In-vivo SILAM ^{13}C Mouse Tissues

Stable isotopic labeling is the most reliable and accurate method for quantitative proteomics and metabolomics. Proteomics studies on uniformly ^{15}N-labeled rats have been previously reported using ^{15}N-labeled algae as protein source for Stable Isotope Metabolic Labeling in Mammals (SILAM).[1]

If the goal is to detect and relatively quantify a large number of metabolites in an untargeted manner, lower organisms can be fed or mammalian cells grown in culture with a diet or media, respectively where ^{12}C is replaced with the stable ^{13}C isotope. The generated tissue or cellular material can then be used as isotope-labeled internal standard reference. This approach has been successfully established for microorganisms and was subsequently extended to plants and small animals.

Silantes ^{13}C-SILAM Diets for *in-vivo* labeling of mice

Silantes has developed a diet to ^{13}C-label mice with the aim of producing isotope-labeled material as reference for metabolomics studies.

Preliminary studies by us have shown (next page) that the partially ^{13}C-labeled mouse reference material can be used for the relative quantification of amino acids in human plasma samples. We envision that this approach can be extended to the relative quantification of other small molecules of interest in metabolomics research.

We have developed 2 diets for metabolic ^{13}C-labeling of mice:

1) Partially ^{13}C-labeled SILAM mouse diet based on *Ralstonia eutropha* biomass with a ^{13}C isotopic enrichment of > 20 atom %.

2) Uniformly ^{13}C-labeled SILAM mouse diet based on *Arthrospira maxima* ("Spirulina") biomass with a ^{13}C isotopic enrichment of > 98 atom %.

[1] Daniel B. McClatchy, Meng-Qiu Dong, Christine C. Wu, John D. Venable, and John R. Yates (2007). 15N Metabolic Labeling of Mammalian Tissue with Slow Protein, Journal of Proteome Research, 6(5), 2005-2010.

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Metabolomics Study using the Partially ^{13}C-labeled SILAM Mouse Diet

We have shown that amino acids in human plasma samples can be relatively quantified using reference material from mice fed for 14 days with the partially ^{13}C-labeled diet.

Mouse amino acids from tissue and blood had up to 75% ^{13}C enrichment levels although the initial diet only had a ^{13}C enrichment of ~20%.

Figure 1 shows the ^{13}C enrichment of free amino acids in mouse plasma, heart tissue and plasma protein hydrolysates mapped on a simplified metabolic pathway. The overall average ^{13}C enrichment was ~ 65%. Essential amino acids showed particularly high ^{13}C isotope enrichment. This is due to the fact that essential amino acids cannot be produced by the organism itself and proteins from the partially ^{13}C-enriched feed serve as the main protein source.

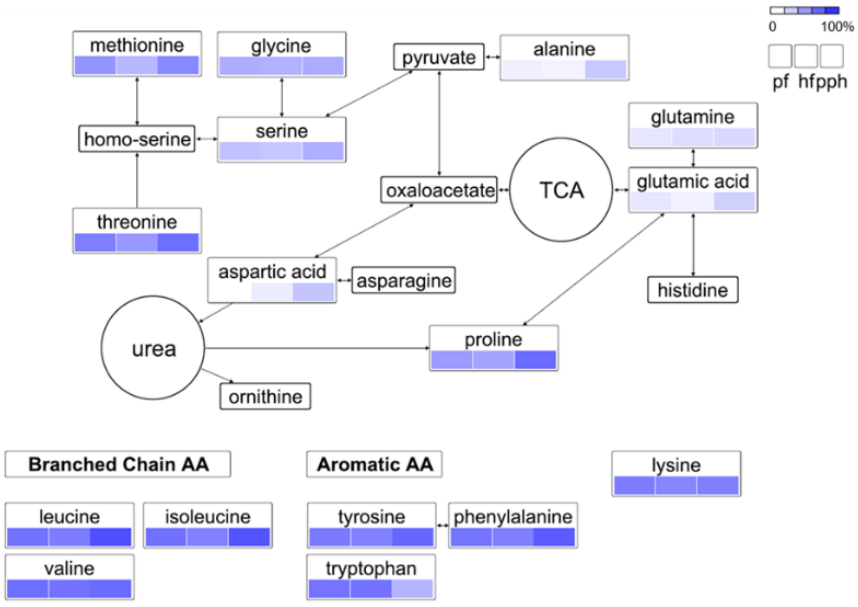

Figure 1:

^{13}C enrichment of free amino acids in mouse tissue and blood. The color code represents the average ^{13}C enrichment of 3 biological replicates (plasma = pf; heart = hf) and 3 technical replicates (plasma protein hydrolysates = pph).

For the relative quantification of amino acids in human plasma samples, the partially ^{13}C-labeled mouse reference turned out to be a very valuable source. Since mice share many metabolic pathways with humans it is conceivable that our approach can be further extended to other small molecules of interest in human metabolomics research.

In addition, mouse ^{13}C-labeled blood and tissue material can serve as valuable reference for metabolomics studies of mouse models, to verify biomarker candidates and aid in their identification.

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