HIGHLY SENSITIVE METABOLIC FLUX ANALYSIS USING ^{14}C MICROTRACERS COMBINED WITH ACCELERATOR MASS SPECTROMETRY

A PROOF OF CONCEPT STUDY ON DE NOVO LIPOGENESIS

Morrison MC, Stevens L, Kleemann R, Verheij ER, Vaes W.

Department of Metabolic Health Research, the Netherlands Organisation for Applied Scientific Research (TNO), Leiden, the Netherlands.

TNO innovation for life

Introduction

Metabolic flux measurements play an important role in advancing our understanding of (patho)physiology, disease mechanisms and the development of new therapeutics. By incorporating stable or radioactive isotopes into specific molecules (tracers), the distribution and fate of the isotope can be followed – thereby providing insight into the movement and metabolic transformation of biomolecules.

An isotope that is frequently used for such analyses is ^{13}C, which is analyzed by isotope ratio mass spectrometry. The high natural abundance of ^{13}C (~1%) requires the use of large amounts of labelled substrates, especially in clinical studies.

An alternative isotope is ^{14}C, which has an extremely low natural abundance (1 in 10^{12}). In combination with analysis by extremely sensitive accelerator mass spectrometry (AMS), this enables the detection of very small amounts of ^{14}C-labeled product/biomarker at very low (microtrace) amounts of labelled substrate administration.

Aim

Provide proof of concept for a ^{14}C-microtracer approach to assess activity of the *de novo* lipogenesis (DNL) pathway. DNL is an important metabolic pathway that is deregulated in various disease states, including metabolic dysfunction-associated steatotic liver disease (MASLD).

Methods

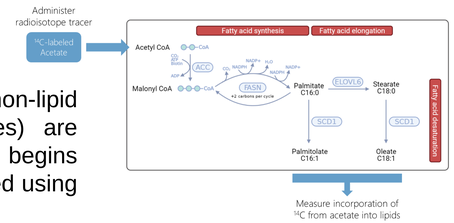
De novo lipogenesis activity was assessed in Ldlr-/-.Leiden MASLD mice[1] fed a standard chow or a MASLD-inducing high-fat diet (HFD) with/without the DNL inhibitor firsocostat (ACCi), as well as in an *ex vivo* porcine liver perfusion model[2].

Incorporation of ^{14}C from ^{14}C-acetate into total lipid extracts (from plasma and liver samples), bile, fatty acids (plasma and liver), and cholesterol was assessed by LC/MS and AMS.

1. Gart et al., Translational characterization of the temporal dynamics of metabolic dysfunctions in liver, adipose tissue and the gut during diet-induced NASH development in Ldlr-/-.Leiden mice. Heliyon. 2023.
2. Stevens et al., Evaluation of Normothermic Machine Perfusion of Porcine Livers as a Novel Preclinical Model to Predict Biliary Clearance and Transporter-Mediated Drug-Drug Interactions Using Statins. Drug Metab Dispos. 2021.

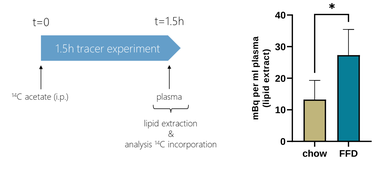
Assessment of DNL using a ^{14}C-acetate microtracer

DNL is a pathway in which non-lipid precursors (primarily carbohydrates) are converted into lipids. The process begins with acetyl-Coa and can be assessed using a ^{14}C-acetate tracer.

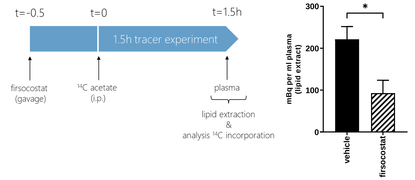


^{14}C from acetate is incorporated into lipid fraction in plasma (and liver) of Ldlr-/-.Leiden MASH mice

Ldlr-/-.Leiden MASH model

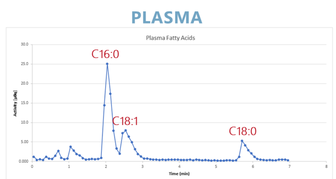


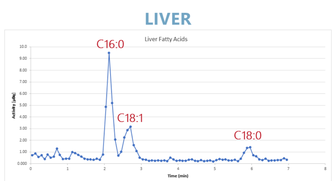
^{14}C incorporation in lipid fraction is increased in Ldlr-/-.Leiden mice with MASH (FFD) relative to chow.



DNL inhibitor firsocostat (inhibits ACC), strongly reduces ^{14}C incorporation in plasma lipid fraction as expected.

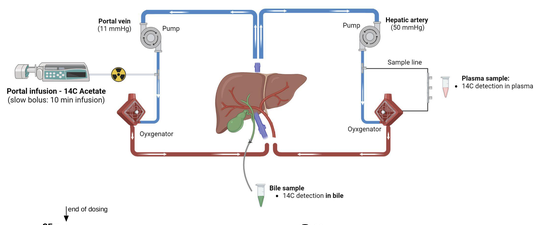
^{14}C fatty acids in plasma and liver reflect DNL activity

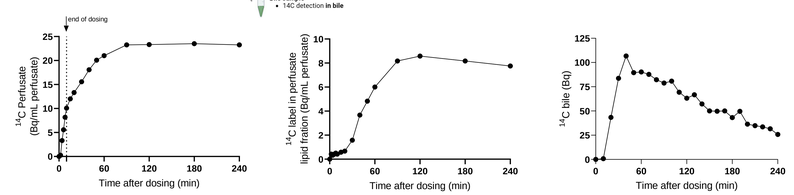




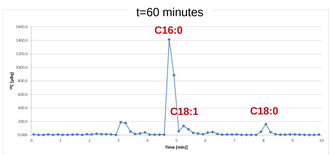
Fatty acid profiling analysis (LC/MS) combined with AMS enrichment analysis showed that the ^{14}C signal from acetate is predominantly found in palmitate (C16:0, the primary end product of DNL) and in lesser amounts also in fatty acids that result from further processing of palmitate (C18:0 and C18:1) thus confirming that the observed incorporation of ^{14}C from acetate into the lipid fraction of plasma and liver is indeed a reflection of DNL.

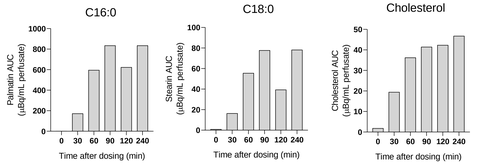
Metabolic flux analysis in *ex vivo* liver for DNL, bile and cholesterol synthesis using ^{14}C microtracers.





Similar to observations in the Ldlr-/-.Leiden MASH mouse model, ^{14}C from acetate was also incorporated into the plasma lipid fraction in the *ex vivo* liver perfusion model. In addition, enrichment of ^{14}C was also observed in the bile collected from the *ex vivo* liver.





LC/MS analysis followed by AMS enrichment analysis showed ^{14}C enrichment in C16:0, C18:0 and C18:1 fatty acids, again confirming DNL. Furthermore, ^{14}C from acetate was also observed in the cholesterol fraction, showing that this methodology can also be applied for analysis of new cholesterol synthesis.

4. Conclusion

This highly sensitive ^{14}C microtracer approach combined with AMS analysis can be used for assessment of metabolic fluxes in various experimental models at much lower amounts of tracer used than in conventional tracer methodologies. This allows study of metabolic pathways without disrupting the pathway of interest by adding large quantities of precursor. This opens up opportunities to study the activity of (metabolic) pathways in which only very small amounts of product are produced.

› martine.morrison@tno.nl