SPOTLIGHT on Deuteromics™:

Exploring Multiple Metabolic Pathways with D_2O

Deuteromics (n) (Doo-teer-oh'-miks) *— Simultaneous exploration of multiple metabolic pathways by the deuterium labeling of several key metabolites.*

INTRODUCTION

Over the last 50 years, many elegant experiments have been designed to elucidate metabolic pathways in health and disease. Among these approaches, stable isotope techniques have emerged to play a significant role. By incorporating ^{13}C, ^{15}N, ^{18}O and ^{2}H into a variety of substrates and coupling this labeling with mass spectrometry; substantial advances have been made in our understanding of complex metabolic processes. Despite these successes, a number of problems, both theoretical and practical, have remained.

In this paper, we introduce a variation on these stable isotope techniques wherein a simple oral dose of deuterium oxide (D_2O) provides near-universal labeling of the metabolome with the stable isotope of hydrogen. The predictable distribution of deuterium atoms across metabolic pathways yields maximal information with minimal expense while significantly simplifying the calculations. We term this novel approach 'Deuteromics'.

Advantages of using Deuterium Oxide

Deuterium oxide or "heavy water" has distinct advantages as a non-radioactive isotope tracer for precursor-product labeling experiments. The major advantages of heavy water labeling are:

- No special precautions needed with small oral dosages of deuterium oxide
- Rapid equilibration with total body water
- Tracer can be given orally to achieve an isotopic plateau
- Deuterium oxide is inexpensive and has a long half-life making it ideal to study synthesis rates of fast or slow turnover substrates
- Multiple metabolic pathways can be investigated simultaneously from a single deuterium oxide tracer
- Dosing with deuterium oxide is not limited to specialized research centers.
- Reduces clinical costs since D_2O is cheaper than other labeled tracers and the use of gas chromatography combustion isotope ratio mass spectrometry (GCC-IRMS) allows even lower levels of isotope to be measured.
- The GCC-IRMS detection method increases potential deuterium oxide labeling applications.

Limitations of Using Deuterium Oxide

Deuterium labeling is useful for measuring synthesis rates or flux through a metabolic pathway. However, the following limitations of the deuterium oxide approach should be noted:

- Substrate oxidation to carbon dioxide (CO_2) cannot be measured with deuterium oxide labeling
- Certain micronutrients such as vitamins are not enriched with deuterium
- Site-specific labeling information is difficult to obtain.

What are the differences between deuterium labeling and using ^{13}C-tracers?

Many researchers have utilized continuous infusion precursor protocols with non-radioactive tracers. Tracers such as ^{13}C-acetate must be constantly infused because of their rapid turnover. Assumptions about the precursor enrichment must be made. In addition, assumptions about the product labeling using complex mathematical modeling such as mass isotopomer distribution analysis (MIDA) must be used to calculate fractional synthesis rates. Di Buono et al have summarized the differences between the MIDA technique and using deuterium oxide and GCC-IRMS analysis (Deuteromics™):

	Deuteromics™	**MIDA and ^{13}C-tracers**
Tracer	D_2O, inexpensive	^{13}C-acetate, expensive
Amount of Tracer dose	Low	High
Study Conditions	Free-living subjects	In-patient, infusions needed
Sampling Protocol	Two blood samples	Serial blood samples, requiring indwelling catheter
Mass Spectrometry	GCC-IRMS	GC/MS
Data Analysis	Simple mathematical equations	Complex mathematical modeling
Precursor pool	NADPH, total body water	Acetyl-CoA
Determination of precursor enrichment	Plasma water (also saliva or urine)	MIDA of product (requires assumptions)

Reproduced from Di Buono, M, Jones PJH, Beaumier, L and Wykes LJ (2000) J. Lipid Res: 41:1516-23.

How does deuterium oxide label pathways?

As deuterium oxide equilibrates in body water, various metabolic pathways incorporate deuterium atoms either by:

- Direct insertion of the deuterium atom – for example in TCA cycle fumarate conversion to malate.
- Via labeling with NADH or NADPH – for example malate + NAD conversion to oxaloacetate + $NADH+D^+$ in TCA cycle
- Via labeling with $FADH_2$ – for example beta oxidation of fatty acids labels $FADH_2$
- One carbon methyl pool – methionine catabolism and recycling labels methyl groups

Deuterium equilibrates across body pools within 30 minutes in rodents and about 1 hour in human subjects.

What Applications can be used with Deuterium Oxide?

Deuterium oxide is a **near-universal label** for biosynthesis of macromolecules as well as many biological compounds. The following table lists some examples of compounds and pathways labeled with deuterium oxide administration:

Compound Labeled with Deuterium Oxide	**Pathway**
Glucose	Gluconeogenesis
Lactate	Glycolysis
Alanine	Plasma and Muscle Protein Synthesis
Glucose in glycogen	Glycogen Synthesis and Catabolism
Palmitate and Glycerol in Triglycerides	De novo Lipogenesis
Cholesterol	Cholesterol Synthesis
Alanine	Collagen Synthesis
3-methyl Histidine	Creatinine Excretion – muscle breakdown
Nucleotides	Cell Proliferation
Glycerol	Glycerolyogenesis

What is a typical Protocol for the use of Deuterium Oxide?

For mice and rat studies, administer 20 µl/gram body weight of deuterium oxide (99.9% D) by intraperitoneal injection. If you collect blood samples longer than 6 hours, administer 2% deuterium oxide in drinking water for the remainder of the study.

For human studies, administer orally a single bolus, 1 gram/kg body weight deuterium oxide (99.9% D). If you collect blood samples longer than 24 hours, administer 0.5% deuterium oxide in drinking water for the remainder of the study.

Blood sampling will depend on the substrate being measured. Generally, a pre-dose sample is collected and then sampling 3-6 hours for fast turnover substrates or 1-3 days for slowly turning over substrates.

How can you take advantage of GCC-IRMS technology?

Although GCC-IRMS instruments are expensive and complex to operate, Metabolic Solutions, Inc. (MSI) makes it easy and inexpensive for researchers to utilize this technology. MSI has helped over 850 researchers throughout the world with measuring stable isotope tracers. Recently, MSI added a Thermo Finnigan Delta V GCC-IRMS instrument with capabilities to detect ^{13}C, ^{15}N, and ^{2}H labeled compounds. The ability to detect deuterium labeled compounds is unique – only a handful of instruments in the world have this capability.

Call Dr. David Wagner to discuss how to apply deuterium oxide tracing to your research projects at 1-866-302-1998. You may email him at david@metsol.com.