

Intracellular localization of AMP deaminase and its novel role in BCAA and lipid metabolism in diabetic cardiomyopathy

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Background: A metabolomic study in the human heart suggested a pivotal role of amino acid (AA) metabolism in fatty acid oxidation, which is dysregulated in type 2 diabetes mellitus (T2DM) and heart failure. We previously reported that aberrant up-regulation of AMP deaminase 3 (AMPD3) impairs cardiac energetics in T2DM hearts, and AMPD3 was recently shown to be activated by fasting and to promote AA metabolism and fatty acid oxidation in skeletal muscle. A sodium glucose cotransporter 2 inhibitor (SGLT2i) has been shown to augment systemic AA metabolism, but its effect on cardiac AA metabolism remains unknown.

Purpose: We hypothesized that AMPD3 has a role in AA and lipid metabolism in cardiomyocytes and that the protective effect of an SGLT2i in diabetic hearts is mediated by modification of AA and lipid metabolism.

Methods and results: Proteomic analyses of AMPD3 immunoprecipitates in rat hearts revealed that AMPD3 interacted with the E1 α and E2 components of the BCKDH complex, a rate-limiting enzyme of branched-chain AA (BCAA) catabolism. Immunoblotting using subcellular fractions revealed that BCKDH localized not only in the mitochondria matrix but also in the cytosol and endoplasmic reticulum (ER) and that AMPD3 interacted with BCKDH in the cytosol and ER. Despite comparable expression of BCKDH components and phosphorylation of E1 α at Ser293, significant accumulation of BCAA was observed in T2DM rats (OLETF; 317 \pm 30 nmol/g) com-

pared to that in control rats (LETO; 213 \pm 16 nmol/g), and the accumulation of BCAA was accompanied by up-regulation of AMPD3 in the cytosol and ER by 98% and 231%, respectively. In cardiomyocytes, disruption of BCAA catabolism by knockdown of BCKDH-E1 α resulted in a 5.8-fold increase in AMPD3 at the transcriptional level and blunted lipid droplet biogenesis in response to a long-chain fatty acid challenge. Next, myocardial infarction (MI) was induced in LETO and OLETF pretreated with empagliflozin (10 mg/kg/day, 14 days) or a vehicle. Pathway analysis of cardiac metabolites revealed arginine biosynthesis and BCAA metabolism as the most significantly changed pathways with empagliflozin, with BCAA (791 \pm 187 nmol/g), glutamate, glutamine and urea being significantly increased. Empagliflozin restored myocardial ATP and survival after MI in OLETF to levels comparable to those in LETO. Electron microscopy showed a significantly higher prevalence of myocardium lipid droplets in OLETF, which was further increased by empagliflozin.

Conclusions: The results support the hypotheses that imbalance of extra-mitochondrial AMPD3-BCKDH interaction underlies dysregulated BCAA metabolism in T2DM hearts and that activation of cardiac AA metabolism by an SGLT2i normalizes fatty acid overload through sequestration into intracellular lipid droplets.