A NEW 2H2O-METABOLIC LABELING METHOD REVEALS HETEROGENEOUS ALTERATIONS OF PROTEIN TURNOVER IN THE FAILING HEART

Poster Contributions
Hall C
Sunday, March 30, 2014, 9:45 a.m.-10:30 a.m.

Session Title: Novels Insights and Approaches to Heart Failure Mechanisms
Abstract Category: 13. Heart Failure and Cardiomyopathies: Basic
Presentation Number: 1187-207

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The altered myocardial protein turnover is considered a major pathogenic factor in heart failure (HF), however studies performed with standard molecular analysis have provided only indirect and discordant evidence of this phenomenon. Direct measurements of the rate of in vivo protein turnover are not available due to the lack of adequate methodologies. To overcome this limitation, we tested a new method based on the administration of 2H2O (deuterated water) and subsequent endogenous labeling of 2H to amino acids. A loading dose of 2H2O was i.v. (15ml/kg), followed by 72h of oral administration in the drinking water (5%) to dogs with advanced pacing-induced congestive HF (left ventricular end-diastolic pressure ≥25mmHg) and to normal controls. Levels of 2H2O were stable, and left ventricular tissue was harvested finally. Myocardial proteins were trypsinized after gel electrophoresis, peptide fragments analyzed by high resolution liquid chromatography-tandem mass spectrometry (MS) and individual synthesis rates was calculated from the time course of the rise in the enrichment with 2H in peptide isotopomers using specialized software. We focused on selected proteins that play an essential role in maintaining cardiomyocyte cytoarchitecture, contractile function and metabolism and are known to be altered in HF. Data are expressed as percent of newly synthesized molecules/hour over their respective total pools. Compared to control, in HF the protein turnover rate changed as follows (n=6/group): among the structural proteins, the heavy chain beta-myosin increased from 0.15±0.04 to 0.20±0.02%/h, while cardiac actin decreased from 0.20±0.08 to 0.10±0.04%/h (all P<0.05) and desmin did not change significantly; among the energy producing enzymes, creatine kinase increased from 0.10±0.03 to 0.15±0.03%/h, while adenine nucleotide translocase-1 decreased from 0.23±0.08 to 0.14 ±0.04%/h (all P<0.05) and ATP synthase did not change significantly. In conclusion, HF causes differential, rather than uniform, changes in the rate of synthesis of myocardial proteins. These results are the first to show the potential of 2H2O-metabolic labeling for quantitative assessment of molecular turnover in HF.