PROTEIN METABOLISM

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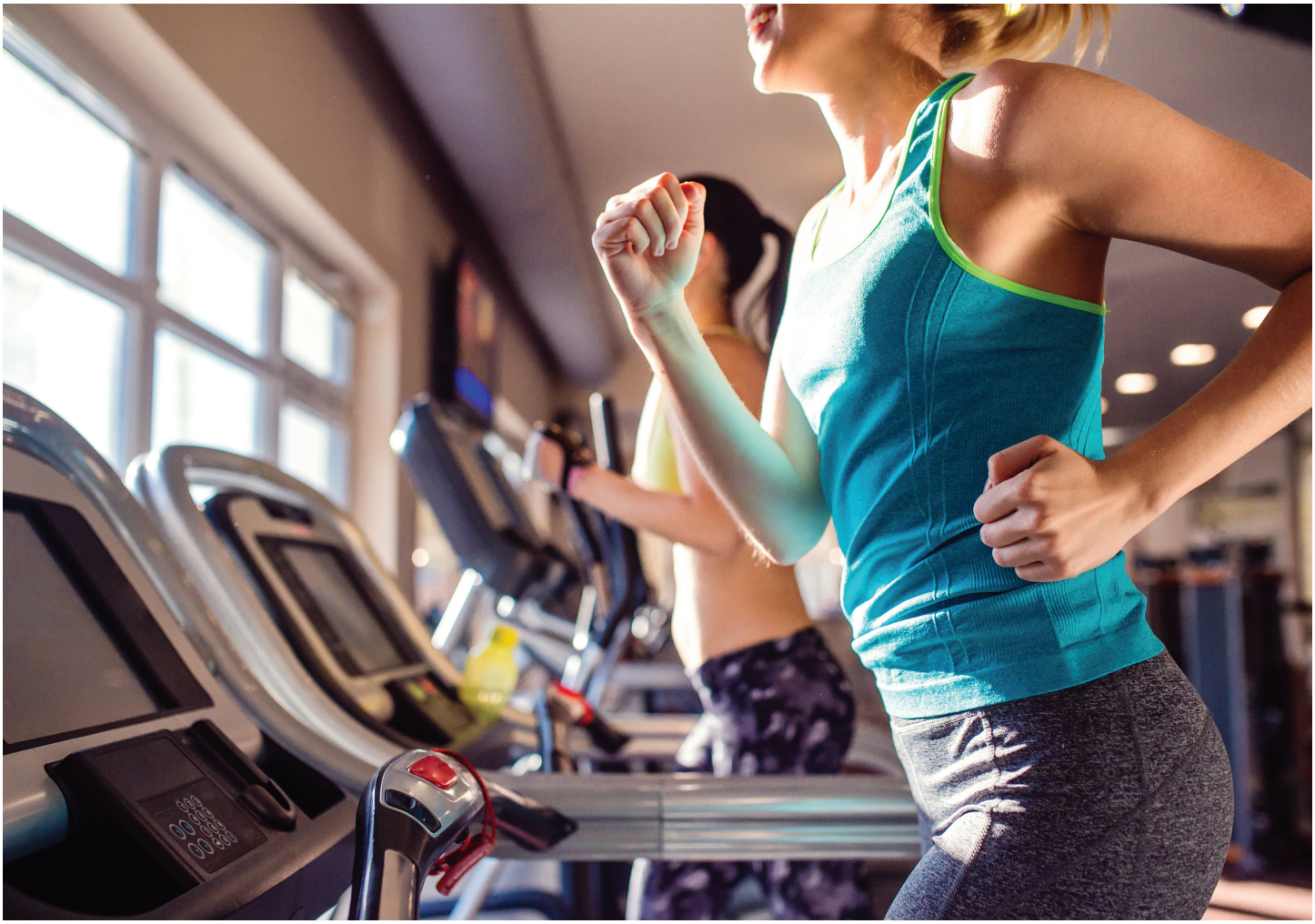
Amino Acid Indicators and Protein Turnover



The Impact of Stable Isotope Tracers on Metabolic Research

Robert R. Wolfe, PhD | Professor, Department of Geriatrics
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Tracer methodology has advanced the field of metabolism by enabling the quantification of metabolic reactions *in vivo*. Stable isotope tracers have been particularly important in this regard, as these tracers have made possible a wide range of studies that would not have been possible with radioactive tracers. Early efforts using stable isotope tracers focused on determining the nature of protein "turnover," or the simultaneous processes of protein synthesis and breakdown. As analytical techniques have developed and a wide variety of isotopic tracers have become available, the scope of tracer studies has widened to the point where it would be impossible to even touch on all possible applications. Some specific examples will be discussed.

Methods using stable isotope tracers fall into two general categories, those in which the use of stable isotopes is a preferable option to the use of the corresponding radioactive tracer for reasons of ease of disposal or analysis, and methods for which there are no radioactive tracers available that would enable quantification of the metabolic pathway under investigation.

The ease of disposal of stable isotopes stems from the fact that, unlike radionuclides, they do not undergo spontaneous decay with resulting emissions that have adverse biological effects (hence the name *stable* isotopes). Stable isotopes are naturally occurring and may be present in significant amounts. For example, slightly more than one percent of all naturally occurring carbon is ^{13}C, and the amount of ^{13}C infused in the context of a tracer study will likely not significantly affect the whole body level of enrichment. Since mice have been raised to have almost entirely ^{13}C in their bodies without apparent adverse effects, we can be quite confident that the experimental use of stable isotopes is safe and that no special procedures are necessary in the disposal of animals given stable isotopes. The potential analytical advantages of stable isotope tracers are two-fold. If mass spectrometry is used to measure enrichment, then the ratio of tracer to tracee is measured directly as opposed to the separate measurement of concentration and decays per minute (dpms) and the calculation of specific activity (the expression of tracer/tracee ratio when radioactive tracers are used). Another advantage of stable isotopes stemming from analysis is that the use of selected ion monitoring with mass spectrometry enables definitive proof that the analyte has been isolated in absolutely pure form for the measurement of stable-isotope enrichment. Also, the measurement of enrichment in specific positions of a molecule is generally much more feasible with mass spectrometry and stable isotopes than the chemical isolation of radioactive atoms in specific positions in a molecule and subsequent determination of dpms of those isolated atoms. The use of D-glucose (6,6-D_2, 99%) to measure the rate of hepatic glucose production is the most common example of a stable isotope providing an alternative to the radioactive analogue.

While factors such as safety and convenience are important, the most exciting advances in metabolism stemming from the use of stable isotope tracers generally involve the quantification of metabolic pathways that realistically could not have been measured otherwise. Nitrogen metabolism is the most obvious example, since there is no radioactive isotope of nitrogen. Since nitrogen is the key element that defines amino acids and protein, a wide variety of applications have been derived to quantify various aspects of nitrogen metabolism in the body using ^{15}N as a tracer. Stable isotopes of carbon and hydrogen have also been used to label amino acids in order to perform novel studies of amino acids. Individual amino acids can be labeled with a variable number of heavy stable isotopes in order to produce molecules of different molecular weight that retain the same metabolic functions (isotopomers). Isotopomers can be useful in a variety of approaches. For example, measurement of muscle-protein synthesis involves infusion or injection of an amino acid tracer and measurement of the rate of incorporation over time into the tissue protein. Collection of the muscle protein requires a muscle

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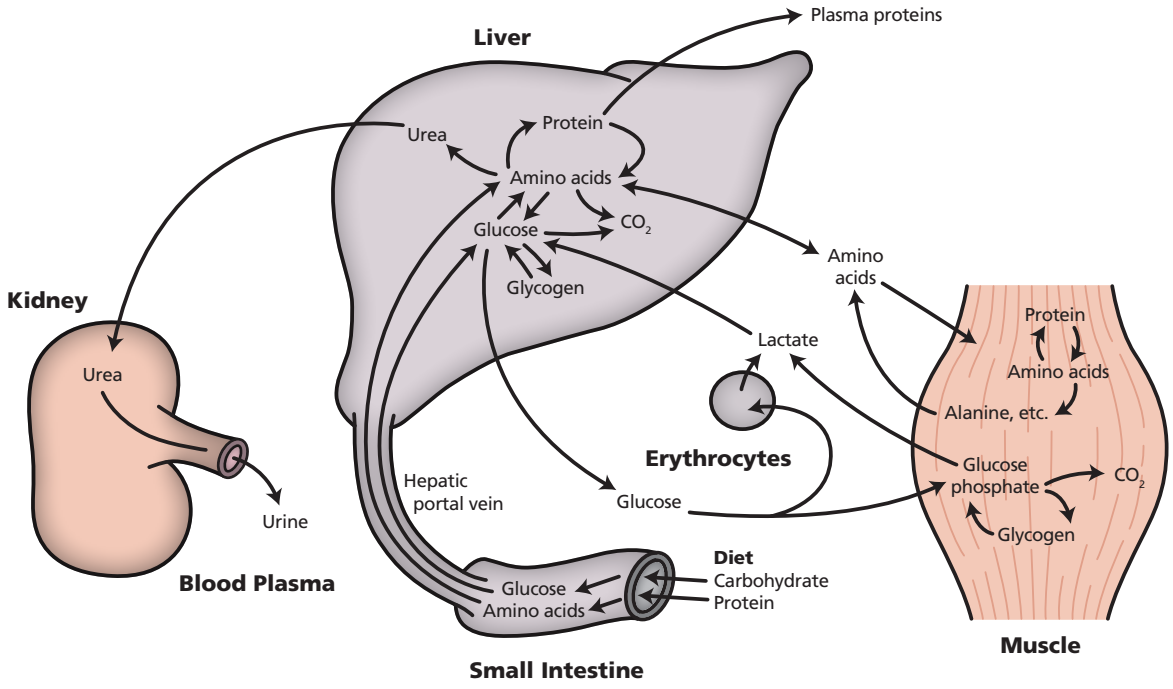
biopsy. By staggering the times of administration of isotopomers of the same amino acid, one single biopsy can suffice to determine the rate of incorporation over time, thereby enabling the calculation of the rate of muscle-protein synthesis.

Stable isotope methodology also enables the concurrent use of the same isotope incorporated into numerous molecules. Since there are 20 different amino acids in the body, it is often important to study the interaction of the kinetics of multiple amino acids. For example, amino acid transmembrane transport differs for specific amino acids, but there is overlap in carrier functions for particular amino acids. It is, therefore, advantageous to quantify transport rates of different amino acids simultaneously. This can be accomplished using stable isotope tracers of the amino acids of interest because the amino acids are separated by gas or liquid chromatography prior to measurement by mass spectrometry. Therefore, different amino acids with the same stable isotope tracer can be distinguished even though the mass increase caused by the tracer is the same in each case.

The unique ability to measure by mass spectrometry the enrichment of a variety of molecules enriched with the same stable isotope tracer has been central to the development of many new approaches in the field of metabolic research. The most popular method involves administering deuterium oxide (D, 99.9%) and measuring the synthetic rates of a wide variety of molecules by determining the rate of incorporation of the deuterium. It is also possible to quantify intracellular reaction rates using both positional and mass isotopomers of the same tracer, most commonly using ^{13}C. Use of positional isotopomers to calculate various intracellular flux rates involves administering a molecule with labeling in a specific position and determining by mass spectrometry the extent of appearance of the stable isotope tracer in other positions of the same molecule, or in specific positions of other molecules. Mass isotopomers have proven useful to determine the enrichment of precursors of the synthesis of polymers such as fatty acids. If multiple labeled precursors (e.g. ^{13}C-acetate) are incorporated into a product that is a polymer of the precursor (e.g. palmitate), then this will be reflected in the mass increase in the product. From the profile of mass increases in the product the precursor for synthesis can be calculated.

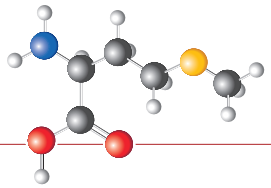
Increased sophistication of mass spectrometry analysis has led to the development of the field of metabolomics. The concentrations of a wide variety of compounds, usually in the blood or urine, are measured to develop a profile distinctive of a particular metabolic state. Stable isotope tracers have played an important role in metabolomics, as their use as internal standards enable quantification of the concentration of any tracee for which a stable isotope tracer is available. Although the metabolomics approach has proven useful in some circumstances, there has been some ambiguity in interpreting metabolomics profiles because they reflect only concentrations. For that reason the field of fluxomics is evolving in which a wide variety of tracers are given to the subject before the blood is sampled so that the metabolomics profile can reflect not only concentrations, but also the flux rates of relevant metabolic pathways.

This brief overview is meant only as a introduction to the varied possibilities possible with the use of stable isotope tracers. A key factor in the development and advancement of these applications has been the increasing availability of a wide variety of stable isotope tracers from Cambridge Isotope Laboratories, Inc. (CIL). The diversity of the CIL products has advanced to the point where the application of new methodologies is limited only by our own insights and creativity.



Graphic: schoolbag.info

Amino Acid Oxidation

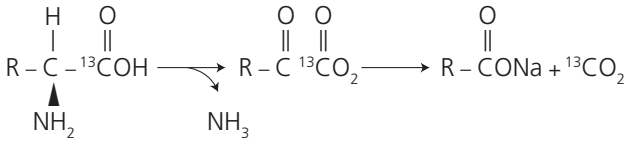


Methionine

The oxidation of amino acids is a very important biochemical process. It can:

- Provide a significant contribution to energy production
- Allow amino acid to participate in gluconeogenesis
- Provide carbon skeleton building blocks for synthesizing other compounds

The amino acid can be labeled, with a ^{13}C, on the first position of the molecule, and the conversion into carbon-13 dioxide can be measured by either isotope ratio mass spectrometry (IRMS) or infrared (IR) spectroscopy to determine the rate of oxidation.



Amino acid **α-Keto acid**

Products of Interest

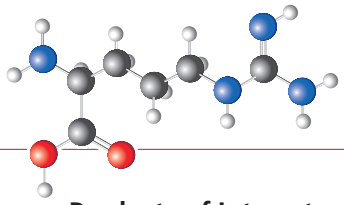
Catalog No.	Description
CLM-468	L-Leucine (1-^{13}C, 99%)
CLM-653	L-Lysine·2HCl (1-^{13}C, 99%)
CLM-3267	L-Methionine (1-^{13}C, 99%)
CLM-762	L-Phenylalanine (1-^{13}C, 99%)
CLM-441	Sodium bicarbonate (^{13}C, 99%)
CLM-778	L-Tryptophan (1-^{13}C, 99%)

Recent Advances in Determining Protein and Amino Acid Requirements in Humans

Elango, R.; Ball, R.O.; Pencharz, P.B.
Child & Family Research Institute, BC Children's Hospital, Vancouver, British Columbia, Canada
2012. *Br J Nutr, 108*. PMID: 23107531

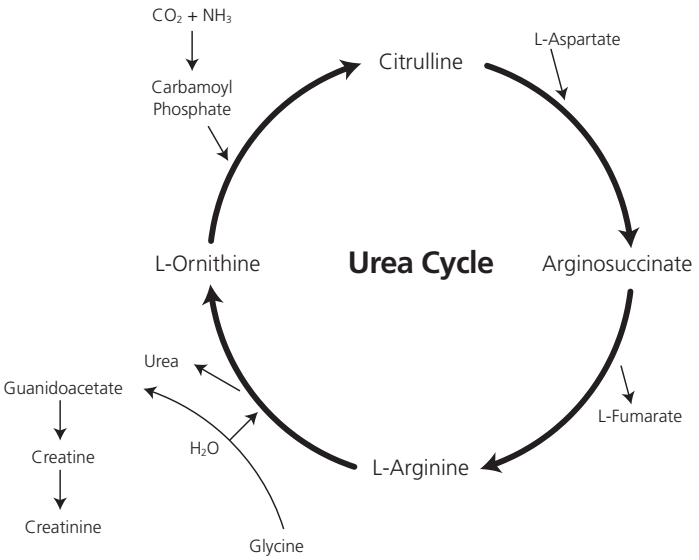
ABSTRACT During the past 25 years a significant amount of research has been conducted to determine amino acid requirements in humans. This is primarily due to advancements in the application of stable isotopes to examine amino acid requirements. The indicator amino acid oxidation (IAAO) method has emerged as a robust and minimally invasive technique to identify requirements. The IAAO method is based on the concept that when one indispensable dietary amino acid (IDAA) is deficient for protein synthesis, then the excess of all other IDAA, including the indicator amino acid, will be oxidized. With increasing intakes of the limiting amino acid, IAAO will decrease, reflecting increasing incorporation into protein. Once the requirement for the limiting amino acid is met there will be no further change in the indicator oxidation. The IAAO method has been systematically applied to determine most IDAA requirements in adults. The estimates are comparable to the values obtained using the more elaborate 24h-indicator amino acid oxidation and balance (24h-IAAO/IAAB) model. Due to its noninvasive nature the IAAO method has also been used to determine requirements for amino acids in neonates, children, and in disease. The IAAO model has recently been applied to determine total protein requirements in humans. The IAAO method is rapid, reliable and has been used to determine amino acid requirements in different species, across the life cycle and in disease. The recent application of IAAO to determine protein requirements in humans is novel and has significant implications for dietary protein intake recommendations globally.

Arginine Cycling – Urea Cycle



Arginine

The main purpose of the urea cycle is the disposal of excess nitrogen, via urea formation. The cycle also produces nitric oxide, which has been shown to be a vasodilator. Arginine is considered a semi-essential amino acid, and depending on health status, arginine supplementation may be required. Arginine and citrulline are involved in inborn errors of metabolism as well. CIL has the widest range of label patterns of citrulline, ornithine, and arginine for tracing the urea cycle.



Products of Interest

Catalog No.	Description
DLM-7476	ADMA·HCl·½H_2O (asymmetric dimethylarginine) (2,3,3,4,4,5,5-D_7, 98%) may be hydrate
CLM-2265-H	L-Arginine·HCl ($^{13}C_6$, 99%)
NLM-395	L-Arginine·HCl (guanido-$^{15}N_2$, 98%+)
NLM-396	L-Arginine·HCl ($^{15}N_4$, 98%)
CNLM-539-H	L-Arginine·HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
CNLM-9007-CA	L-Argininosuccinic acid barium salt·2H_2O (arginine-$^{13}C_6$, 99%; $^{15}N_4$, 99%) CP 90%+
CLM-4899	L-Citrulline (ureido-^{13}C, 99%)
DLM-6039	L-Citrulline (4,4,5,5-D_4, 95%)
CDLM-8808	L-Citrulline (ureido-^{13}C, 99%; 3,3,4-D_3, 98%)
CLM-7933	Creatine (guanidino-^{13}C, 99%)
DLM-1302	Creatine (methyl-D_3, 98%)
DLM-3653	Creatinine (*N*-methyl-D_3, 98%)
NLM-823	Nitric oxide (^{15}N, 98%+)
CLM-4724	L-Ornithine·HCl ($^{13}C_5$, 98%)
DLM-6046	L-Ornithine·HCl (4,4,5,5-D_4, 95%)
DLM-2969	L-Ornithine·HCl (3,3,4,4,5,5-D_6, 95%)
NLM-3610	L-Ornithine·HCl ($^{15}N_2$, 98%)
CDLM-3873	L-Ornithine·HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
CNLM-7578	L-Ornithine·HCl ($^{13}C_5$, 98%; $^{15}N_2$, 98%)
CLM-311	Urea (^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%+)
CNLM-234	Urea (^{13}C, 99%; $^{15}N_2$, 98%+)

Arginine Appearance and Nitric Oxide Synthesis in Critically Ill Infants Can Be Increased with a Protein-Energy-Enriched Enteral Formula

de Betue, C.; Joosten, K.; Deutz, N.; Vreugdenhil, A.; Waardenburg, D.
Department of Pediatrics, Maastricht University Medical Center, Maastricht, Netherlands
2013. *Am J Clin Nutr, 98(4)*, 907-916. PMID: 23945723

ABSTRACT Background: Arginine is considered an essential amino acid during critical illness in children, and supplementation of arginine has been proposed to improve arginine availability to facilitate nitric oxide (NO) synthesis. Protein-energy-enriched enteral formulas (PE-formulas) can improve nutrient intake and promote anabolism in critically ill infants. However, the effect of increased protein and energy intake on arginine metabolism is not known.

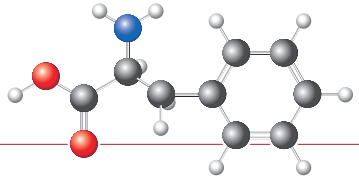
Objective: We investigated the effect of a PE-formula compared with that of a standard infant formula (S-formula) on arginine kinetics in critically ill infants.

Design: A two-hour stable-isotope tracer protocol was conducted in two groups of critically ill infants with respiratory failure because of viral bronchiolitis, who received either a PE-formula (n = 8) or S-formula (n = 10) in a randomized, blinded, controlled setting. Data were reported as means ± SDs.

Results: The intake of a PE-formula in critically ill infants (aged 0.23 ± 0.14 y) resulted in an increased arginine appearance (PE-formula: 248 ± 114 μmol · kg(-1) · h(-1); S-formula: 130 ± 53 μmol · kg(-1) · h(-1); P = 0.012) and NO synthesis (PE-formula: 1.92 ± 0.99 μmol · kg(-1) · h(-1); S-formula: 0.84 ± 0.36 μmol · kg(-1) · h(-1); P = 0.003), whereas citrulline production and plasma arginine concentrations were unaffected.

Conclusion: In critically ill infants with respiratory failure because of viral bronchiolitis, the intake of a PE-formula increases arginine availability by increasing arginine appearance, which leads to increased NO synthesis, independent of plasma arginine concentrations. This trial was registered at www.trialregister.nl as NTR515.

Protein Turnover

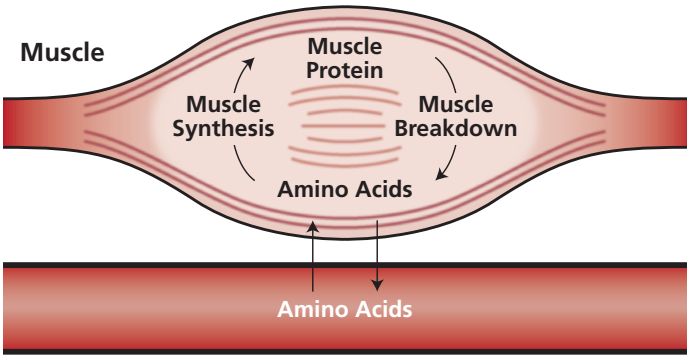


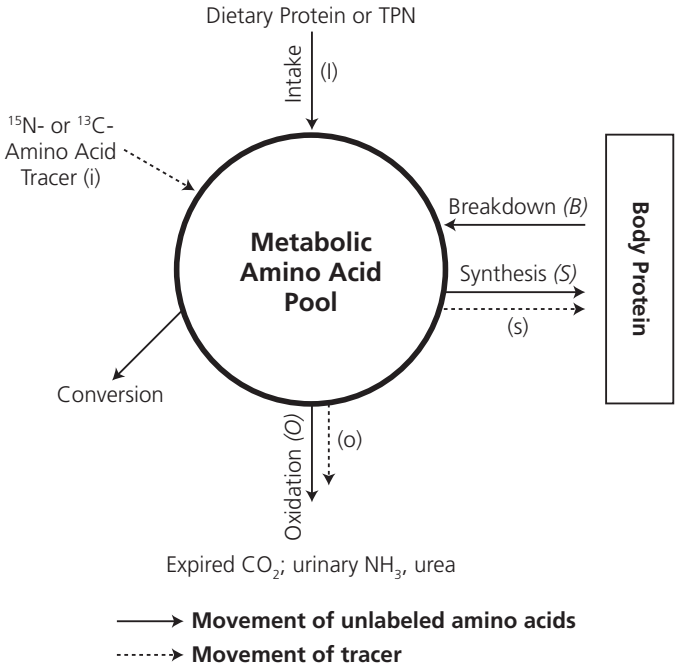
Phenylalanine

Protein turnover occurs between the fractional synthesis rate (FSR) and the fractional breakdown rate (FBR).
Other considerations:

- Essential amino acids are required (e.g., leucine and phenylalanine)
- Protein turnover can provide valuable information for a treatment response
- It is usually measured using a GC-MS, IRMS, or LC-MS

There are many different label patterns of amino acids available that incorporate deuterium, carbon-13, nitrogen-15, and/or oxygen-18 to probe protein turnover.





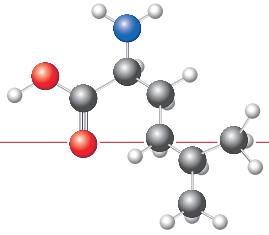
In vivo Measurement of Synthesis Rate of Multiple Plasma Proteins in Humans

Jaleel, A.; Nehra, V.; Persson, X.M.; Boirie, Y.; Bigelow, M.; Nair, K.S.
Division of Endocrinology, Diabetes, Metabolism, and Nutrition; Mayo Clinic, Rochester, Minnesota USA
2007. *Am J Physiol Endocrinol Metab, 292(1),* E190-197. PMID: 16449301

ABSTRACT Advances in quantitative proteomics have facilitated the measurement of large-scale protein quantification, which represents net changes in protein synthesis and breakdown. However, measuring the rate of protein synthesis is the only way to determine the translational rate of gene transcripts. Here, we report a technique to measure the rate of incorporation of amino acids from ingested protein labeled with stable isotope into individual plasma proteins. This approach involves three steps: 1) production of stable isotope-labeled milk whey protein, oral administration of this intrinsically labeled protein, and subsequent collection of blood samples; 2) fractionation of the plasma and separation of the individual plasma proteins by a combination of anion exchange high-pressure liquid chromatography and gel electrophoresis; and 3) identification of individual plasma proteins by tandem mass spectrometry and measurement of stable isotopic enrichment of these proteins by gas chromatography-mass spectrometry. This method allowed the measurement of the fractional synthesis rate (FSR) of 29 different plasma proteins by using the same precursor pool. We noted a 30-fold difference in FSR of different plasma proteins with a wide range of physiological functions. This approach offers a tremendous opportunity to study the regulation of plasma proteins in humans in many physiological and pathological states.

Graphics: imgrum.net; Stable Isotopes in Human Nutrition: Laboratory Methods and Research Applications

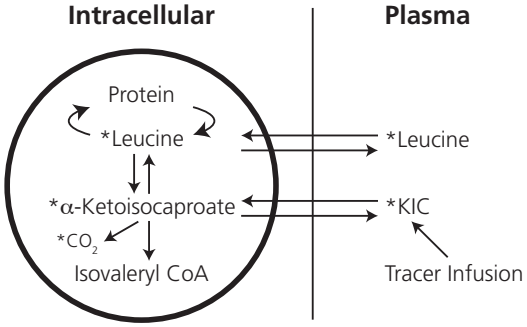
Leucine



Since leucine is an essential amino acid, it makes it a useful probe of metabolism. Leucine is important because it is:

- A ketogenic amino acid
- A signaling compound effective for the mTOR pathway
- Involved in inherited genetic disorders like maple syrup urine disease
- A possible link between diseases such as obesity and diabetes

There are many different label patterns of leucine available that incorporate deuterium, carbon-13 and/or nitrogen-15 to probe the pathway of choice.



Products of Interest

Catalog No.	Description
CLM-2093	α-Ketoisocaproic acid, sodium salt (1-^{13}C, 99%)
DLM-1944	α-Ketoisocaproic acid, sodium salt (methyl-D_3, 98%)
DLM-4214	α-Ketoisocaproic acid, sodium salt (isopropyl-D_7, 98%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
CLM-468	L-Leucine (1-^{13}C, 99%)
CLM-3524	L-Leucine (1,2-$^{13}C_2$, 99%)
DLM-567	L-Leucine (D_{10}, 98%)
DLM-4212	L-Leucine (isopropyl-D_7, 98%)
DLM-1259	L-Leucine (5,5,5-D_3, 99%)
CNLM-615	L-Leucine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-3450	L-Leucine (2-^{13}C, 99%; ^{15}N, 95-99%)
CLM-441	Sodium bicarbonate (^{13}C, 99%)

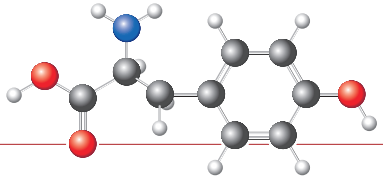
Compartmental Model of Leucine Kinetics in Humans

Cobelli, C.; Saccomani, M.P.; Tessari, P.; Biolo, G.; Luzi, L.; Matthews, D.E.
Department of Electronics and Informatics, University of Padua, Italy
1991. *Am J Physiol, 261*, E539-550. PMID: 1928344

ABSTRACT The complexity of amino acid and protein metabolism has limited the development of comprehensive, accurate whole body kinetic models. For leucine, simplified approaches are in use to measure *in vivo* leucine fluxes, but their domain of validity is uncertain. We propose here a comprehensive compartmental model of the kinetics of leucine and α-ketoisocaproate (KIC) in humans. Data from a multiple-tracer administration were generated with a two-stage (I and II) experiment. Six normal subjects were studied. In experiment I, labeled leucine and KIC were simultaneously injected into plasma. Four plasma leucine and KIC tracer concentration curves and label in the expired CO_2 were measured. In experiment II, labeled bicarbonate was injected into plasma, and labeled CO_2 in the expired air was measured. Radioactive (L-[1-^{14}C]leucine, [4,5-^{3}H]KIC, [^{14}C]bicarbonate) and stable isotope (L-[1-^{13}C]leucine, [5,5,5-$^{2}H_3$]KIC, [^{13}C]bicarbonate) tracers were employed. The input format was a bolus (impulse) dose in the radioactive case and a constant infusion in the stable isotope case. A number of physiologically based, linear time-invariant compartmental models were proposed and tested against the data. The model finally chosen for leucine-KIC kinetics has 10 compartments: four for leucine, three for KIC, and three for bicarbonate. The model is a priori uniquely identifiable, and its parameters were estimated with precision from the five curves of experiment I. The separate assessment of bicarbonate kinetics (experiment II) was shown to be unnecessary. The model defines masses and fluxes of leucine in the organism, in particular its intracellular appearance from protein breakdown, its oxidation, and its incorporation into proteins. An important feature of the model is its ability to estimate leucine oxidation by resolving the bicarbonate model in each individual subject. Finally, the model allows the assessment of the domain of validity of the simpler commonly used models.

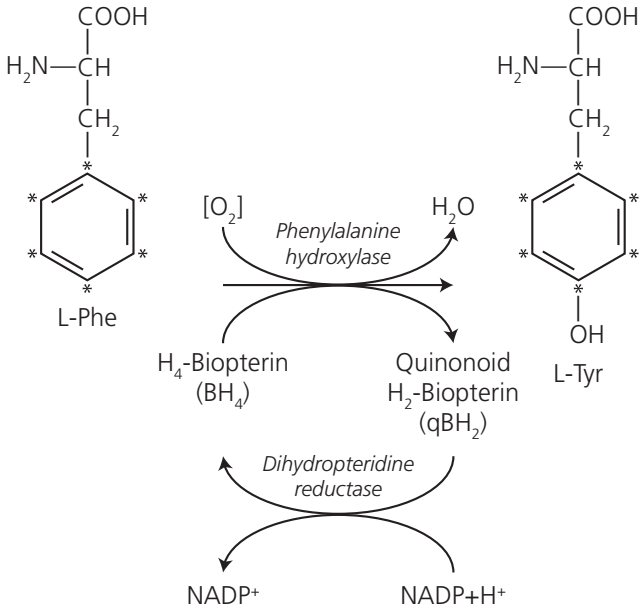
Graphic: Isotope Tracers in Metabolic Research: Principles and Practice Kinetic Analysis

Phenylalanine/Tyrosine



Tyrosine

Another tracer that is commonly used to measure FSR and FBR is the essential amino acid phenylalanine. When using it as a tracer, the experiment needs to account for the conversion of phenylalanine into tyrosine, which is accomplished by using a different mass tyrosine tracer. A consideration for the experimental design is that phenylalanine appears less often in proteins compared to leucine, and phenylalanine has a higher mass than leucine. Unlike leucine, phenylalanine calculations do not have to account for oxidation. There are many different label patterns of phenylalanine and tyrosine.



Products of Interest

Catalog No.	Description
CLM-1055	L-Phenylalanine (ring-$^{13}C_6$, 99%)
CLM-2250-H	L-Phenylalanine ($^{13}C_9$, 99%)
DLM-1258	L-Phenylalanine (ring-D_5, 98%)
NLM-108	L-Phenylalanine (^{15}N, 98%)
CNLM-575-H	L-Phenylalanine ($^{13}C_9$, 99%; ^{15}N, 99%)
CLM-1542	L-Tyrosine (ring-$^{13}C_6$, 99%)
CLM-2263-H	L-Tyrosine ($^{13}C_9$, 99%)
CLM-623	L-Tyrosine (phenol-3,5-$^{13}C_2$, 95-99%)
DLM-451	L-Tyrosine (ring-D_4, 98%)
NLM-590	L-Tyrosine (^{15}N, 99%)
CNLM-439	L-Tyrosine ($^{13}C_9$, 99%; ^{15}N, 99%)

Higher Muscle Protein Synthesis in Women than Men Across the Lifespan, and Failure of Androgen Administration to Amend Age-Related Decrements

Henderson, G.C.; Dhatariya, K.; Ford, G.C.; Klaus, K.; Basu, R.; Rizza, R.A.; Jensen, M.D.; Khosla, S.; O'Brien, P.; Nair, S.[1]
1. Division of Endocrinology, Mayo Clinic, Rochester, Minnesota USA
2009. *FASEB J, 23(2)*, 631-641. PMID: 18827019

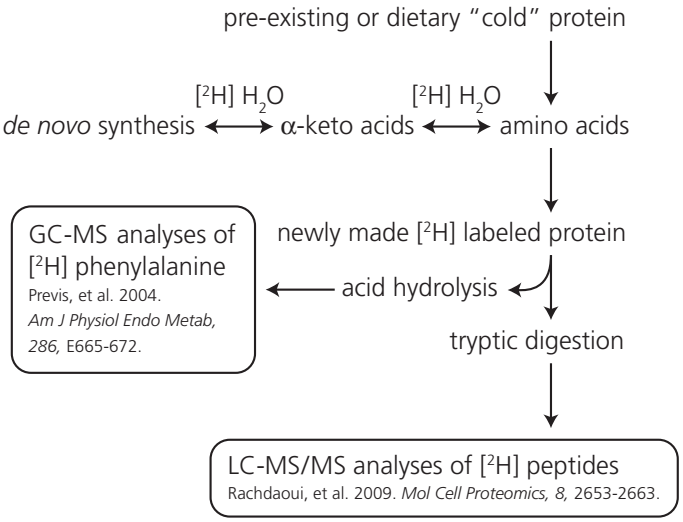
ABSTRACT We investigated age and sex effects and determined whether androgen replacement in elderly individuals (≥60 yr) could augment protein synthesis. Thirty young men and 32 young women (18-31 yr) were studied once, whereas 87 elderly men were studied before and after 1 yr of treatment with 5 mg/day testosterone (T), 75 mg/day dehydroepiandrosterone (DHEA), or placebo (P); and 57 elderly women were studied before and after 1 yr of treatment with 50 mg/day DHEA or P. [^{15}N]phenylalanine and [$^2H_4$]tyrosine tracers were infused, with measurements in plasma and vastus lateralis muscle. Whole-body protein synthesis per fat-free mass and muscle protein fractional synthesis rate (FSR) were lower in elderly than in young individuals (P<0.001), not significantly affected by hormone treatments, and higher in women than in men (P<0.0001), with no sex × age interaction. In regression analyses, peak O_2 consumption (VO_2peak), resting energy expenditure (REE), and sex were independently associated with muscle FSR, as were VO_2peak, REE, and interactions of sex with insulin-like growth factor-II and insulin for whole-body protein synthesis. Women maintain higher protein synthesis than men across the lifespan as rates decline in both sexes, and neither full replacement of DHEA (in elderly men and women) nor partial replacement of bioavailable T (in elderly men) is able to amend the age-related declines.

Graphic: WordPress.com

Deuterium Oxide

Deuterium oxide

Deuterium oxide can be used to measure FSR and FBR. Enriching the total water pool with deuterium incorporates deuterium into amino acids, glucose, cholesterol, DNA, and lipids. The rates of flux through these pathways can be determined in one experiment. Using the deuterium oxide will require a more complex modeling and mass isotopomer distribution analysis (MIDA) for analyzing the data. Because the technique is based on labeling the total water pool, it will take a period of time for the enrichment to return to natural abundance or "washout," which needs to be accounted for in the experimental design.



Products of Interest

Catalog No.	Description
DLM-4-99	Deuterium oxide (D, 99%)
DLM-4-70	Deuterium oxide (D, 70%)
DLM-2259	Deuterium oxide (D, 99.8%) microbiological tested
DLM-2259-70	Deuterium oxide (D, 70%) microbiological tested
DLM-4	Deuterium oxide (D, 99.9%)
DLM-4-99.8	Deuterium oxide (D, 99.8%)

Quantifying Rates of Protein Synthesis in Humans by Use of ^2H_2O: Application to Patients with End-Stage Renal Disease

Previs, S.F.;[1] Fatica, R.; Chandramouli, V.; Alexander, J.C.; Brunengraber, H.; Landau, B.R.
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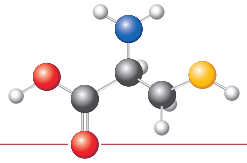
2004. *Am J Physiol Endocrinol Metab, 286(4)*, E665-672. PMID: 14693509

ABSTRACT A method is introduced for quantitating protein synthetic rates in humans by use of ^2H_2O. Its validity was tested in subjects with end-stage renal disease. Six clinically stable subjects, hemodialyzed three times weekly, ingested ^2H_2O to a body water ^2H enrichment of approximately 0.4%. On dialysis, body water enrichment declined to approximately 0.1%. Enrichment of the alpha-hydrogen of plasma-free alanine was also approximately 0.4% before and approximately 0.1% after dialysis. Beta-hydrogen enrichment was approximately 80-100% of alpha-hydrogen enrichment. ^2H_2O was ingested to replace ^2H_2O removed after each dialysis for 15-51 days, returning enrichment to approximately 0.4%. Enrichment of alanine from plasma albumin gradually increased, with again approximately 80-100% as much ^2H in beta – as in alpha-hydrogens. With continued dialyses, without ^2H_2O replacement, alanine from albumin enrichment gradually declined, whereas free alanine and water enrichments were negligible. The fractional albumin synthesis rate, calculated from the increase in enrichment in alanine from albumin, was 4.0 +/- 0.5%/day, and from the decrease, 4.6 +/- 0.2%/day. Thus, body water enrichment in a subject given ^2H_2O can be maintained constant long term. A rapid exchange, essentially complete, occurs between the hydrogens of alanine and body water. An integrated measure over a long period of albumin's synthetic rate can be estimated from both the rise in enrichment of alanine from the protein during ^2H_2O ingestion and fall on ^2H_2O withdrawal, while the subject's living routine is uninterrupted. Estimates are in subjects with renal disease, but the method should be applicable to estimates of protein synthetic rates in normal subjects and in other pathological states.

Graphic courtesy of Stephen Previs

Methionine/Cysteine Metabolism



Cysteine

Methionine is considered an essential amino acid, while cysteine is considered semi-essential because it is synthesized from methionine. Methionine plays a primary role as a methyl donor. Once the methionine is converted to the S-adenosyl-methionine (SAM), the methyl group on the SAM can be donated to an oxygen, nitrogen, or aromatic system. Cysteine also plays an important role in oxidation. Because of its thiol functional group, it is very easily oxidized and becomes a major source of antioxidants.

Products of Interest

Catalog No.	Description
CLM-8906	S-Adenosyl-L-homocysteine (adenosine-$^{13}C_{10}$, 98%)
CLM-4320	L-Cysteine ($^{13}C_3$, 99%)
CLM-3852	L-Cysteine (1-^{13}C, 99%)
DLM-769	L-Cysteine (3-3-D_2, 98%)
DLM-6901	L-Cysteine (2,3,3-D_3, 98%)
NLM-2295	L-Cysteine (^{15}N, 98%)
CNLM-3871-H	L-Cysteine ($^{13}C_3$, 99%; ^{15}N, 99%)
CLM-893-H	L-Methionine ($^{13}C_5$, 99%)
CLM-3267	L-Methionine (1-^{13}C, 99%)
CLM-206	L-Methionine (methyl-^{13}C, 99%)
DLM-431	L-Methionine (methyl-D_3, 98%)
NLM-752	L-Methionine (^{15}N, 96-98%)
CDLM-760	L-Methionine (1-^{13}C, 99%; methyl-D_3, 87%)
CNLM-795-H	L-Methionine ($^{13}C_5$, 99%; ^{15}N, 99%)
DLM-8259	DL-Homocysteine (3,3,4,4-D_4, 98%)

Effects of Randomized Supplementation of Methionine or Alanine on Cysteine and Glutathione Production During the Early Phase of Treatment of Children with Edematous Malnutrition

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1. Tropical Metabolism Research Unit, Tropical Medicine Research Institute, University of the West Indies, Kingston, Jamaica
2. USDA/Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX USA

2014. *Am J Clin Nutr, 99(5)*, 1052-1058. PMID: 24598154

ABSTRACT **Background:** We have shown that a low glutathione concentration and synthesis rate in erythrocytes are associated with a shortage of protein-derived cysteine in children with edematous severe acute malnutrition (SAM).

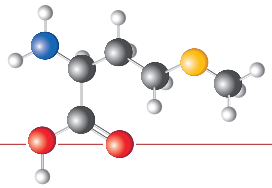
Objective: We tested the hypothesis that methionine supplementation may increase protein-derived cysteine and upregulate cysteine synthesis, thereby improving glutathione synthesis during the early treatment of edematous SAM.

Design: The cysteine flux, its *de novo* synthesis and release from protein breakdown, and erythrocyte glutathione synthesis rate were measured in 12 children with edematous SAM in the fed state by using stable isotope tracers at three clinical phases as follows: 3 ± 1 d (±SE) [clinical phase 1 (CP1)], 8 ± 1 d [clinical phase 2 (CP2)], and 14 ± 2 d (clinical phase 3) after admission. Subjects were randomly assigned to receive equimolar supplements (0.5 mmol · kg(-1) · d(-1)) of methionine or alanine (control) immediately after CP1.

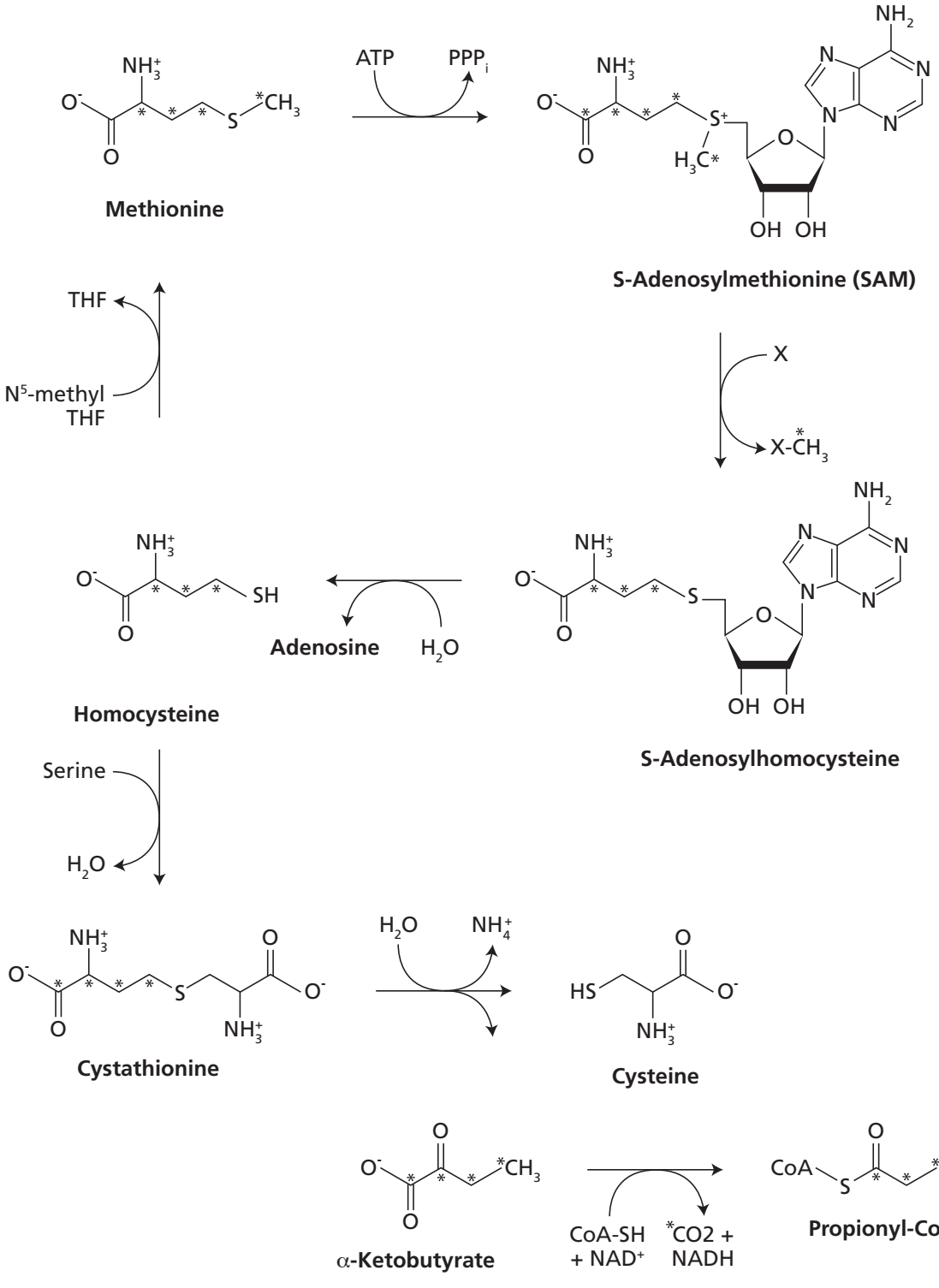
Results: In the methionine compared with the alanine group, cysteine flux derived from protein breakdown was faster at CP2 than CP1 ($P < 0.05$), and the change in plasma cysteine concentration from CP1 to CP2 was greater ($P < 0.05$). However, there was no evidence of a difference in cysteine *de novo* synthesis and its total flux or erythrocyte glutathione synthesis rate and concentration between groups.

Conclusions: Methionine supplementation increased cysteine flux from body protein but had no significant effect on glutathione synthesis rates. Although cysteine is made from methionine, increased dietary cysteine may be necessary to partially fulfill its demand in edematous SAM because glutathione synthesis rates and concentrations were less than previous values shown at full recovery. This study was registered at clinicaltrials.gov as NCT00473031.

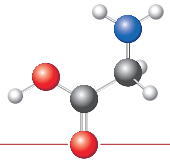
The Fates of Methionine



Methionine

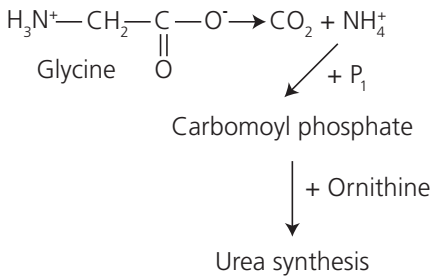


Nitrogen Balance



Glycine

The nitrogen balance method normally involves using an amino acid that is labeled with nitrogen-15. The amino acid is metabolized and the ^{15}N label is converted into urea-^{15}N. Based off the urea-^{15}N enrichment, the amino acid oxidation is calculated. A challenge for this method is that the tracer takes a long time to reach a steady-state enrichment level, and there may be recycling of the ^{15}N isotope.



Products of Interest

Catalog No.	Description
NLM-718	L-Aspartic acid (^{15}N, 98%)
NLM-135	L-Glutamic acid (^{15}N, 98%)
NLM-202	Glycine (^{15}N, 98%)
NLM-142	L-Leucine (^{15}N, 98%)
NLM-108	L-Phenylalanine (^{15}N, 98%)

The Measurement of Total Protein Synthesis and Catabolism and Nitrogen Turnover in Infants in Different Nutritional States and Receiving Different Amounts of Dietary Protein

Picou, D.; Taylor-Roberts, T.

Medical Research Council, Metabolic Research Unit, The University of the West Indies, Mona, Jamaica

1969. *Clin Sci, 36(2)*, 283-296. PMID: 5772104

ABSTRACT Nine Jamaican infants aged 10 to 20 months and with or recovering from malnutrition were given at a constant rate glycine-^{15}N containing 34 atoms percent excess ^{15}N by vein in a 5% glucose solution for 27 to 30 h or intragastrically in a milk mixture for 28 to 31 h. Some of the infants also had by gastric infusion ^{15}N-enriched egg protein. Each infant was given a total of 1 mg ^{15}N per kg bodyweight. Infants receiving an intravenous infusion were given milk mixtures 4-hourly.

Faecal excretion of ^{15}N was independent of route of administration, protein intake and nature of labelled compound and amounted to 1.49 to 4.88% of total ^{15}N infused. The percentage enrichment of ^{15}N in urinary urea was constant after 18 h in infants taking enough protein and after 24 h in infants on a low-protein diet. The percentage ^{15}N excreted as urea, the calculated turnover rate of amino N and calculated rate of synthesis of tissue protein in an infant with daily protein intake of about 5.3 g and given glycine-^{15}N by vein did not differ by more than 15% from corresponding values when he was given ^{15}N intragastrically, but the calculated catabolic rate of tissue protein was much less with intragastric infusion. The percentage ^{15}N in urea in an infant on a daily protein intake of about 2.5 g was higher during intravenous infusion than during intragastric infusion of glycine-^{15}N, but turnover rate of amino N and calculated rates of catabolism and synthesis of tissue protein were similar during both types of infusion.

When well-nourished infants were given ^{15}N intragastrically as glycine or egg protein, turnover rate of amino N and calculated rate of synthesis and catabolism of tissue proteins were similar. Five infants were given glycine-^{15}N on admission to hospital and after they were completely recovered. Protein intake per kg bodyweight during both periods was similar but recovered infants were given a higher energy intake. Rates of amino N turnover, synthesis and catabolism of tissue protein were higher in the malnourished state than after recovery but net synthesis of protein was similar. A larger proportion of N entering the metabolic pool was synthesised into protein in malnourished than in well-nourished children.

Infants recovered from malnutrition were given a diet providing daily 1.2 g protein and 120 kcal per kg bodyweight or an isocaloric diet providing 5.2 g protein per kg. From intragastric infusion studies with glycine (0.8 mg ^{15}N per kg bodyweight) the tissue protein catabolic rate was lower with the high-protein diet, but protein synthetic rate and amino N turnover were similar. The percentage of amino N entering the pool and synthesised to protein was much higher when infants were given the low-protein diet.

Graphic: Isotope Tracers in Metabolic Research: Principles and Practice Kinetic Analysis

Research Use of CIL Products

CIL manufactures highly pure research biochemicals that are produced for research applications. As a service to our customers, some of these materials have been tested for the presence of *S.aureus, P. aeruginosa, E. coli, Salmonella sp,* aerobic bacteria, yeast and mold as well as, the presence of endotoxin in the bulk material by taking a random sample of the bulk product. Subsequent aliquots are not retested. Presence of endotoxin is assessed by determining endotoxin content following established protocols and standardized limulus amebocyte lysate (LAL) reagents. These tests are provided at no charge for any materials listed in our catalog or website that is designated as "MPT" (microbiologically and pyrogen tested) in the item product number (i.e, DLM-349-MPT).

CIL is able to provide microbiological testing for other products. Depending on the compound and the quantity ordered, an additional charge may apply. Please note that microbiological-tested products are not guaranteed to be sterile and pyrogen free when received by the customer, and microbiological testing does not imply suitability for any desired use. If the product must be sterile and pyrogen-free for a desired application, CIL recommends that the product be packaged or formulated into its ultimate dose form by the customer or appropriate local facility. The product should always be tested by a qualified pharmacy/facility prior to actual use.

CIL research products are labeled "For Research Use Only. Not for use in diagnostic procedures." Persons intending to use CIL products in applications involving humans are responsible for complying with all applicable laws and regulations including but not limited to the US FDA, other local regulatory authorities and institutional review boards concerning their specific application or desired use.

It may be necessary to obtain approval for using these research products in humans from the US FDA or the comparable governmental agency in the country of use. CIL will provide supporting information, such as lot-specific analytical data and test method protocols, to assist medical research groups in obtaining approval for the desired use. An Enhanced Technical Data Package (EDP) is also available (see page 15 for more information).

CIL will allocate a specific lot of a product to customers who are starting long-term projects requiring large amounts of material. Benefits from this type of arrangement include experimental consistency arising from use of only one lot, no delay in shipments, and guaranteed stock. Please note that some CIL products have a specific shelf life and cannot be held indefinitely. If interested, please contact your sales manager for further details.

Because of increasing regulatory requirements, CIL manufactures different grades of materials to help researchers with those requirements. Listed below are the grades of materials that CIL currently manufactures:

Catalog No.	Description
CLM-XXX-**0**	Research grade
CLM-XXX-**MPT**	Microbiologically and Pyrogen Tested
CLM-XXX-**CTM**	Manufactured following ICH Q7, Section XIX
CLM-XXX-**GMP**	Good Manufacturing Practices grade

For more information on controls in manufacturing and testing of the different grades, go to: Search → Literature → Product Quality Designations from the isotope.com home page.

CIL's cGMP Production Capabilities

With increasing requirements from institutional review boards (IRBs) and governmental agencies, partnering with CIL for your next stable isotope cGMP (current good manufacturing practices) project can help ensure your regulatory compliance. With the world's largest ^{13}C and ^{18}O isotope-separation plants, CIL is able to provide the raw materials necessary for your project. Your compound of interest most likely already appears in CIL's extensive list of research compounds – if not, CIL's team of PhD chemists can determine the best method of synthesis for incorporating ^{13}C, ^{15}N, D, ^{17}O, and/or ^{18}O into your compound.

CIL has manufactured bulk active pharmaceutical ingredients (APIs) since 1994. It recently added a 15,000-square-foot, state-of-the-art cGMP facility to complement its existing cGMP facilities. An additional team of experts – specializing in synthetic chemistry, customer support, quality control, and quality assurance – serves to provide technical guidance from beginning to end of your project.

Partner with CIL to help you meet your increasing regulatory compliance requirements.

Products of Interest

Catalog No.	Description
CLM-804-CTM	Cholesterol (3,4-$^{13}C_2$)
DLM-4-700-CTM	Deuterium oxide (D, 70%)
CLM-1396-CST	D-Glucose ($^{13}C_6$)
CLM-420-CST	D-Glucose (1-^{13}C)
CLM-349-CTM	D-Glucose (6,6-D_2)
DLM-1229-CST	Glycerol (1,1,2,3,3-D_5)
CLM-2262-CTM	L-Leucine ($^{13}C_6$)
DLM-1259-CTM	L-Leucine (5,5,5-D_3)
CLM-761-CTM	L-Phenylalanine (1-^{13}C)
CLM-156-CTM	Sodium acetate (1-^{13}C)
CLM-440-CTM	Sodium acetate (1,2-$^{13}C_2$)
CLM-3276-GMP	Uracil (2-^{13}C)
CLM-311-GMP	Urea (^{13}C)

Other products may be available as CTM/cGMP. Please inquire for details.

Manufacturing Capabilities

- Dedicated development facility
- Five production and two isolation suites
- Dedicated packaging room
- Production scale from milligrams to multikilograms
- Clinical trials to bulk API
- Customizable projects to meet your needs

Analytical Services

- Fully equipped analytical facility
- Method development and validation
- Raw material and final product testing
- Wet chemistry and compendial methods
- Stability studies and chambers
- Dedicated cGMP instruments and facility
- Analytical instrumentation:
  - High-field NMR (^1H, D, ^{13}C, ^{15}N, multinuclear)
  - HPLC with UV, RI, ELSD, DA, Pickering, and MS detection
  - GC with FID, ECD, and MS detection
  - KF
  - FT-IR
  - Polarimetery
  - TOC

Quality and Compliance

- Drug master files
- FDA-audited facility
- QA release of API product
- Follows FDA and ICH guidances
- CMC sections for NDA or IND

Enhanced Technical Data Package (EDP)

CIL offers the option of an Enhanced Technical Data Package (EDP). This data package is available for most MPT products. It includes all of the data currently included with the MPT products, as well as the additional information listed below. You have the option of purchasing this package at the time of order or at a later date.

Please note that if you choose to purchase at a later date, some of the information listed below may not be available. Also, the EDP may not be available for all lots. In some cases, only a partial EDP may be available. Please confirm availability and content prior to order.

Enhanced Technical Data Package Contents

- Additional testing data; products with an EDP have been tested to the specifications/monograph similar to those detailed in the USP or EP, but not using compendia methods.
- Product description: structural formula, stereochemical description, molecular formula.
- Product physical properties: melting point, pH, optical rotation (mix of literature or measured values).
- Outline of the synthesis route, including details of solvents used.
- Data used to confirm structure and chemical purity.
- Impurities: available data on impurities detected and identified together with the method of detection and the cutoff applied.
- Residual solvents: measured residual solvents from the final synthetic step and purification.
- Certificates of Analysis if raw materials where appropriate.
- Informal stability data: estimated and measured.
  - This will be either actual shelf life data, if it can be obtained from CIL history or by analysis of in-stock batches, or
  - If no data is available, CIL will commit to assaying the batch provided after six months and one year. Data will be provided after one year, unless the batch fails assay after six months. This option will not be available if the Enhanced Data Package is ordered at a later date.

CIL products are labeled "For Research Use Only. Not for use in diagnostic procedures."

The references in this document were chosen to represent the wide range of applications that CIL products support. Papers not referenced in this document do not imply a lesser impact on the scientific community.

These articles do not imply that materials produced by CIL are suitable for any intended purpose.

Additional Products of Interest

Catalog No.	Description
CLM-116	L-Alanine (1-^{13}C, 99%)
DLM-248	L-Alanine (3,3,3-D_3, 99%)
NLM-454	L-Alanine (^{15}N, 99%)
CNLM-534-H	L-Alanine ($^{13}C_3$, 99%; ^{15}N, 99%)
CLM-8699	L-Asparagine·H_2O ($^{13}C_4$, 99%)
NLM-3286	L-Asparagine·H_2O ($^{15}N_2$, 98%)
CNLM-3819-H	L-Asparagine·H_2O ($^{13}C_4$, 99%; $^{15}N_2$, 99%)
CLM-1801-H	L-Aspartic acid ($^{13}C_4$, 99%)
CLM-3616	L-Aspartic acid (1-^{13}C, 99%)
DLM-546	L-Aspartic acid (2,3,3-D_3, 98%)
NLM-718	L-Aspartic acid (^{15}N, 98%)
CNLM-544-H	L-Aspartic acid ($^{13}C_4$, 99%; ^{15}N, 99%)
CLM-1800-H	L-Glutamic acid ($^{13}C_5$, 99%)
CLM-674	L-Glutamic acid (1-^{13}C, 99%)
DLM-556	L-Glutamic acid (2,3,3,4,4-D_5, 97-98%)
NLM-135	L-Glutamic acid (^{15}N, 98%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
CLM-3612	L-Glutamine (1-^{13}C, 99%)
CLM-2001	L-Glutamine (1,2-$^{13}C_2$, 99%)
NLM-557	L-Glutamine (amide-^{15}N, 98%+)
NLM-1328	L-Glutamine ($^{15}N_2$, 98%)
CNLM-1275	L-Glutamine ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CLM-422	Glycine (1-^{13}C, 99%)
CLM-1017	Glycine (1,2-$^{13}C_2$, 97-99%)
DLM-280	Glycine (D_5, 98%)
DLM-1674	Glycine (2,2-D_2, 98%)
NLM-202	Glycine (^{15}N, 98%)
CNLM-1673	Glycine ($^{13}C_2$, 99%; ^{15}N, 99%)
CLM-2264	L-Histidine·HCl·H_2O ($^{13}C_6$, 97-99%) <5% D
NLM-1513	L-Histidine·HCl·H_2O ($^{15}N_3$, 98%) <5% D
CNLM-758	L-Histidine·HCl·H_2O ($^{13}C_6$, 97-99%; $^{15}N_3$, 97-99%) <5% D
DLM-2949	τ-methyl-L-Histidine (methyl-D_3, 98%)
CLM-2248-H	L-Isoleucine ($^{13}C_6$, 99%)
CLM-1026	L-Isoleucine (1-^{13}C, 99%)
DLM-141	L-Isoleucine (D_{10}, 98%)

Catalog No.	Description
NLM-292	L-Isoleucine (^{15}N, 98%)
CNLM-561-H	L-Isoleucine ($^{13}C_6$, 99%; ^{15}N, 99%)
CLM-2247-H	L-Lysine·2HCl ($^{13}C_6$, 99%)
CLM-653	L-Lysine·2HCl (1-^{13}C, 99%)
DLM-2640	L-Lysine·2HCl (4,4,5,5-D_4, 96-98%)
NLM-1554	L-Lysine·2HCl ($^{15}N_2$, 98%)
CNLM-291-H	L-Lysine·2HCl ($^{13}C_6$, 99%; $^{15}N_2$, 99%)
CLM-2260-H	L-Proline ($^{13}C_5$, 99%)
CLM-510	L-Proline (1-^{13}C, 99%)
DLM-487	L-Proline (D_7, 97-98%)
NLM-835	L-Proline (^{15}N, 98%)
CNLM-436-H	L-Proline ($^{13}C_5$, 99%; ^{15}N, 99%)
CLM-1574-H	L-Serine ($^{13}C_3$, 99%)
CLM-1573	L-Serine (1-^{13}C, 99%)
DLM-582	L-Serine (2,3,3-D_3, 98%)
NLM-2036	L-Serine (^{15}N, 98%)
CNLM-474	L-Serine ($^{13}C_3$, 99%; ^{15}N, 99%)
CLM-2261	L-Threonine ($^{13}C_4$, 97-99%)
CLM-447	L-Threonine (1-^{13}C, 99%)
DLM-1693	L-Threonine (D_5, 98%)
NLM-742	L-Threonine (^{15}N, 98%)
CNLM-587	L-Threonine ($^{13}C_4$, 97-99%; ^{15}N, 97-99%)
CLM-4290-H	L-Tryptophan ($^{13}C_{11}$, 99%)
CLM-778	L-Tryptophan (1-^{13}C, 99%)
DLM-1092	L-Tryptophan (indole-D_5, 98%)
NLM-800	L-Tryptophan ($^{15}N_2$, 98%)
CNLM-2475	L-Tryptophan ($^{13}C_{11}$, 99%; $^{15}N_2$, 99%)
CLM-2249-H	L-Valine ($^{13}C_5$, 99%)
CLM-470	L-Valine (1-^{13}C, 99%)
DLM-488	L-Valine (D_8, 98%)
NLM-316	L-Valine (^{15}N, 98%)
CNLM-3466	L-Valine (1-^{13}C, 99%; ^{15}N, 98%)
CNLM-8678	L-Valine (2-^{13}C, 99%; ^{15}N, 98%)
CNLM-442-H	L-Valine ($^{13}C_5$, 99%; ^{15}N, 99%)

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