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REVIEWS

Forum

Distinct Roles of the NAD$^+$-Sirt1 and FAD-LSD1 Pathways in Metabolic Response and Tissue Development

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Various nutritional signals are transduced by two epigenetic pathways: NAD-dependent sirtuin Sirt1 (NAD$^+$-Sirt1) deacetylase and flavin adenine dinucleotide–dependent lysine-specific demethylase 1 (FAD-LSD1). These pathways are controlled by dietary vitamins and nutrient-responsive hormones such as glucocorticoids and insulin, resulting in endocrine–metabolism–epigenome cooperation in adipocyte and skeletal muscle development.

The nutritional environment affects cellular function, growth, and development by influencing the expression of developmental and metabolic genes via the epigenome. Various nutrients and their metabolites that are used for chemical modifications of nucleic acids and proteins such as histones are also essential as cofactors of the modifying enzymes [1]. In this Forum article, we focus on mammalian sirtuin Sirt1 deacetylase and lysine-specific demethylase 1 (LSD1), which are unique regulatory proteins that use vitamin-derived metabolites, and discuss how they connect nutritional states to the epigenome of metabolic tissues.

NAD$^+$-Sirt1 and FAD-LSD1 Pathways

NAD-dependent Sirt1 (NAD$^+$–Sirt1) and flavin adenine dinucleotide-dependent LSD1 (FAD–LSD1) play pivotal roles in transcriptional and epigenetic regulation (Figure 1) [1]. Sirt1 is the mammalian ortholog of yeast *Sir2* deacetylase that promotes energy consumption under calorie restriction, by stimulating mitochondrial oxidative phosphorylation (OXPHOS) in adipocytes. Regarding the control of the function of the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), Sirt1 activates PGC-1α protein under fasting through deacetylation of its lysine residues in an NAD$^+$-dependent manner [1,2]. In contrast, LSD1 promotes the storage of excess energy under high-fat-diet conditions in adipocytes, by suppressing the expression of the *PGC-1α* gene through demethylation of histone H3 at lysine 4 at the promoter region in a FAD-dependent manner [1,3]. However, the role of LSD1 in controlling energy metabolism is highly context dependent, because it maintains OXPHOS activity under normal diet conditions [3], and it activates OXPHOS-related genes with nuclear respiratory factor 1 under cold exposure or β3-adrenergic stimulation [4]. Because PGC-1α is a key transcription regulator that promotes mitochondrial biogenesis and OXPHOS, Sirt1 and LSD1 function using cellular NAD$^+$ and FAD, respectively, to regulate the metabolic response and energy balance.

Among the dietary vitamin B group, nicotinamide is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (Nampt), and then to NAD$^+$ by nicotinamide mononucleotide adenylyltransferase (Nmnat) [5]. The other NAD$^+$ precursors include niacin-derived nicotinic acid and nicotinamide riboside. In contrast, riboflavin (vitamin B_2) is converted to flavin mononucleotide (FMN) by riboflavin kinase (RFK), and then to FAD by FAD synthetase (FADS) [6]. Thus, the cellular NAD$^+$ and FAD concentrations fluctuate depending on the activity of these enzymes as well as nutritional inputs due to energy availability. Since NAD$^+$ and FAD are used as cofactors in specific oxidative reactions where they are reduced to NADH and $FADH_2$, respectively, intracellular levels of them are metabolically controlled by the balance between their synthesis and consumption. Notably, the circadian transcription factors CLOCK/BMAL1 were found to directly regulate Nampt expression, resulting in circadian oscillations of NAD$^+$ levels [5]. It is interesting that, among the core CLOCK proteins, CRY proteins are stabilized by the FAD binding through competing with the ubiquitin E3 ligase FBXL3 [7], suggesting the presence of a crosstalk between NAD$^+$- and FAD-mediated pathways.

Muscle Fiber-Type Specificity in Health and Disease

In mammals, skeletal muscles are heterogeneous in constitution and number of muscle fibers, as well as in morphology and function at the anatomical positions within the body. Skeletal muscle fibers are classified into slow-twitch myofibers (type I) and fast-twitch myofibers (type II), based on the expression patterns of myosin heavy chain (MyHC) isoforms and metabolic properties (Figure 2) [8]. Slow-twitch fibers contribute to persistent endurance contraction with large OXPHOS capacity and mitochondria enrichment. In contrast, fast-twitch fibers, with high glycolytic bias, help powerful rapid contraction, but they easily develop fatigue. During developmental processes, myogenic cells can exclusively differentiate into either fiber type, partly under the influence of nutritional and hormonal conditions [9]; fasting (glucocorticoids action) and feeding (insulin action) promote slow-twitch and fast-twitch fiber formation, respectively. However, the mechanism of fiber-type selection during myogenesis is still unclear.

An appropriate proportion of muscle fiber types is essential for human health and pathophysiology. Of importance, muscle wasting and atrophy occur differentially in specific fiber types, resulting in a transition

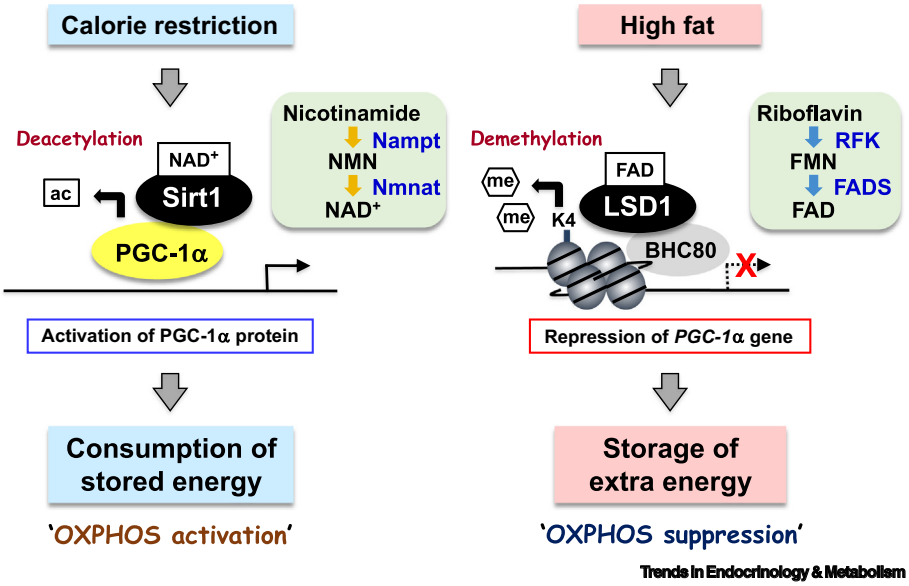

Figure 1. NAD$^+$-Dependent Sirt1 and FAD-Dependent LSD1 Pathways. Sirt1 deacetylase and lysine-specific demethylase 1 (LSD1) distinctly function in energy homeostasis in adipocytes. Sirt1 promotes consumption of stored energy under calorie restriction and fasting by activating peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) via deacetylation of this protein [2]. In contrast, LSD1 induces storage of extra energy under a high-fat diet by suppressing the expression of the *PGC-1α* gene through the demethylation of histone H3 at lysine 4 at the gene promoter [3]. PGC-1α is a transcription regulator that promotes mitochondrial biogenesis and oxidative phosphorylation (OXPHOS). NAD$^+$ and flavin adenine dinucleotide (FAD) are used as a coenzyme for Sirt1 and LSD1, respectively. NAD$^+$ is mainly synthesized from nicotinamide by nicotinamide phosphoribosyltransferase (Nampt) and nicotinamide mononucleotide adenylyltransferase (Nmnat), whereas FAD is produced from riboflavin by riboflavin kinase (RFK) and FAD synthase (FADS) [5,6]. Abbreviations: FMN, flavin mononucleotide; NMN, nicotinamide mononucleotide.

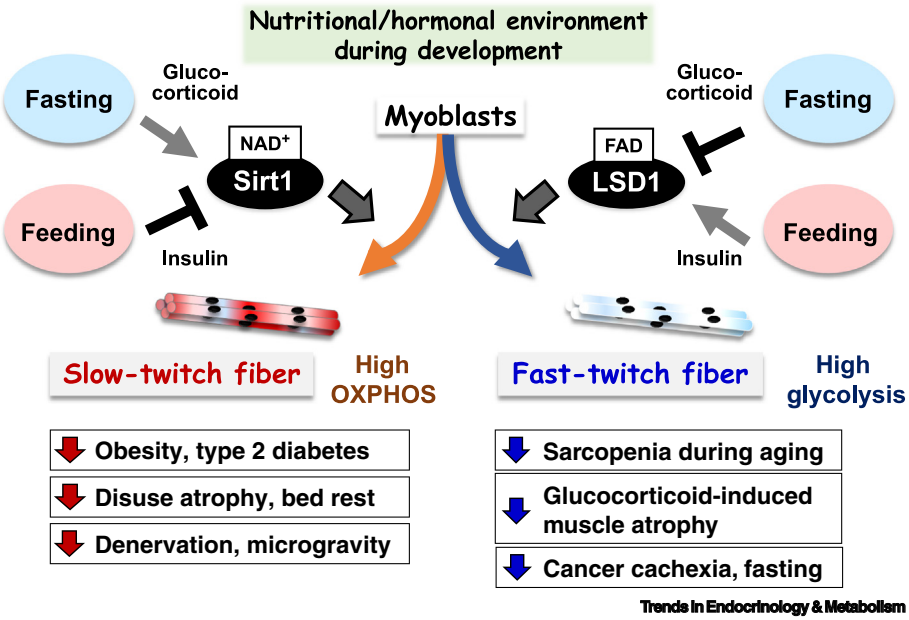

Figure 2. Possible Involvement of Sirt1 and LSD1 in Muscle Fiber-Type Specificity under Hormonal Actions. In skeletal muscles, slow-twitch fibers contribute to persistent endurance contraction, with large oxidative phosphorylation (OXPHOS) capacity, while fast-twitch fibers, with high glycolytic metabolism, enable powerful rapid contraction [8]. During development, skeletal myoblasts can exclusively differentiate into either fiber type under fasting (glucocorticoids action) and feeding (insulin action) conditions [9]. The Sirt1 pathway promotes slow-twitch fiber formation and OXPHOS activity, under the control of the hormones [10–13]. In contrast, the lysine-specific demethylase 1 (LSD1) pathway facilitates fast-twitch fibers and glycolytic activity, which is regulated by these hormones [14]. Muscle atrophy in specific fiber types and the resultant fiber-type transition occur under pathophysiological conditions [8]. Preferential decreases of slow-twitch fibers are found in obesity, type 2 diabetes, and muscle disuse, which cause a slow-to-fast fiber type shift. Opposite changes are seen in sarcopenia during aging and cancer cachexia.

of fiber-type profile [8]. Slow-twitch fibers are predominantly decreased during muscle disuse due to bed rest, denervation, and microgravity, which cause muscle atrophy with a slow-to-fast fiber type shift involving changes in MyHC isoforms. A slow-to-fast fiber type shift has also been reported in obesity and type 2 diabetes, which may arise from the impairment of PGC-1α function in slow oxidative muscle fibers. Conversely, a fast-to-slow fiber type shift with preferential reduction in fast-twitch fibers is found in sarcopenia during aging, cancer cachexia, fasting, and glucocorticoid-induced muscle atrophy. Most fast-to-slow shifts possibly contribute to malnutrition and chronic inflammation with increased levels of cytokines, such as tumor necrosis factor α, where forkhead box O (FOXO) and nuclear factor κB (NF-κB) are involved [8].

Sirt1 and LSD1 Distinctly Regulate Myogenesis under Hormonal Actions

Many studies have shown that glucocorticoids and insulin respond to nutrient availability, fasting, and feeding, and oppositely act on various tissues [9]. Glucocorticoids are steroid hormones secreted from the adrenal cortex and assume a catabolic role in the cells through the glucocorticoid receptor under fasting stress conditions. In contrast, insulin is a polypeptide hormone produced by the β cells of the pancreatic islets and has, under feeding conditions, its anabolic effects through the insulin receptor by augmenting intracellular uptake of fuels. Notably, these hormones distinctively regulate the NAD^+–Sirt1 and FAD–LSD1 pathways in skeletal muscles (Figure 2).

Cohen *et al.* have shown that Sirt1 expression was induced in a variety of rat tissues under calorie restriction as well as in cultured cells that were treated with serum from these animals [10]. Later studies have indicated that calorie restriction increased Sirt1 levels in mice and humans as well. In contrast, addition of insulin or insulin-like growth factor-1 to the serum reduced Sirt1 expression *in vitro* [10]. Recently, it has been demonstrated that insulin signaling represses the NAD^+-dependent SIRT1 activity by promoting its binding to a sorting protein, PACS-2 [11].

Ljubicic *et al.* have reported that the natural polyphenolic compound, resveratrol, induced the expression of the slow muscle fibers in the *mdx* mouse model of Duchenne muscular dystrophy (DMD), which are known to be more resistant to the dystrophic changes in DMD patients, through enhanced activity of the Sirt1–PGC-1α axis [12]. Furthermore, Chalkiadaki *et al.* have found that muscle-specific Sirt1 overexpression increased slow-twitch fibers and alleviated the dystrophic phenotype in *mdx* mice, by increasing the levels of PGC-1α and decreasing the expression of muscle atrophy genes [13]. In addition, muscle-specific Sirt1-knockout mice showed reduced endurance exercise capacity such as distance run, together with no significant influence on the gastrocnemius fast muscle. These experiments demonstrated that genetic gain or loss of Sirt1 in the muscle either promotes or suppresses the formation of slow-twitch fibers and OXPHOS capacity [13]. Considering the nutrient- and hormone-sensing nature of Sirt1, it is plausible that the control of Sirt1 activity by environmental factors is directly linked to fiber type plasticity.

Anan *et al.* have revealed that loss of LSD1 activity during myogenic differentiation enhances a fast-to-slow fiber shift and the OXPHOS capacity of myotubes, suggesting a role of LSD1 in fast-twitch fiber formation [14]. In differentiating myoblasts and gastrocnemius muscle from mice, LSD1 protein is degraded by treatment with the glucocorticoid dexamethasone (Dex), which has been known to direct slow fiber formation over the fast. Dex treatment induces expression of ubiquitin E3 ligase, JADE-2, resulting in proteasomal degradation of LSD1. Furthermore, in differentiating myoblasts, the combination of Dex and the chemical LSD1 inhibitor, S2101, synergistically derepress slow fiber-type genes, resulting in an enhanced switch of fast-to-slow fibers. These observations suggest that LSD1 links the nutritional condition to metabolic reprogramming during myogenesis. Given the opposing roles of Sirt1 and LSD1 in the regulation of slow-fiber traits, it is tempting to examine how different hormonal inputs organize the functions of these proteins in fiber-type specification.

Considering the Mechanistic Models of the Developmental Origins Theory

We finally discuss the possible involvement of the Sirt1 and LSD1 pathways in metabolic phenotypes under the nutritional environment. Epidemiological studies and animal experiments have shown that undernutrition during pregnancy causes low birth weight of newborns with increased risk of obesity, type 2 diabetes, and heart disease in adulthood [15]. Developmental origins of health and disease (DOHaD), initially called fetal origins of adult disease, was proposed by Barker *et al.* in the 1980s [15]. Although the molecular mechanism of this theory remains undetermined, this parent–child environment may be based on at least a two-step response. Under starvation, an immediate response saves the life of the embryo by consuming stored nutrients within the body, resulting in low body weight and lean phenotype. Then, an adaptive response builds a defense to future starvation by storing extra nutrients. This adaptation can later be fit for similar undernutrition, but unfit for high-fat diet and overnutrition, because the resultant obese phenotype causes the above-mentioned adult diseases.

Taken together, we propose a sequential involvement of the NAD^+–Sirt1 and FAD–

LSD1 pathways in DOHaD. In the immediate response, Sirt1 promotes OXPHOS activity and slow-twitch fiber formation under catabolic action. In the following adaptive response, LSD1 enhances energy storage and fast-twitch fiber formation, possibly under anabolic conditions. This order of events appears to be critical for the perinatal survival strategy against undernutrition. Obviously, other metabolic tissues, such as the liver, pancreas, kidney, adipose, and brain, are also involved. However, such developmental adaptation could be advantageous or disadvantageous depending on later nutrient availability. Further research on the NAD^+–Sirt1 and FAD–LSD1 pathways is required to understand the long-term effects of the nutritional environment on the epigenome and metabolic phenotypes, which are linked to subsequent disease susceptibility.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, AMED-CREST, and Takeda Science Foundation (to M.N. and S.H.). We thank our laboratory members for discussion and cooperation.

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https://doi.org/10.1016/j.tem.2019.04.010

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References

1. Etchegaray, J.P. *et al.* (2016) Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. *Mol. Cell* 62, 695–711
2. Rodgers, J.T. *et al.* (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature* 434, 113–118
3. Hino, S. *et al.* (2012) FAD-dependent lysine demethylase LSD1 regulates cellular energy expenditure. *Nat. Commun.* 3, 758
4. Duteil, D. *et al.* (2014) LSD1 promotes oxidative metabolism of white adipose tissue. *Nat. Commun.* 5, 4093
5. Imai, S. *et al.* (2014) NAD^+ and sirtuins in aging and disease. *Trends Cell Biol.* 24, 464–471
6. Powers, H.J. (2003) Riboflavin (vitamin B-2) and health. *Am. J. Clin. Nutr.* 77, 1352–1360
7. Hirano, A. *et al.* (2017) FAD regulates CRYPTOCHROME protein stability and circadian clock in mice. *Cell Rep.* 19, 255–266
8. Ciciliot, S. *et al.* (2013) Muscle type and fiber type specificity in muscle wasting. *Int. J. Biochem. Cell Biol.* 45, 2191–2199
9. Goldstein, I. *et al.* (2015) Transcriptional and chromatin regulation during fasting – The genomic era. *Trends Endocrinol. Metab.* 26, 699–710
10. Cohen, H.Y. *et al.* (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390–392
11. Krzysiak, T.C. *et al.* (2018) An insulin-responsive sensor in the SIRT1 disordered region binds DBC1 and PACS-2 to control enzyme activity. *Mol. Cell* 72, 985–998
12. Ljubicic, V. *et al.* (2014) Resveratrol induces expression of the slow, oxidative phenotype in mdx mouse muscle together with enhanced activity of the SIRT1-PGC-1α axis. *Am. J. Physiol. Cell Physiol.* 307, C66–C82
13. Chalkiadaki, A. *et al.* (2014) Muscle-specific SIRT1 gain-of-function increases slow-twitch fibers and ameliorates pathophysiology in a mouse model of Duchenne muscular dystrophy. *PLoS Genet.* 10, e1004490
14. Anan, K. *et al.* (2018) LSD1 mediates metabolic reprogramming by glucocorticoids during myogenic differentiation. *Nucleic Acids Res.* 46, 5441–5454
15. Barker, D.J. (2007) The origins of the developmental origins theory. *J. Intern. Med.* 261, 412–417

Forum

The Whys and Hows of Calculating Total Cellular ATP Production Rate

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Quantifying total cellular ATP production rate has become easier with recent technology and is essential to understanding energy metabolism in cells and tissues. We review fundamental concepts for determining total cellular ATP production rate from measurements of oxygen consumption and acidification rates and discuss their application to answering biological questions.

Cells Leverage Network Properties to Control ATP Production and Consumption

Current technologies for measuring rates associated with ATP production are increasingly accessible. Some instruments, such as the Agilent Seahorse XF Analyzer, simultaneously measure extracellular acidification and oxygen consumption, which together can be used to calculate total cellular ATP production rate [1]. Others, such as the Oroboros O2k [2], can be configured to include pH measurements for the same goal. These platforms and methods complement a renewed interest in using bioenergetic analysis to explore mechanisms of carcinogenesis and cancer progression, diabetes [3], cellular differentiation [4], and neurodegeneration [5] among others. Here, we review the calculation of cellular ATP production rate and its applications to these fields.

The task of meeting cellular ATP demand in cells, tissues, and organisms is shared between mitochondrial oxidative phosphorylation and glycolytic ATP production. Basal and maximal rates of cellular ATP production, their partitioning between glycolysis and oxidative phosphorylation, and what meaning can be drawn from these measurements are issues of intense current interest. Calculating ATP production rates can reveal the efficiency of energy transduction, and therefore what a cell or organism needs to survive and function. It can reveal the adaptability of ATP production to stresses, and therefore how energetically sensitive (or resistant) biological targets are to drugs or toxins. Combined with control analysis, it can provide new mechanistic insights into complex diseases [3].

Cellular ATP production is driven by a network of linked reactions that achieve stable steady-state fluxes. The principles that govern glycolytic and oxidative ATP production are the same ones that govern other steady-state networks. At the timescale of most measurements, seconds to minutes, these networks operate by and are explained by the interactions between internal forces (levels) and fluxes (rates) [6]. They have internal control; an imposed