USE OF STABLE ISOTOPOMERS AND LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY (LC/MS) FOR CARDIAC SUBSTRATE PREFERENCE ANALYSIS IN MICE

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OBJECTIVES: Heart is an organ characterized by prominent metabolic flexibility for utilizing all carbon substrates. It is well known that cardiac substrate preference could be coordinated by factors such as the availability of individual substrates, hormonal activity but also might be deregulated in pathological conditions. However, so far there is no simple *in vivo* method to investigate changes in substrates use in cardiac metabolism in mice. Thus, the aim of the study was to develop method for the analysis of cardiac substrate preference using stable ^{13}C glucose, ^{13}C valine and ^{13}C leucine isotopomers as well as LC/MS.

METHODS: **Our assay investigated the D-Glucose-1,6-$^{13}C_2$ flux to Pyruvate-3-^{13}C as well as the entrance of the Acetyl-CoA-3-^{13}C (formed from D-Glucose-1,6-$^{13}C_2$ and L-Leucine-3-^{13}C) and Sucinnyl-CoA-2,3,4-$^{13}C_3$ (formed from L-Valine-$^{13}C_5$) to the Krebs cycle by analysis of Alanine-3- ^{13}C as well as Glutamate-4-^{13}C and Glutamate-1-^{13}C enrichment in mice heart (Scheme).**

RESULTS AND CONCLUSIONS: After 90 min (D-Glucose-1,6-$^{13}C_2$) or 60 min (L-Leucine-3-^{13}C and L-Valine-$^{13}C_5$) of administration mice were anesthetized, intubated and ventillated. Hearts were then freeze-clamped. Blood samples were collected from a tail vein before and after isotopomer injection. Hearts were extracted with perchloric acid while the blood extraction was performed with acetone. The method was validated with inhibitors of glucose and fatty acids metabolism that demonstrated expected shifts in cardiac metabolic substrate use (Figure).

Our method allows fast and simple estimation of cardiac glucose and BCAA use in mice and has potential for analysis of changes in pathology and after pharmacological treatments.

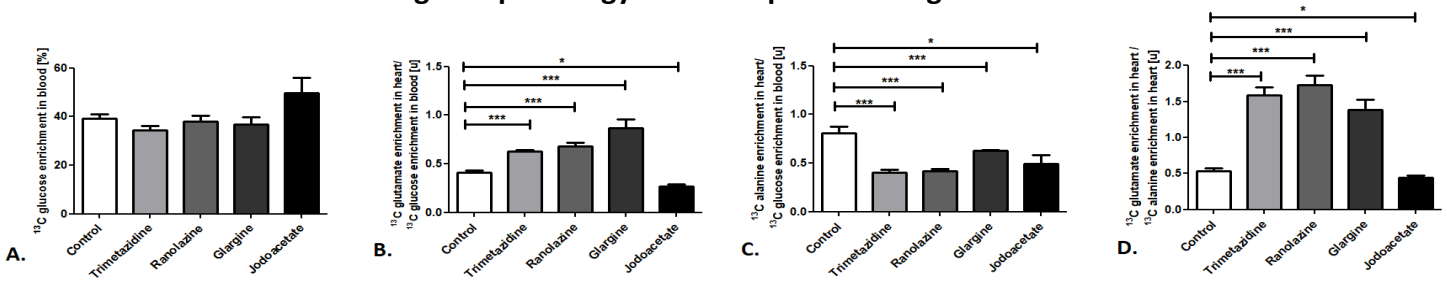
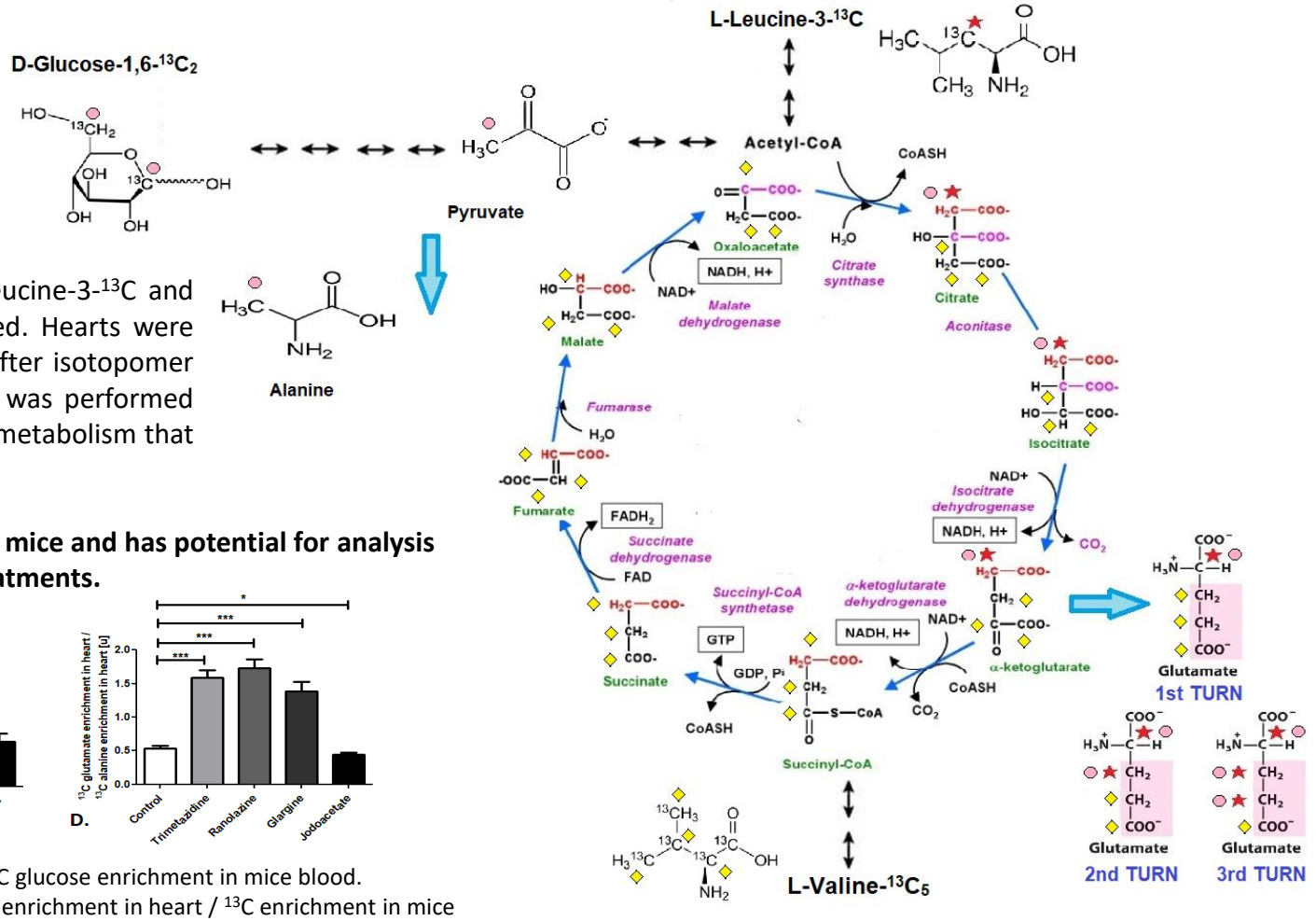


Figure. Glucose use in mice cardiac metabolism after pharmacological treatment A. ^{13}C glucose enrichment in mice blood. B. ^{13}C glutamate enrichment in heart/ ^{13}C glucose enrichment in mice blood ratio. C. ^{13}C alanine enrichment in heart / ^{13}C enrichment in mice blood ratio. D. ^{13}C glutamate/ ^{13}C alanine ratio in mice heart. Results presented as mean ± SEM, n=5, * p<0.05, **p<0.01, ***p<0.001.



Scheme of cardiac substate preference method.