular NAD^+ can be elevated through inhibition of other enzymes which use NAD^+. In particular, the poly(ADP-ribose) polymerases (PARPs), which metabolize NAD^+ to form polymers of ADP-ribose (PAR) on other proteins, are major consumers of NAD^+ 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^+ levels in skeletal muscle with the expected outcome of increased SIRT1 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^+ consumer (6). Thus, PARP-1 deletion presumably affects SIRT1 primarily due to increased NAD^+ levels (8). In contrast, even though PARP-2 null mice also show increased NAD^+ levels (7), PARP-2 also acts as a transcriptional repressor of SIRT1 (7).

Modulation of PARP activity provides a novel therapeutic strategy to increase SIRT1 expression and activity in skeletal muscle, and represents an alternative to pharmacological activation of SIRT1 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 and colleagues published in this issue of *Diabetes*, 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, in the new study by Mohamed, elevated PARP-2 expression was associated with lower NAD^+ levels and reduced SIRT1 expression, together with a reduction in mitochondrial oxidative function. Depletion of intracellular NAD^+ 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 SIRT1 (7), also interact with other transcriptional regulators such as forkhead box O1 (FoxO1) (12) and peroxisome proliferator-activated receptor γ (PPARγ) (13). Further, direct PARylation of mitochondrial proteins has also been demonstrated to reduce oxidative function (14), suggesting that the connection between PARP-2 and mitochondrial dysfunction in skeletal muscle is multifaceted. In a series of elegant experiments, the authors of the new report investigated the long elusive mechanism behind increased PARP-2 expression in HFD-fed mice by performing a miRNA screen in skeletal muscle. Several differentially regulated miRNAs were discovered, of which two (miR-149 and miR712-3p) were significantly down-regulated 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^+ levels, increased SIRT1 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 β-cell dysfunction and insulinopenia (7). Furthermore, it will be important to elucidate whether the miR-149/PARP-2/SIRT1-axis postulated here in a mouse model is also dysregulated in human obesity, and thus a valid therapeutic target in patients.

Mohamed and colleagues have identified a novel miRNA that modulates oxidative function in skeletal muscle by affecting the intracellular levels of the metabolite NAD^+. Besides miR-149, other miRNAs such as miR-494 (15), miR-23 (16) and miR-696 (17) 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 (15-17). Since both exercise and caloric restriction are linked to increased SIRT1 activity and improved mitochondrial function, it will be

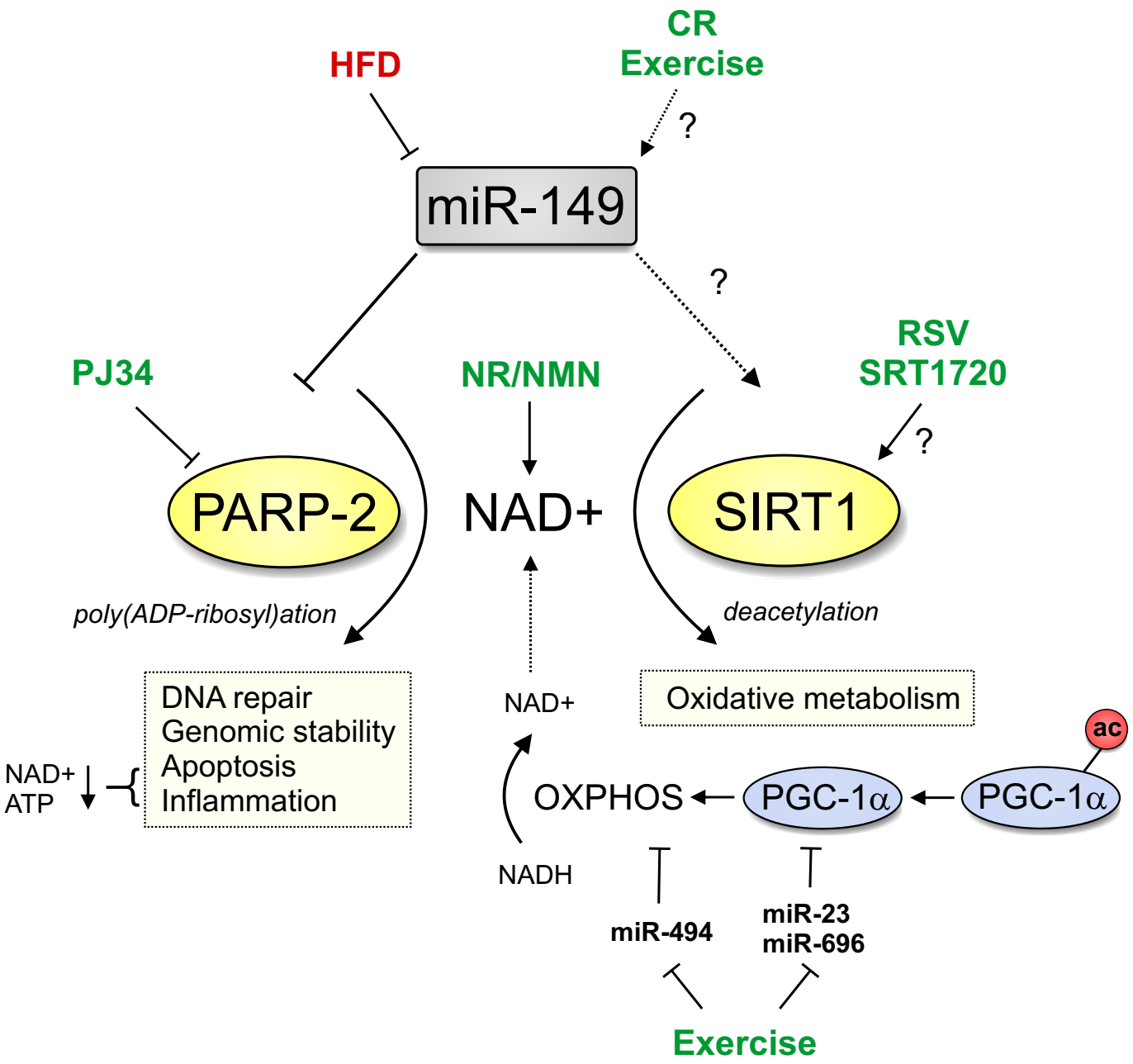
interesting to investigate whether miR-149 and in extension also PARP-2 are differentially regulated in these metabolically beneficial states. In conclusion, this study provides novel evidence of dysregulated PARP-2 during obesity, and proposes an important role of miR-149 in the control of intracellular NAD^+ levels, thus providing another example of a miRNA that acts as a major regulator of cellular metabolism. The relevance of this paper is underscored by the emerging role of PARPs in metabolic regulation (6), and thus provides a framework for futures studies on the connection between PARP activity and mitochondrial function. Moreover, these findings put an emphasis on the clinical relevance of PARP-inhibitors, not only in the field of oncology (18), but also as an incipient therapeutic strategy for treatment of obesity, and obesity-related metabolic disorders.

References

1. Williams CB, Gurd BJ: Skeletal muscle SIRT1 and the genetics of metabolic health: therapeutic activation by pharmaceuticals and exercise. Appl Clin Genet 2012;5:81-91
2. Vinciguerra M, Fulco M, Ladurner A, Sartorelli V, Rosenthal N: SirT1 in muscle physiology and disease: lessons from mouse models. Dis Model Mech 2010;3:298-303
3. Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J: The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 2012;15:838-847
4. Yoshino J, Mills KF, Yoon MJ, Imai S: Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 2011;14:528-536
5. Krishnakumar R, Kraus WL: The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell 2010;39:8-24
6. Bai P, Canto C: The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. Cell Metab 2012;16:290-295
7. Bai P, Canto C, Brunyanszki A, Huber A, Szanto M, Cen Y, Yamamoto H, Houten SM, Kiss B, Oudart H, Gergely P, Menissier-de Murcia J, Schreiber V, Sauve AA, Auwerx J: PARP-2 regulates SIRT1 expression and whole-body energy expenditure. Cell Metab 2011;13:450-460
8. Bai P, Canto C, Oudart H, Brunyanszki A, Cen Y, Thomas C, Yamamoto H, Huber A, Kiss B, Houtkooper RH, Schoonjans K, Schreiber V, Sauve AA, Menissier-de Murcia J, Auwerx J: PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. Cell Metab 2011;13:461-468
9. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J: Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109-1122
10. Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J: Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab 2008;8:347-358
11. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, Grant R: Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. PLoS One 2011;6:e19194
12. Sakamaki J, Daitoku H, Yoshimochi K, Miwa M, Fukamizu A: Regulation of FOXO1-mediated transcription and cell proliferation by PARP-1. Biochem Biophys Res Commun 2009;382:497-502
13. Bai P, Houten SM, Huber A, Schreiber V, Watanabe M, Kiss B, de Murcia G, Auwerx J, Menissier-de Murcia J: Poly(ADP-ribose) polymerase-2 [corrected] controls adipocyte differentiation and adipose tissue function through the regulation of the activity of the retinoid X receptor/peroxisome proliferator-activated receptor-gamma [corrected] heterodimer. J Biol Chem 2007;282:37738-37746
14. Lai Y, Chen Y, Watkins SC, Nathaniel PD, Guo F, Kochanek PM, Jenkins LW, Szabo C, Clark RS: Identification of poly-ADP-ribosylated mitochondrial proteins after traumatic brain injury. J Neurochem 2008;104:1700-1711
15. Yamamoto H, Morino K, Nishio Y, Ugi S, Yoshizaki T, Kashiwagi A, Maegawa H: MicroRNA-494 regulates mitochondrial biogenesis in skeletal muscle through mitochondrial transcription factor A and Forkhead box j3. Am J Physiol Endocrinol Metab 2012;303:E1419-1427
16. Safdar A, Abadi A, Akhtar M, Hettinga BP, Tarnopolsky MA: miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. PLoS One 2009;4:e5610
17. Aoi W, Naito Y, Mizushima K, Takanami Y, Kawai Y, Ichikawa H, Yoshikawa T: The microRNA miR-696 regulates PGC-1{alpha} in mouse skeletal muscle in response to physical activity. Am J Physiol Endocrinol Metab 2010;298:E799-806
18. Underhill C, Toulmonde M, Bonnefoi H: A review of PARP inhibitors: from bench to bedside. Ann Oncol 2011;22:268-279

Figure Legend

Figure 1. MicroRNA-controlled regulation of metabolic reprogramming of skeletal muscle cells by the metabolite NAD+. Expression of the microRNA miR-149 is regulated by high fat diet (HFD) feeding and potentially by caloric restriction (CR) as well as exercise. The poly(ADP-ribosyl)transferase PARP-2 is inhibited by miR-149 while directly or indirectly, this miRNA increases the activity of the protein deacetylase SIRT1. Thus, by shifting the balance between two major NAD+ consumers, miR-149 determines the metabolic fate of muscle cells and promotes SIRT1-dependent deacetylation of the transcriptional co-activator PGC-1α. 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 microRNAs. 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+ and ATP. This dichotomy in the regulation of cellular metabolism in muscle can furthermore be modulated by pharmacological interventions aimed at PARP-2 and SIRT1 activities as well as elevation of cellular NAD+ levels using PJ34, resveratrol (RSV), SRT1720, nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), respectively.


HFD
CR
Exercise
?
miR-149
?
PJ34
NR/NMN
RSV
SRT1720
?
PARP-2
NAD+
SIRT1
poly(ADP-ribosyl)ation
deacetylation
DNA repair
Genomic stability
Apoptosis
Inflammation
NAD+
ATP
NAD+
Oxidative metabolism
ac
OXPHOS
PGC-1α
PGC-1α
NADH
miR-494
miR-23
miR-696
Exercise