SNP #10: Stable Isotopes – Transcript

Alan Flanagan: Hello and welcome to this segment of Understanding Nutrition Science. Today we're going to be discussing the use of stable isotopes and stable isotope tracers in nutrition research. You may have heard the term stable isotope tracer before. I imagine you have lightly come across the term doubly labeled water at some point as a means of measuring energy expenditure, and you've possibly heard that it's the gold standard for measuring energy expenditure in free living humans because we can't bring people into a lab all the time to measure energy expenditure in a respiratory chamber or otherwise.

Well, doubly labeled water uses stable isotope trace. As its method of ultimately then providing such a calculation of energy expenditure and free living humans and doubly labeled water is merely one application of the use of stable isotopes in nutrition research. Stable isotopes provide the means to measure absorption and metabolism of macronutrients.

Of micronutrients and indeed of trace elements, it allows for the study of metabolic flux of metabolites through different compartments in the body. For example, you could trace the metabolic fate of dietary fatty acids through absorption into the circulation, into the liver, and even ultimately, if, for example, they were packaged into triglyceride and into very low density lipoprotein in the liver, you would be able to trace that process of the journey of the fatty acid in its postprandial metabolism.

And so stable isotopes as a approach or methodology provides nutrition with a very refined and precise methodological approach to understanding at a very technical level and indeed at a very precise level. The absorption, for example, of calcium or as we mentioned, energy expenditure, and body compositio, and the metabolic fate of proteins, carbohydrates and fats, muscle protein synthesis, and otherwise, this is particularly important for a field where much of the criticism is in relation to the accuracy and reliability of methods used in nutrition research. And while that's largely directed at epidemiology and stable isotopes by virtue of their technicality and the requirement for laboratory measurements like gas chromatography, mass spectrometry and indeed the expense of the isotopes themselves for the use in research and the methods to analyze, require blood sampling, for example, or the collection of urine samples. And otherwise, these methodologies are typically used in more controlled studies.

Nevertheless, they do provide a means for nutrition to have a level of precision and accuracy that is really important for the overall understanding of various aspects of metabolism, particularly postprandial metabolism. This is, of course, quite a complicated overall area. It is very technical in many respects, and goes could potentially go into a lot of detail on the various methodologies used, but I think that's superfluous to requirements for your understanding of the use and application of stable isotopes in nutrition research.

And so the purpose of today's segment, Is to come away with a conceptual understanding highlighted by way of a number of specific examples of what exactly a stable isotope tracer, for example, is why they allow us this level of accuracy and precision in measuring metabolism and absorption of various aspects of diet, and understanding the potential application of stable isotopes in nutrition research. So for example, I'm not going to go into detail about the use of mass spectrometry and the detail in relation to that. We're not going to. Get bogged down in too much of the chemistry side of it. Although for proper understanding of what a stable isotope is, we are going to have to dust off our chemistry textbooks and mention things like atoms, protons, neutrons, and electrons, but we won't burden that.

The aim is to just have an understanding of what it is that defines these compounds and why it is then that they can be of incredible use to nutrition research methodology. So we're going to start at the level of what is a stable isotope. That is where we will have to. Briefly dust off the chemistry cobwebs in our brains and collectively think about atoms and atomic mass again.

And then we'll discuss why and how a stable isotope tracer is used. And what it allows you to actually look at in the body as far as the metabolic fate of. Dietary substrates, whether macro or micronutrients or otherwise. And then we'll highlight a number of choice examples of the application of stable isotopes in nutrition research.

At the outset, I want to give a shout out to my supervisor at Dr. Barbara Fielding, whose work in stable isotope research and methodology has been foundational. And indeed she is, along with Professor Margo Umpleby at the University of Surrey the co-author together, both of them of the chapter on stable isotopes in nutrition research, that was in the first edition of Nutrition Research Methodologies published in 2015 and indeed a chapter that really saved my life with my own application of stable isotope tracers during one of my PhD studies, which I will use as one of the examples of the application of stable isotope tracers in nutrition research.

So first off, let's dust off the chemistry books. So all matter in our natural environment is made up of atoms. And you will recall, or maybe not, that all atoms are comprised primarily of three particles, protons, neutrons, and electrons. And those names give away certain chemical properties insofar as protons are positively charged, neutrons are not charged. Hence they're neutral charge and electrons are negatively. Now of every chemical element in the environment, the major chemical elements in our environment are hydrogen, nitrogen, oxygen, and carbon. So what is an isotope in this? So what we mean by an isotope; "iso" meaning "equal" and "tope" derived from the Greek word meaning "place".

What this really means is that we can have a chemical element that actually has a number of different types. So the atomic number is the same. So remember, if we were to write out on the periodic table a chemical element, it would have an atomic number. And then it would have an atomic mass. The atomic number is the number of protons, and then the atomic mass would be the balance of neutrons made up in the nucleus of that chemical element.

And so what an isotope is and what characterizes the difference within the same chemical element. Is a difference in the atomic mass. In reality, what that means is that the number of neutrons differs. For example, we just mentioned the most abundant major elements in the environment. Our hydrogen, carbon, nitrogen, and oxygen. But they're corresponding atomic mass is one for hydrogen, 12 for carbon, 14 for nitrogen, and 16 for oxygen. And the reason these compounds are called 'stable' isotopes is because of their lack of perceptibility to reactions to change in the environment. And this is actually, when it comes to one of the applications of stable isotopes in nutrition research, which is the ability to quantify through mathematical modeling, the potential dietary parameters of a fossil, for example, so this is where a lot of our understanding from paleontology research comes in relation to the possible compositions of the ancestral human. Okay, so we know that an isotope is a different form of the same chemical element, so carbon, but it has a difference in the number of neutrons i e, it's atomic mass is different.

So it's the same chemical element. It has the same atomic number. Which would be six for carbon, but it has a difference in its atomic mass. So the isotope describes the fact that it's just simply the same chemical element with different forms, different types of that chemical element. And the reason it differs is because of the number of neutrons i.e. It differs in its atomic mass.

And isotopes are stable because they are stable in the environment because they do not react or are susceptible to change. So that's what stable isotopes are

themselves. What are stable isotope tracers? Well, a word that you will have heard me use. Thus far is the word abundant, and this is important because we have of these chemical elements, for example, their abundance, their natural abundance becomes important for then using a correspondingly, much less abundant form that because of its less abundance is easier to identify. Okay, so what do we mean by this? Okay, so like we said, the most abundant chemical elements in our environment are hydrogen. Within atomic mass of one carbon within atomic mass of 12 nitrogen was an atomic mass of 14, and oxygen was an atomic mass of 16, and they are stable.

But there are isotope versions of each of those elements that have an abundance of only 0.02 to 1.1% in the natural environment. So they're very, very rare. And that would be hydrogen with an atomic mass of two carbon with an atomic mass of 13 nitrogen with an atomic mass of 15, an oxygen with an atomic mass of.

So what a tracer is, or it's a process known as labeling. This is usually conducted in a laboratory to create the quote unquote label or to create the tracer. The labeling is where you would take a. Let's say for example, we're interested in studying the fate of a fatty acid, we could take that natural fatty acid molecule and in that fatty acid, the carbon in that fatty acid would be the natural abundance form of carbon.

Carbon within atomic mass of 12. And you could substitute that chemically for the less abundant form of carbon. So you've now swapped that 12 carbon for the 13 carbon that is known as labeling. The 13 carbon is now your tracer. So it's now, it's now your stable isotope tracer. This has the same chemical properties as the original molecule. It's still carbon. It's still the same chemical element. It's chemically identical. It's just a slightly different mass, and this now allows you, because of the rarity of this compound, and it's obvious lack of abundance, both in the body and in a particular food, for example, to trace where that goes in the body.

So for example, if you're measuring something in blood, it's going to be obvious to you with the use of these methods that this very rare, abundant isotope. Is appearing in the blood, in the circulation, for example, or is appearing in expired air and you're taking breath samples or is appearing in urine.

In the case of doubly-labeled water, which is how that's collected. So this allows you to create this tracer and the reason that it's called a tracer is simply because it's lack of abundance allows you to quite literally 'trace' the metabolic fate of that compound. So you could administer a tracer orally, or you could administer a tracer intravenously, sometimes you can use and combine both methods,

which is known as the dual isotope method, which we will discuss. And this, these approaches again, allow you to measure the metabolic pathways of carbohydrates, proteins, and fatty acids. They allow you to investigate the absorption metabol. And excretion of vitamins and they allow you to look at the absorption and postprandial metabolism and kinetics of both minerals, but also trace elements as well.

So what are some of the potential applications for these? Compounds. The first is that because we are applying these stable isotope tracers, these less abundant tracers in the body, the background natural abundance of stable isotopes in the body are measured as well. And this gives an overall understanding of the ability to trace the actual less abundant fatty acid or the, for example, a fatty acid or otherwise a less abundant isotope through the body in terms of its digestion, absorption, and a, and assimilation, and a metabolism and excretion. So we will discuss a number of those methodologies as it relates to vitamins and minerals and as it relates to macronutrients, but I thought it would be interesting to just highlight that there's also another use so to speak, of stable isotopes in nutrition research. And that's to actually measure the Presence of stable isotopes in the natural environment, for example, in fossils or in bones that are discovered.

And this is because that in the natural environment, there is a certain degree of enrichment in some of the less abundant Stable isotopes specifically. 13 carbon, and we know that plants have different isotopic signatures, so to speak, whether they're derived from land or the sea, whether they're terrestrial or marine, we know that fish differ to land mammals and we know that plants themselves can differ relative to the environment that they're growing in. So there's a range of stable isotope, natural abundance of carbon, both in the environment, in air but also in food sources. And this allows the measurement of carbon stable isotope abundance in, for example, cows grazing or in different types of plants or in fish.

What it also provides is a means to apply this technique to determine differences. For example, it can be determined whether an animal was a carnivore or a herbivore. It can be determined the relative contributions of food sources using isotopes and. Is the level of intricacy that stable isotopes have provided, that have allowed for measurements distinguishing between whether an animal consumed more of a marine fish based diet or a meat based diet.

And this is particularly important because this is provided the primary basis for the ability. To understand the potential composition of the ancestral human diet. So stable isotope research and analysis has been hugely important for

paleontology and anthropology. And while that may not necessarily be any of our respective fields it is, I think, interesting and indeed obviously important in the context of ongoing debates of the optimal human. That the methods that are used, although they do rely heavily on complex mathematical modeling, but these methods are particularly advanced. And do allow for a degree of insight into our evolutionary past and the likely composition of foods consumed that are analyzed through the recovery of human or indeed other hominoid fossil remains.

Well, that's typically not going to be the context of the application that we will I believe likely read or come across the use of techniques as we read research papers. , there's different techniques that can be used. And there's three in particular that I want to highlight before we go on to discuss by way of example, some of the application as it relates to macronutrients and micronutrients trends.

The selection of a given stable top tracer technique will depend on the question. To be answered as far as what it is exactly that we are trying to know, there's three main methods that you will likely come across, or four main methods that you will likely come across in nutrition research. One is known as stable isotope dilution. The other is using stable isotopes to measure oxidation rates. The third is the dual isotope method, and the fourth and final is the precursor product method. So let's discuss each of these in turn.

The isotope dilution method is used to assess body pools, for example, a specific tissue compartment, and can be used to measure what is known as flu. So that's the flux of a given stable isotope tracer from one tissue compartment into another. For example, from the circulation to the liver and perhaps even into synthesis into a triglyceride or into packaged into very low density lipoprotein or VLDL. So isotope dilution in this context, or the measurement of flux or exchange of, for example, fatty acids between body pools or glucose, is typically used by intravenous infusion.

What usually happens is people are hooked up to an IV and the stable isotope tracer is infused into the circulation at a constant rate, and they're aiming to achieve what is known as steady state. And this will be achieved over a certain amount of time. But what the steady state is describing is where the tracer and the more abundant version of that tracer, which in this context we will call the "tracee", are in balance. So the loss of the tracer from the pool that you are investigating, let's say for example, plasma is lost at the same rate as the tracee. So that is where a steady state has been achieved. And what you're ultimately

capable of doing is looking at the metabolic clearance of the trace and that gives you an estimate of the efficiency of its removal.

So, for example, this steady state achievement with the isotope dilution technique can be used to assess the size of a body pool or a body store of a given nutrient. For example, this technique has been used to quantify the whole body level of vitamin A by using a stable isotope tracer of retinol.

The second technique is the use of oxidation rates. This specifically uses 13 carbon labeled tracers, and the reason that it uses this is because when 13 carbon appears in carbon dioxide, the rate of appearance of 13 carbon in that carbon dioxide in expired air can provide a measure of the oxidation rate of the metabolite that you're interested in.

This is a really useful method because it's non-invasive. The 13 carbon labeled stable isotope tracer can be administered in food. And participants are only required to give breath samples, which are sealed, and then the breath is analyzed to determine the levels of 13 carbon in the expired air, in carbon dioxide. And this was a method that we used in one of the studies for my PhD, we used a 13 carbon labeled octanoic acid.

So octanoic acid is a medium chain fatty. It was labeled with 13 carbon, so this very less abundant isotope form or type of carbon. And because it was a fatty acid, it bound with fat, we added a tiny amount to mix in with eggs at breakfast, and this was administered to the participants. Now because it's not broken down in the stomach, the rate of appearance of this 13 carbon tracer in expired air can be used to measure the rate of gastric emptying, and that was exactly the purpose for which we were employing stable isotope tracer analysis in that particular study. So participants expired, breathed into breath tubes prior to eating the egg breakfast, which was laced or fortified with this stable isotope tracer fatty acid. They then ace the breakfast and then for five hours, 300 minutes after that breakfast was consumed every 30 minutes they provided more breath samples. And over time you were able to model the rate of appearance of that 13 carbon in the carbon dioxide, thus providing a measure of the rate of gastric emptying. That is a technique that has been used widely. The administration of 13 carbon, not necessarily always in a fatty acid form.

Again, it depends on the question being asked. This method can also be used for overall gastrointestinal transit time. It can be used to look at fat and protein digestion and clinically. This use of stable isotope tracers can also be used for the diagnosis of conditions like *h pylori*, infection in the stomach. So this is a

method that is looking at the oxidation rates and is primarily measured using breath samples and is noninvasive.

The other two methods that are worthy of discussion are the dual isotope methods and precursor product methods. The dual isotope methods is a technique that's used for measuring absorption from oral intake through diet. So this, and the reason it is called dual isotope method, the dual here is that a stable isotope tracer is provided with the meal for oral ingest. That's going to be digested and absorbed in the ordinary manner through the gastrointestinal tract. And then what makes this method a dual technique is the simultaneous provision of another stable isotope tracer, not the same one that has been given in the meal, one that's distinguishable from.

The stable isotope tracer that was administered with the food for oral consumption. This second tracer is administered intravenously, and if you recall, this reflects the dilution method where you're able to determine the body pool or storage pool of a given metabolite. And so you're able to then correct for the absorption that is observed from the tracer in the meal relative to the levels of that body pool that a given individual has, which would likely differ from person to person based on factors like body size and otherwise. So it allows you to correct for that between person variation.

I'm going to use again, each of these, highlighted by way of example, for the dual isotope method, we'll use an example of measuring what's known as the calcium fractional absorption rate. And then the final method is known as, precursor product method and that is used for measuring synthesis rates, for example, muscle protein synthesis. And this is looking at absolute synthesis rates or incorporation of tracers into tissue. So let's think about the application of these techniques to the study of macronutrients, micronutrients, trace elements in relation to the measurement of energy expenditure or body composition.

This can be used using the dilution technique. So the dilution technique where there is administration of a stable isotope tracer to determine a steady state and to then calculate the body pool or storage of a given, for example, nutrient. But it can also be used to determine compartments or tissue compartments of body, including fat free mass.

So with this example, doubly labeled water is used. So this is H2O where there are less abundant forms of both hydrogen and oxygen used hydrogen with an atomic mass of two, oxygen with a atomic mass of 18, in contrast to their more abundant H1 and O16 or 16O and 1H. So that is doubly labeled water.

And using the dilution method where this is administered orally into body water, the methods provides a means to measure total body water. and use a calculation to derive in cal a, a score for fat free mass. And so you are able to study participants fasting to provide a dose of the labeled water to wait for the steady state period, and then take another sample and using mathematical models, determine what the fat free mass is. So body composition can be calculated using this stable isotope tracer technique. An example of the use of oxidation rates I discussed in relation to the gastric emptying measurement method that we used in our research where we provided a 13 carbon stable isotope fatty acid and used that measuring the rate of appearance of that stable isotope in expired air from breath samples taken from participants to determine the rate of gastric emptying because this particular fat was not broken down in the stomach. So it only begins to be broken down once it's passed through the stomach. And it's rate of appearance in oxidation as a result can be determined as a measure of gastric emptying in this particular case. But there are other applications of using oxidation rates to calculate other aspects of metabolism. The dual isotope technique, remember where a stable isotope is given orally and also a second. A stable isotope is given intravenously can be used to determine, for example, fractional calcium absorption.

This is where a particular stable isotope of calcium is given with a meal and ingested orally. And another calcium stable isotope is given intravenously as an infusion. The intravenous administrative stable isotope tracer allows for the assessment of the actual body pool of calcium and that allows for the correction of between person or inter individual differences in that calcium pool mass that would relate to body size to be determined and then urinary collections over 24 hours are made and the enrichment of both the stable isotope tracers, the one administered orally and the one administered intravenously, can be used to calculate the fractional absorption of calcium that was administered with that. And that is calculated as the ratio of the oral cal, the stable isotope tracer calcium stable isotope tracer to the intravenous calcium stable isotope tracer that are recovered in that 24 hour urine sample.

There is the capacity to use the technique we discussed known as precursor product method to look at muscle protein synthesis. This specifically uses the amino acid leucine commonly as the tracer. It's known that leucine, for example, only has two metabolic fats. It's either oxidized or it's incorporated into protein.

So the measurement of leucine, again, this can be done with the use of breath samples, the appearance of leucine that is labeled with 13 carbon and the appearance of that leucine 13 carbon in expired air can be used to provide a

measurement of the balance of whole body protein synthesis and protein breakdown. And leucine stable isotope 13 carbon tracers can also be used to look at muscle protein synthesis, specifically, which can be determined by the provision of those stable isotope tracer labeled amino acids, and then the sampling of a muscle biopsy, to look at the enrichment of that stable isotope tracer into muscle protein taken from that biopsy.

There is the capacity to use stable isotope tracers to look at the kinetics of postprandial lipemia for example, lipoprotein metabolism. One example here would be the use of a form of glycerol; a stable isotope tracer of glycerol to look at triglycerides kinetics in a postprandial period, or even in a fasting period, recall that a triglyceride is formed of three fatty acids and a glycerol backbone.

So the provision of a glycerol stable isotope tracer allows for the tracing of the metabolic fat of that tracer and the rise and ultimate decline of the tracer in triglycerides over eight to 12 hours after its administration. This can be used to distinguish between triglyceride that's incorporated into chylomicrons i.e., the lipoprotein large lipoproteins that take in dietary fat consumed through the diet and absorb through the intestines.

And it can also be used to separate the quantification of triglycerides from very low density lipoprotein particles that are synthesized in the liver. So it provides a means of distinguishing between endogenous and exogenous triglyceride synthesis and intake. And there are a number of other potential applications of stable isotope tracers.

The metabolic fate, this term that I may have used before, flux this idea that actually when we consume foods and digest and process them into their component glucose and fatty acid and amino acid forms, we can trace the flux of these substrates. Through different tissues in the body. So stable isotope tracers can be used, for example, to look at the partitioning of fatty acids in the liver. And these are just a number of examples that can be used for stable isotope tracer application in terms of studying macro and micronutrients.

The final one, because it is a measurement that is perhaps more commonly discussed is doubly labeled water. So in humans we know that total energy expenditure is comprised of three main components, your resting metabolic rate, the thermic effect of food, and physical activity level. And while we can measure this from oxygen consumption and expired air in laboratory circumstances using, for example, a respiratory chamber, we can't do that in a free living context. And we also are typically measuring energy expenditure in a

lab context with people who are lying, prone in a bed, for example, to minimize inputs.

So it's not a effective measure of free living, total energy expenditure and the doubly labeled water method provides that this was first used in the 1980s. It's the gold standard for measuring free living, total energy expenditure in humans. And the reason it is called doubly labeled water is because it uses two stable isotope tracers: a 18 oxygen and two hydrogen; oxygen with an atomic massive 18 and hydrogen with an atomic mass of 2.

And this method has been incredibly useful for determining free living energy expenditure in humans. It's also the method that showed that with increasing adiposity, there's an increase in total energy expenditure, such that an individual with class two obesity, for example, will have a significantly higher total energy expenditure compared to someone with a lean body composition. So in some, the use of stable isotope tracers in nutrition research provides for a very precise ability to quantify the metabolic face of macronutrient. The digestion, metabolism, absorption, and kinetics of micronutrients, and particularly both of vitamins and trace elements and minerals.

It provides a method of effective and accurate quantification of total energy, expenditure, and free living individuals. And the methods of using stable isotopes in research can also be used in the context of paleontology and anthropology to attempt to discern the composition of the diet derived from fossils.

So there is a broad application of this technique to nutrition. Ideally I'd like you to come away from today understanding a) what a stable isotope tracer is, i.e. the taking of a abundant chemical element and the substitution of that abundant chemical element for its less abundant but chemically identical counterpart.

The provision of that less abundant, but chemically identical counterpart in, for example, a protein like leucine, an amino acid like leucine or in a fatty acid, or with, for example, glucose, or even at the level of vitamins like vitamin A or minerals like calcium. This has broad application to allow the precise tracing of the metabolism of these macro and micronutrients.

In addition to the other applications, as we discussed and provides nutrition research with a very precise ability to determine the metabolism and indeed the fate and flux of nutrients through tissues and indeed extending to the impact of diet on, for example, postprandial, lipoprotein metabolism and otherwise.

So I hope that was helpful. I hope conceptually you understand: A) what a stable isotope is. B) what a stable isotope tracer. And C) have some examples of the application and the broad application of this methodology for nutrition research. This is, I appreciate, a complex methodology in many respects, and so as always, with any follow up questions, queries or otherwise that you may have having listened to this please submit them in an email and I'll be more than happy to answer them in due course, and until the next time, take care and stay reading.