Development of biomarkers for the protein restriction using *Slc6a19* knock out mice

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Mutation in B^0AT1 (Slc6a19) lead to an autosomal recessive disorder known as Hartnup disorder that is mainly characterized by reduced absorption of amino acids in the intestine and renal aminoaciduria. The characterization of *Slc6a19* knock out mouse model has revealed signs of protein restriction such as elevated levels of FGF21, but also higher levels of GLP-1 due to increase in amino acid load in the intestine. The combination of both effects improve glycaemic control and makes this transporter a potential target for the treatment of type 2 diabetes. Our lab is currently screening for chemical compounds that can block this transporter effectively. To evaluate the efficacy of these compounds, there is a need to develop biomarkers that can easily detect the successful inhibition of B^0 AT1 in mice and to detect protein restriction in general.

Increased levels of amino acids in urine demonstrate the malfunction of B^0AT1 in the kidney. To quantify the reduced absorption of amino acids in the intestine, we developed a simple method in which mice voluntarily eat a diet that incorporates a small amount of C-14 labelled amino acids. The results showed reduced absorption of methionine and leucine in the knock out mice. The difference in the rate of absorption of different amino acids provides direct information about the preference of substrates for B^0AT1 in the intestine.

To find biomarkers that can detect the inhibition of B^0AT1 in intestine and correlate with protein restriction and malabsorption, a non-targeted metabolomics approach was designed to identify metabolites in urine, fecal and breath samples. A particularly interesting group of metabolites are fermentation products of amino acids that may show proportionality to protein load. The microflora of the intestine ferments amino acids into short chain fatty acids and other metabolites. In urine samples, a significant increase in neutral amino acids and some metabolites of bacterial origin was seen in the knock out mice. This shows the potential of qualitative metabolomics to identify biomarkers for protein restriction and malabsorption.