prosciento®

ADVISE • ADVANCE • ACHIEVE

FACT SHEET

Hepatic De Novo Lipogenesis Assessment

Hepatic de novo lipogenesis (DNL) is the synthesis of fatty acids in the liver from their metabolic precursor acetyl-CoA and is an important pathway reflecting metabolic health. Abnormal regulation of hepatic DNL has been observed in insulin resistance, type 2 diabetes and nonalcoholic fatty liver disease (NAFLD) (1,2), which makes it an appealing target for the treatment of metabolic diseases. Quantifying DNL using stable isotopes, therefore, is a valuable pharmacodynamic (PD) marker in early clinical trials. ProSciento scientists have extensive experience in the use of stable-isotope tracer methodologies, including the measurement of DNL in phase 1 and phase 2 clinical trials.

Stable Isotope Methods and Considerations

DNL measurements are performed using stable isotope labeling with either ^{13}C-acetate (IV) or deuterated water (oral), depending on the objective of the study.

- ^{13}C-acetate labeling is the preferred option for short-term repeat testing (see reference (3,4) as examples).
- Deuterated water labeling is useful for long-term and integrated analyses (see references (1,2) as examples).

The simple oral route of deuterated water administration unburdens the patient and research site from overnight ^{13}C-acetate intravenous infusions, and the ability to administer deuterated water for weeks allows the labeling of slow-turnover lipid pools in the liver and the measurement of steady-state DNL. However, because deuterated water has a long half-life in the body and deuterium carry-over from visit to visit is challenging to correct for, ^{13}C-acetate is more suitable for short-term repeat testing of DNL. ^{13}C-acetate labeling has been shown to be a robust and reproducible method when combined with fructose feeding (Figure 1) (3). ProSciento has established relationships with producers of stable isotopes, GMP manufacturers for the preparation of sterile solutions for IV administration, and analytical laboratories.

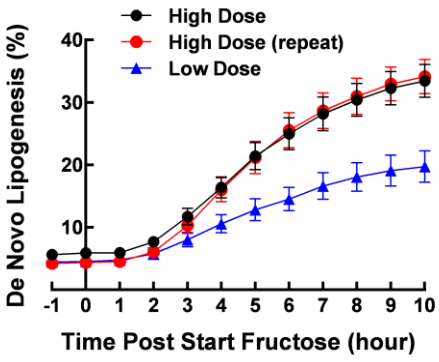

Figure 1: Time course of hepatic de novo lipogenesis (DNL) in response to acute fructose feeding in healthy subjects. Fractional DNL represents the percent of newly synthesized palmitate in VLDL-triglyceride measured during an infusion of [1-$^{13}C_1$]acetate. In two of the three study periods, subjects received 10 mg fructose/kg FFM/min (high dose and high dose repeat), and, in a third period, subjects received 5 mg fructose/kg FFM/min (low dose). Data (mean ± SEM) is from 10 subjects who completed all three study periods. Details of the method are described by Beysen et al. (4). Reproduced with permission (4).

Subjects and Procedures

Subjects receive standardized meals followed by an overnight fast prior to the DNL assessment. Limitations are often placed on weight, dietary and exercise changes, and alcohol, simple sugar and caffeine intake; all of which may influence de novo lipogenesis.

- ***Steady-state DNL using deuterated water labeling:*** These types of studies generally consist of several short outpatient visits for blood (for DNL measurement) and saliva or urine (for body water enrichment) collection, while

the deuterated water is consumed daily at home. An overnight admission to the clinical research facility prior to the DNL assessment may be considered to help ensure compliance with nutrition and exercise. The deuterated water labeling protocol depends on the specific study design, however, a typical labeling paradigm consists of subjects drinking 50 mL of 70% deuterated water three times a day for several days or weeks.

- ***Stimulated DNL using ^{13}C-acetate labeling and fructose feeding:*** A continuous infusion of ^{13}C-acetate is started the evening before the DNL assessments and continued until the last blood sample is collected the next day. After an overnight fast, a blood sample is collected to measure baseline DNL, followed by the frequent administration of small fructose drinks and regular blood sampling. A minimum of five days is required for sufficient label washout prior to repeat testing. Oral administration of deuterated water may be used instead of an ^{13}C-acetate infusion, however, a longer washout period (weeks) is required for repeat testing.

- ***Sample Processing and Analyses:*** Triglycerides are isolated from plasma or very-low density lipoproteins. Fractional DNL is then determined by measuring the incorporation of ^{13}C or deuterium in palmitate and the precursor pool using gas chromatography–mass spectrometry (GC-MS) and mass isotopomer distribution analyses (MIDA) (5) or isotope ratio mass spectrometry (IRMS).

Applications in Clinical Drug Development

Direct measurement of DNL can be very valuable in making early decisions in cardiometabolic drug development. Fructose-stimulated DNL, for example, has successfully been used in phase 1 and 2 clinical trials to assess the PD effects of lipogenesis inhibitors for the treatment of NASH (4,6,7). Deuterated water labeling has been applied in patients with NASH to reveal significantly greater DNL in this population compared to healthy subjects (1,2) and to demonstrate significant reductions in integrated DNL after 12 weeks acetyl-CoA carboxylase inhibition (2).

References

1. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology. 2014 Mar;146(3):726–35.
2. Lawitz EJ, Coste A, Poordad F, Alkhouri N, Loo N, McColgan BJ, et al. Acetyl-CoA Carboxylase Inhibitor GS-0976 for 12 Weeks Reduces Hepatic De Novo Lipogenesis and Steatosis in Patients with Nonalcoholic Steatohepatitis. Clin Gastroenterol Hepatol. 2018 Apr 26;
3. Beysen C, Ruddy M, Stoch A, Mixson LA, Rosko K, Riiff T, et al. Dose-dependent quantitative effects of acute fructose administration on hepatic de novo lipogenesis in healthy humans. Am J Physiol Endocrinol Metab. 2018 Mar 20;
4. Stiede K, Miao W, Blanchette HS, Beysen C, Harriman G, Harwood HJ, et al. Acetyl-coenzyme A carboxylase inhibition reduces de novo lipogenesis in overweight male subjects: A randomized, double-blind, crossover study. Hepatology. 2017 Aug;66(2):324–34.
5. Hellerstein MK, Neese RA. Mass isotopomer distribution analysis at eight years: theoretical, analytic, and experimental considerations. Am J Physiol. 1999 Jun;276(6 Pt 1):E1146-1170.
6. Kim C-W, Addy C, Kusunoki J, Anderson NN, Deja S, Fu X, et al. Acetyl CoA Carboxylase Inhibition Reduces Hepatic Steatosis but Elevates Plasma Triglycerides in Mice and Humans: A Bedside to Bench Investigation. Cell Metab. 2017 Aug 1;26(2):394-406.e6.
7. Griffith DA, Kung DW, Esler WP, Amor PA, Bagley SW, Beysen C, et al. Decreasing the rate of metabolic ketone reduction in the discovery of a clinical acetyl-CoA carboxylase inhibitor for the treatment of diabetes. J Med Chem. 2014 Dec 26;57(24):10512–26.

ProSciento Methodology Fact Sheets

Introduction to Glucose Clamps – Automated Versus Manual Techniques | Euglycemic Time-Action Profile Clamps | Hyperinsulinemic Euglycemic Insulin Sensitivity Clamp | Hypoglycemic Clamp Studies | Oral Glucose Tolerance and Mixed Meal Tolerance Tests | Graded Glucose Infusion for Assessment of β-Cell Function | MRI for Assessment of NAFLD/NASH | Hepatic De Novo Lipogenesis

About ProSciento Methodology Fact Sheets

Each fact sheet describes one select technique from our wide scope of methodologies for metabolic clinical research. Contact bd@prosciento.com for fact sheets highlighting additional techniques.

Contact Us

Business Development and Scientific Services
+1 (619) 419-2038
bd@prosciento.com
www.prosciento.com

ProSciento, Inc.
855 3rd Avenue Suite 3340
Chula Vista, CA 91911. USA