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EXACERBATION OF MYOCARDIAL ISCHEMIA/REPERFUSION INJURY INDUCED BY HIGH-FAT-HIGH-FRUCTOSE (HFHF) DIET: ROLE OF NLRP3 INFLAMMASOME

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Objectives: The diet-induced metabolic overload initiates a low-grade, chronic inflammatory response, known as metaflammation, which promotes cardiometabolic diseases. A recently identified pathways involved in metaflammation is the NLRP3 inflammasome, a large multimeric danger-sensing platform that promotes autocatalytic activation of the cysteine protease caspase-1 and mediates the cleavage of inactive pro-IL-1β, among other proteins, into its active form. Recently we demonstrated that the NLRP3 inflammasome activation is present in hepatic cell lipotoxicity due to high concentration of the palmitic acid. We have also shown that a fructose-enriched diet induces upregulation of renal NLRP3 expression linked to renal dysfunction. In the present study we have studied the potential role of NLRP3 inflammasome complex in presence of a standard diet (SD) or a HFHF diet in determining the heart response to ischemia/reperfusion (I/R).

Materials and methods: C57Bl/6 male mice were subjected two different diets (SD or HFHF) for 12 weeks, at the end of which the *ex vivo* hearts were exposed to I/R protocol. Western blot analysis on heart homogenates were realized to evaluate NLRP3, IRS-2 and metabolic markers (CPT-1m and SDH) and immuno staining/histochemistry for evaluation of lipid accumulation.

Results: Dietary manipulation evoked the shift of myosin heavy chain isoform content from α to β and an increased expression of markers of oxidative metabolism, increased intramyocellular lipid accumulation as shown by Oil Red O staining. Immunohistochemistry analysis showed that HFHF reduced translocation of GLUT-4 from cytosol to membranes, and increased phosphorylation rate of IRS-2 that inactivates insulin signaling, thus indicating a diet-induced insulin resistance of the cardiomyocytes. The I/R protocol in HFHF mice hearts showed greater infarct size and LDH release in comparison with SD mice. I/R induced a significant upregulation of NLRP3 and caspase-1 either in hearts from mice fed SD or in those from mice fed HFHF. Yet, pre-ischemic expression levels of NLRP3 and activated caspase-1 were drastically higher in HFHF mouse hearts in comparison to those recorded in SD hearts.

Conclusions: This study demonstrates that the upregulation of NLRP3 protein evoked by I/R injury is drastically higher in the presence of a diet-induced metabolic derangement. These findings suggest a potential association between increased activity of NLRP3 and enhanced susceptibility to a myocardial ischemic insult, thus suggesting a potential role of the NLRP3 as innovative pharmacological target to counteract the development of cardiovascular disorders linked to metabolic diseases.