RESEARCH HIGHLIGHT

Lactylation constrains OXPHOS under hypoxia

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Cells sense and adapt to oxygen availability. A new study published in *Cell Research* reports that hypoxia-induced mitochondrial protein lactylation halts oxidative phosphorylation, underscoring the swift cellular response to low oxygen.

Hypoxia impacts diverse physiological processes, including cellular metabolism.[1] Cells respond to oxygen deficiency by suppressing mitochondrial oxidative phosphorylation (OXPHOS) to mitigate the generation of reactive oxygen species (ROS) and oxidative damage. This metabolic adaptation involves hypoxia-inducible factors (HIFs) that increase the expression of pyruvate dehydrogenase kinases, leading to the phosphorylation and inhibition of Pyruvate Dehydrogenase E1 Subunit Alpha 1 (PDHA1) within the pyruvate dehydrogenase complex — a crucial enzyme for producing mitochondrial acetyl-CoA.[2,3] However, the gradual transcription-dependent regulation falls short of a full explanation for the swift cessation of OXPHOS during hypoxic conditions, such as that occurring during muscle contraction. Now a new study by Mao et al. uncovers that hypoxia rapidly triggers lactylation of the key mitochondrial acetyl-CoA providers (PDHA1 and carnitine palmitoyl transferase 2 (CPT2)), effectively halting OXPHOS.[4]

Protein lysine L-lactylation, referred to as lactylation, is a newly identified protein post-translational modification.[5] Driven by L-lactate, the end-product of glycolysis, lactylation is linked to hypoxia and the Warburg effect, connecting nutrient metabolism to various physiological and pathophysiological processes, such as development, cancer, and immunity.[6] While nucleocytosolic acylations like lactylation and acetylation are believed to be enzymatic, mitochondrial acylations are assumed to be a result of chemical reactions.[7] This presumption was partially based on the apparent lack of transferases identified within this organelle. The study by Mao et al. challenges this view and unveils a new function for mitochondrial alanyl-tRNA synthetase 2 (AARS2), which catalyzes mitochondrial protein lactylation.

Mao et al. postulated that AARS1 and AARS2 could potentially act as lactyltransferases due to the structural similarity between lactate and their cognate substrate, alanine. They set out to find that mitochondrial AARS2, in contrast to cytosolic AARS1, contains proline residues within a prolyl hydroxylase domain (PHD, a known regulator of HIF1α during hypoxia)-recognized sequence. Mao et al. demonstrated that only AARS2 accumulation was induced by hypoxia, which was dependent on proline 377 and the oxygen-sensitive PHD2. Using mass spectrometry, they confirmed the presence of hydroxylated proline 377 (P377OH) on AARS2 in cell cultures. Subsequently, they developed an antibody specifically targeting P377OH. Utilizing this reagent, Mao et al. revealed the regulatory mechanism governing AARS2 in the presence of oxygen: oxygen-sensitive PHD2 hydroxylates the P377 site on AARS2, thereby enhancing its interaction with the E3 ligase von Hippel–Lindau (VHL), which leads to ubiquitination and subsequent proteasomal degradation of AARS2 (Fig. 1).

The authors next sought to understand the physiological roles of AARS2 during hypoxia. While mitochondrial OXPHOS generates most cellular ATP, it must be restrained to limit ROS production and associated damage in response to hypoxia. Mao et al. found that AARS2 accumulation under hypoxia reduced cellular oxygen-consuming rate (OCR), thus inhibiting respiration, whereas AARS2-deficient cells maintained higher OCR. Glucose, fatty acids, and glutamine generate acetyl-CoA for subsequent OXPHOS. Mao et al. found that AARS2 curbs the functions of two pivotal enzymes involved in acetyl-CoA production: PDHA1 in pyruvate oxidation and CPT2 in fatty acid oxidation, leading to OXPHOS suppression.

The primary role of aminoacyl tRNA synthetases, including AARS2, is to synthesize aminoacyl-tRNAs for protein translation, although they have also been found to catalyze protein aminoacylations.[8] Mao et al. demonstrated that alanine levels did not impact PDHA1, CPT2, or OXPHOS activities. Intriguingly, molecular docking and isothermal titration calorimetry revealed lactate binding to AARS2. The authors next showed that AARS2 is actually a lactyltransferase, capable of adding a lactyl group to PDHA1 and CPT2. Biochemical measurements indicated that AARS2's K_m for lactate was 5.21 mM, aligning with physiological mitochondrial lactate levels. Furthermore, Mao et al. established that AARS2-mediated lactylation of PDHA1 K336 and CPT2 K457/8 suppressed their enzymatic activities, and the lactylation on these sites was elevated in hypoxic cells and running-induced hypoxic mouse leg skeletal muscles. Notably, lactylation at PDHA1 K336 did not affect phosphorylation levels of serines 232, 293, and 300, suggesting a HIF-independent regulatory mechanism. Dynamic control of lactylation involves delactylases for removal.[9] Mao et al. showed that mitochondrial Sirtuin 3 (SIRT3) efficiently removed lactylation from PDHA1 and CPT2, thereby restoring their functions. Upon re-oxygenation, the lactylation of PDHA1 and CPT2 decreased due to the absence of AARS2 and the combined delactylation activity of SIRT3.

The decline in oxygen levels in skeletal muscles during exercise is a consequence of heightened oxygen consumption.[10] Mao et al. found that elevated OXPHOS activity led to the accumulation of AARS2 and the induction of PDHA1 and CPT2 lactylation. This observation points to a regulatory feedback loop linking cellular OXPHOS activity and hypoxia. In mouse models, the authors demonstrated that type I skeletal muscles, rich in mitochondria

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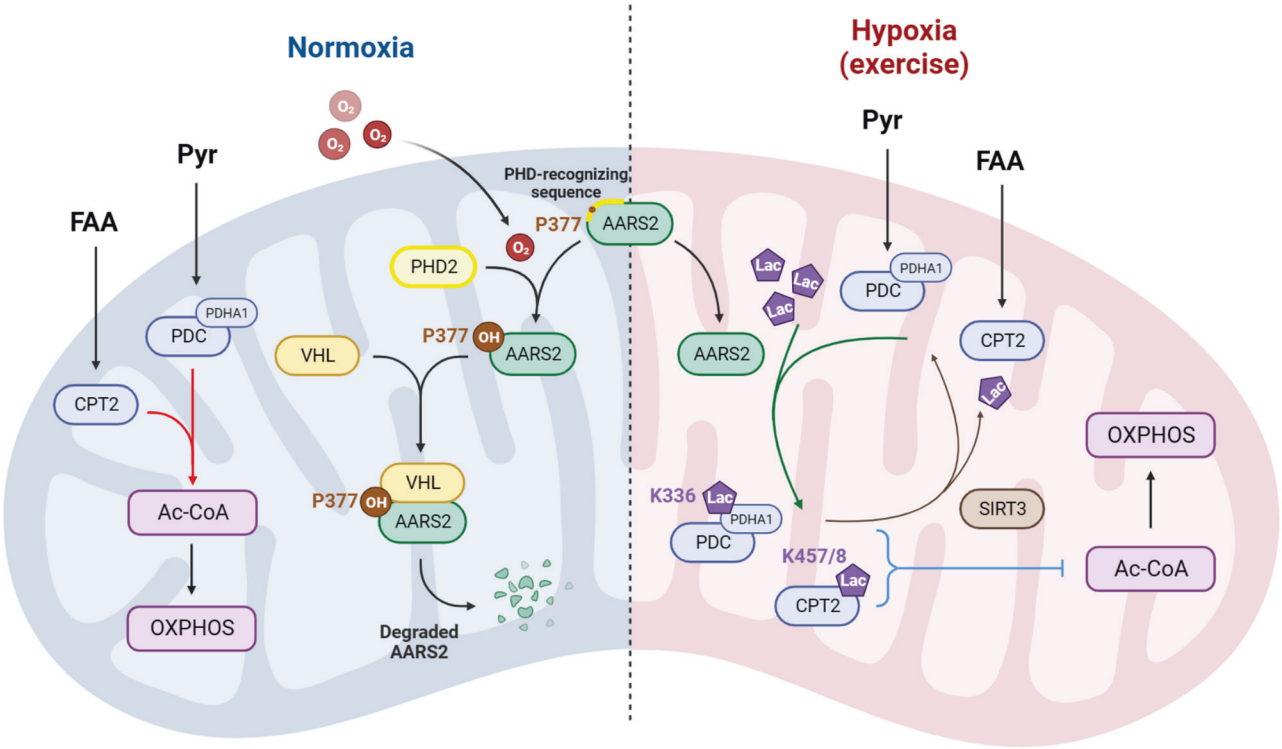

Fig. 1 Lactylation of PDHA1 and CPT2 inhibits OXPHOS. Under normoxia, PHD2 hydroxylates AARS2 at P377, enhancing its interaction with VHL and prompting its degradation. OXPHOS is unaffected, as acetyl-CoA production from pyruvate and fatty acid proceeds normally. However, during hypoxia, exemplified by muscle contraction during exercise, AARS2 accumulates and functions as a lactyltransferase. This activity curtails acetyl-CoA production through the lactylation of PDHA1 and CPT2, ultimately leading to the suppression of OXPHOS. SIRT3 can counteract this effect by removing lactylation from PDHA1 and CPT2, and restoring OXPHOS activity. This figure was created with BioRender.com.

and responsible for endurance, exhibited higher AARS2 accumulation and lactylation after exercise compared to type II muscles with fewer mitochondria. Additionally, mice overexpressing AARS2 displayed diminished endurance in extensive running compared to *Aars2*$^{-/-}$ mice due to reduced OXPHOS. However, they exhibited lower levels of ROS and oxidative damage after exhaustive running. This underscores the potential role of mitochondrial lactylation as a protective mechanism against oxidative stress during endurance exercise.

Together, Mao et al.'s study highlights the pivotal role of protein lactylation in governing mitochondrial OXPHOS, illustrating how cells adapt metabolically under hypoxia to minimize ROS generation and associated damage. The recognition of AARS2 as a lactyltransferase not only unveils an unprecedented facet of its function but also opens avenues for uncovering novel lactyltransferases. These findings highlight the influence of cellular states on metabolism, emphasizing the significance of metabolites like lactate in modulating cellular functions through feedback mechanisms. This research once again suggests that substances once deemed 'metabolic wastes' indeed play crucial roles.

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ADDITIONAL INFORMATION

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