The integrated stress response to the rescue of the starved heart

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The heart consumes large amounts of adenosine triphosphate (ATP) to sustain the processes of excitation-contraction coupling, and the flexible utilization of different substrates for ATP production ensures a dynamic adaptation to changing energetic demands in response to changes in the hormonal milieu, inotropic stimulation, and substrate availabilities. Because fatty acids (FA) are the primary fuel for oxidative phosphorylation in the human heart, it is not surprising that a varying degree of cardiac involvement is as a hallmark of inherited disorders of FA oxidation. As an example, pathogenic mutations in the gene encoding the very long-chain acyl-CoA dehydrogenase (VLCAD), which catalyzes the first step in mitochondrial β-oxidation of very long-chain FA, manifest with cardiomyopathy and skeletal myopathy during infancy.

Patients with VLCAD deficiency show a high respiratory exchange ratio during low-intensity exercise, indicating that impaired FA oxidation is compensated by enhanced muscular glycogenolysis and increased carbohydrate utilization.

Mouse models of genetic ablation of key FA oxidation enzymes recapitulate some of the features of human VLCAD deficiency and other FA oxidation disorders. Mice with deletion of the long-chain acyl-CoA dehydrogenase gene (LCAD KO) exhibit mild cardiac hypertrophy, but develop cardiac dysfunction and severe metabolic derangements when subjected to fasting. Under physiological conditions, myocardial glucose uptake progressively declines during fasting, sparing glucose derived from hepatic glycogen mobilization and gluconeogenesis to support brain metabolism. Meanwhile, cardiac ATP production is fueled by FA oxidation. In LCAD KO mice, defective FA oxidation increases systemic glucose consumption during fasting, thus rapidly draining hepatic glycogen stores. Once glycogen is depleted, blood glucose levels are exclusively sustained by hepatic gluconeogenesis, which however is impaired by a shortage in the supply of glucogenic precursors. In fact, the liver normally relies on lactate and alanine derived from pyruvate in the skeletal muscle to sustain gluconeogenesis, but circulating levels of these metabolites are decreased in LCAD KO mice. As a result, akin to patients with VLCAD deficiency, LCAD KO mice develop life-threatening hypoglycemia after a relatively short period of fasting. At the same time, insufficient supply of substrates for oxidative phosphorylation and myocardial accumulation of toxic lipid species lead to cardiac dysfunction.

In the current issue of *Cardiovascular Research*, Ranea-Robles and colleagues show that in the LCAD KO mouse model, depletion of circulating amino acids induced by fasting activates the integrated stress response (ISR) in the heart. The ISR is an evolutionarily conserved
homeostatic response mediated by specialized kinases that respond to different cellular stressors by phosphorylating the eukaryotic translation initiation factor eIF2α. Reduced amino acid availability is a known inducer of the ISR via the endoplasmic reticulum (ER) resident stress-induced kinase GCN2. Although the authors did not provide direct evidence of GCN2 activation, they observe the accumulation of uncharged tRNA accompanied by increased phosphorylation of eIF2α in LCAD KO mice. Phosphorylation of eIF2α decelerates translation of mRNAs containing a 5' methylguanine Cap (5'-Cap), which results in a general inhibition of protein synthesis that reduces the anabolic demand for amino acids. At the same time, the slow translation rates engage the preferred translation of rare mRNAs with the short inhibitory upstream open reading frames (uORF) structure. The most prominent gene upregulated via this mechanism is the transcription factor ATF4, which in turn induces expression of a wide set of genes involved in amino acid biogenesis.

The study by Ranea-Robles and colleagues offers important insights into the complex clockwork of cardiac metabolism while leaving some open questions that will foster future research. First, it reinforces the concept that disruption of FA oxidation has important repercussions on cardiac amino acid metabolism. Although the underlying mechanism has not been resolved yet, it can be speculated that the increased reliance on pyruvate oxidation for ATP production in fasted LCAD KO mice hinders cardiac protein degradation by rendering the heart unable to safely dispose of ammonium ions generated by the breakdown of amino acids (Figure). Cardiac proteolysis, which occurs physiologically during fasting, requires removal of nitrogen via its transamination to pyruvate. The product of this reaction, alanine, is shuttled to the liver, where the reverse reaction produces pyruvate for gluconeogenesis and nitrogen that enters the urea cycle. In fasted LCAD KO mice, increased utilization of pyruvate for ATP production might decrease its availability for transamination, ultimately halting protein turnover. This model explains the insufficient supply of glucogenic precursors to the liver and the shortage of amino acids required for tRNA loading observed in the LCAD KO mouse model during fasting.

Furthermore, the study by Ranea-Robles et al. adds to the emerging evidence that the eIF2α-ATF4 signaling axis plays an important homeostatic role in the heart. By inducing a general reduction in mRNA translation, the ISR likely represents an adaptive response in the short term, aiming to reestablish the balance between protein synthesis and degradation. However, it is still unclear whether prolonged ISR activation can have detrimental consequences on cardiac function, as adaptive and maladaptive responses induced by the ISR seem to be strongly
context-dependent. ATF4 upregulates enzymes involved in one-carbon metabolism, a metabolic process that serves to activate and transfer one-carbon units required for a variety of biosynthetic processes, including the synthesis of glutathione (Figure). A recent study revealed that activation of the ATF4-driven transcriptional program protects the heart from pressure overload by promoting glutathione biosynthesis and NADPH production, thereby conferring additional protection against oxidative stress. Whether other biosynthetic pathways linked to one-carbon metabolism are also activated and play a relevant role in this context remains unknown. The flip side of the coin is that one-carbon units required to support these processes are primarily derived from serine, which in turn is synthesized from the glycolytic intermediate 3-phosphoglycerate (Figure). Therefore, ATF4-driven upregulation of serine biosynthesis and one-carbon metabolism comes at the cost of a further increase in glucose utilization, potentially aggravating the metabolic imbalance that induced its activation in the first place. Furthermore, the eIF2α-ATF4 signaling axis is an important inducer of cellular apoptosis by upregulating the transcription factor CHOP during glucose deprivation. While this has not been tested in the heart, it is possible that prolonged activation of the ISR ultimately leads to cardiac myocyte apoptosis in LCAD KO mice.

Altogether, the work by Ranea-Robles and colleagues sheds new light on the homeostatic mechanisms that mediate the response to defective FA oxidation in the heart. However, the role of the ISR and amino acid metabolism in this context is far from being understood. Future studies should address the context-specific consequences of the eIF2α-ATF4 signaling pathway in cardiac myocytes.

Conflict of interest: none declared.
Figure

Overview of fasting-induced metabolic alterations in the long-chain acyl-CoA dehydrogenase knockout (LCAD KO) mouse model. Pathways upregulated by the eIF2α-ATF4 signaling axis are highlighted in blue. Abbreviations: 1C, one-carbon; 3-PG, 3-phosphoglycerate; NAD(P)H, reduced form of the nicotinamide adenine dinucleotide (phosphate).
References