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Application Note

Quantification of DNL with Stable Isotope Techniques

Metabolic Research

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Stable Isotopes: An Approach to Quantifying the Conversion of Carbohydrate to Fat in Humans

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The recent completion of the Human Genome Project and the progress in proteomics have contributed to our current understanding of signal transduction pathways that control and modulate metabolism and, ultimately, determine human health. Transgenic animal model studies have shown, however, that the metabolic consequences of a gene alteration are often unpredictable, because they often fail to take into account the complex intertwine of metabolic redundancies in intact living organisms. These limitations have emphasized the need to develop new methodologies, able to monitor and quantify the dynamics of metabolic fluxes *in vivo*: dynamic metabolomics. Ultimately, the quantification of metabolic controls in whole body systems will dictate therapeutic intervention, based on a better understanding of the roots of metabolic abnormalities.

In this context, a new generation of tracer methodologies using stable isotopes has opened infinite options to study biosynthetic processes *in vivo*.[1] One application of this technique is to quantify the hepatic conversion of carbohydrate (CHO) to fat, de novo lipogenesis (DNL). Carbohydrates breakdown to produce 2-carbon units (monomers) used to synthesize fatty acid polymers. For example, eight monomers are assembled to make the 16-carbon, most common fatty acid, palmitate. With this approach, the monomer pool is first labeled with stable isotopes. Quantification, by gas chromatography/mass spectrometry (GC/MS), of the labeled monomer material incorporated into the polymers then allows the calculation of the proportion of newly synthesized fatty acids. In humans, the contribution of endogenous CHO to *de novo* fat synthesis was, until recently, always considered insignificant. However, over the past decade new evidence, based upon the use of stable isotopes to measure dynamic metabolic fluxes, suggest that hepatic DNL is not always trivial.

Using stable isotopes to study CHO and fat metabolism, we and others have found that the interplay between hepatic DNL and glucose production varies considerably depending on the diet and the health status of the subjects studied. The realization that hepatic DNL may be quantitatively significant under various nutritional and metabolic conditions is not only a shift from previous paradigms, but also points to a potential mechanism linking DNL to VLDL-triglyceride levels and liver fat content. Our ability to quantify DNL in humans may provide mechanistic insight regarding the contribution of DNL to such conditions as hypertriglyceridemia and non-alcoholic steatohepatitis, which are becoming increasingly prevalent in overweight and obese populations.[2]

Effect of dietary carbohydrates on hepatic DNL in healthy volunteers

Based on studies using MIDA (mass isotopomer distribution analysis) to quantify hepatic DNL, it is now generally accepted that in healthy individuals eating a Western high-fat diet, hepatic DNL is minimal in the fasting state but increases multiple-fold postprandially because of the rise in lipogenic precursors that occurs after the consumption of a meal.[3] In contrast, DNL is present even in the fasting state, and becomes quantitatively significant with high CHO feeding,[4] and after overfeeding with simple CHO[5] or fructose.[6] Hence, the use of stable isotope methodologies has allowed researchers to establish that the consumption of a diet rich in total or

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simple CHO is highly lipogenic, producing elevations in DNL that persist even after an overnight fast. In addition to increasing hepatic DNL, such diets concomitantly increase TG (triacylglycerol) levels, supporting the contribution of DNL to hypertriglyceridemia (Schwarz et al. 2003). It is noteworthy, however, that CHO-induced DNL appears to be specific to simple CHO. Indeed when simple sugars are restricted and a high-complex CHO diet is fed, DNL is trivial after an overnight fast (Parks et al., 1999).

Effect of hyperinsulinemia on hepatic DNL

We have also examined DNL in normo- and hyperinsulinemic obese volunteers and found that when fed a Western high-fat diet, hyperinsulinemic individuals have a higher rate of DNL compared to weight-matched normoinsulinemic obese subjects,[4] suggesting that in the absence of high CHO feeding, insulin resistance alone is sufficient to stimulate DNL. Similarly, hyperinsulinemic viscerally obese HIV-positive patients, as well as critically ill insulin resistant patients all have higher hepatic DNL compared to healthy subjects, irrespective of dietary CHO intake.[7-8] Taken together, these findings in hyperinsulinemic obese, critically ill, and HIV-infected subjects suggest that hyperinsulinemia per se stimulates DNL, independently from the macronutrient composition of the diet (**Figure 1**).

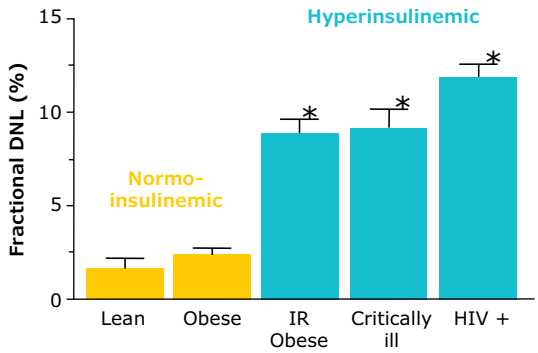

Figure 1. Hepatic DNL measured after an overnight fast in hyperinsulinemic patients. DNL in the fasting state during a Western diet is significantly higher when compared to normoinsulinemic lean or obese subjects (see ref. in text).

The application of stable isotope techniques has also allowed researchers to investigate the relationship between DNL and hepatic steatosis in non-alcoholic fatty liver disease (NAFLD). Interestingly, hyperinsulinemic subjects with NAFLD have been found to have elevated hepatic DNL in the fasting state, and analysis of their liver biopsies show that 26% of their liver fat is derived from CHO.[9] These findings support the hypothesis that hepatic DNL may also be an important contributor to liver fat accumulation. Ultimately, these observations may provide a therapeutic target, focused on modulating DNL by therapeutic lifestyle changes or pharmaceutical intervention, to improve metabolic outcomes in these patients.

Characterizing a phenotype and distinguishing normal physiology from pathophysiology require tools able to assess kinetic metabolic fluxes within whole biological system. We believe that stable isotope techniques represent a powerful non-invasive way to explore and monitor metabolism. These approaches are highly sensitive and hold promise for use as routine clinical diagnostic tools because of their ability to foresee metabolic derailment and because of their capacity to unravel the impact of genetic and environmental factors on the phenotype of human disease.

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