

# A SMALL PART CAN REVEAL THE WHOLE: HOW ISOTOPE TECHNIQUES HELP NUTRITION



**Fig. 1: Determining the amount of fat in the human body by measuring total body water (TBW) with isotopes.**

Stable isotopes can be used to measure the amount of water or other nutrients in the body or the amount of an ingested nutrient that is absorbed and metabolized or excreted. They can be also used to measure the rate of absorption, utilization or synthesis of proteins, fats or carbohydrates.

Stable isotopes of carbon, hydrogen, oxygen, nitrogen, iron and zinc can be used in studies assessing nutritional status, energy expenditure, breastfeeding practices, micronutrient status and the absorption of nutrients from the foods we eat.

Commonly used stable isotopes include deuterium (hydrogen-2), oxygen-18, carbon-13 and nitrogen-15. Isotopes of iron include iron-57 and iron-58, and isotopes of zinc include zinc-67, zinc-68 and zinc-70. All stable isotopes occur naturally, but elements or compounds can be synthesized that are enriched compared to the naturally occurring amount. These isotopes or isotope labelled compounds are metabolized by the body in the same manner as the natural kind, but with the added benefit of being trackable. Stable isotopes are not radioactive and are therefore risk-free for people of all ages.

Water is composed of isotopes of hydrogen and oxygen. Natural water is composed mainly of  $^1\text{H}$  and  $^{16}\text{O}$ , but contains a very small amount of  $^2\text{H}$  (deuterium) and  $^{18}\text{O}$ . However, water can be made to contain a much higher proportion of

deuterium or oxygen-18 compared to natural water. We say that this water is enriched. Deuterium oxide ( $\text{D}_2\text{O}$ ) is enriched water in which 99.8% of the hydrogen atoms are in the form of hydrogen-2.

## Assessment of Body Composition

Determining the amount of fat in the human body can be done by measuring total body water (TBW) with isotopes. The human body can be thought of as being composed of two categories: fat mass, and fat-free mass. There is no water in fat mass, whereas 73–80% of fat-free mass consists of water. The fat-free mass of a newborn baby contains 80% water, and this gradually decreases to 73% in adults. This means fat-free mass can be determined by measuring TBW and then using an appropriate hydration factor. Fat mass is the difference between body weight and fat-free mass. Sometimes the results are expressed as a percentage of total body weight.

The deuterium dilution technique (Fig. 1) involves measuring a person's saliva and/or urine just before they consume a dose of deuterium labelled water and repeating the process 3 to 5 hours later. The increased level of deuterium shows in the person's saliva and urine samples.

Urine or saliva samples gathered from the test subject after isotope equilibration show

increased levels of deuterium. The deuterium is evenly distributed throughout the body after 3 to 5 hours.

The person's pre-dose samples of urine or saliva are compared with the post-dose samples to calculate TBW, fat-free mass and ultimately the amount of fat in the body. Body composition is a good indication of health. Too much fat or too little fat-free mass raises the risk of serious health conditions.

### Assessment of Breastfeeding Practices

Nutrition plays a vital role in early child development. Isotope techniques can help determine if a baby is exclusively



**Fig. 2: Dose to Mother Technique**

The mother consumes deuterium oxide. The deuterium mixes with water in her body including the milk the baby drinks. The saliva of both the mother and child is enriched with deuterium. This can be measured with sensitive equipment.

breastfed or not, as well as how much human milk the baby consumes. Conventional methods to determine the quantity of milk consumed by a baby can be time-consuming. They can also disturb a baby's feeding pattern, as these methods require that the baby is weighed before and after each feed. A more accurate and very informative alternative technique is known as the deuterium oxide dose-to-mother technique. This is the only way to determine whether a baby is exclusively breastfed or not.

A lactating mother drinks a dose of deuterium oxide that is distributed throughout her body and is incorporated into her milk (Fig. 2). Over a period of 14 days, samples of saliva or urine are collected from the mother and child, revealing the changes in isotope concentration. This gives insight into the baby's intake of human milk and whether the baby has consumed water from other sources, as well as the body composition of the mother.

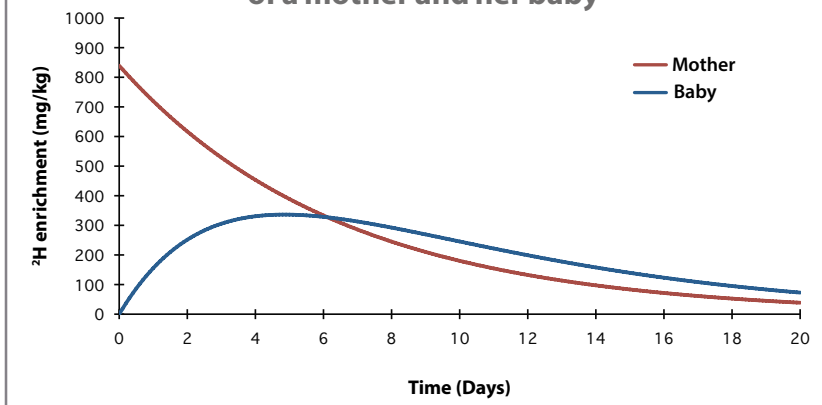
After the mother has taken the dose of deuterium oxide, the deuterium gradually disappears from her body and appears in the body of the baby (Fig. 3). Deuterium in the baby's body comes only from the milk consumed during breastfeeding. As the deuterium is eliminated from the mother's body, the enrichment in her milk declines and therefore the enrichment in the baby's body also falls. A mathematical model is used to determine how much of the deuterium given to the mother appears in the baby's saliva. This is related to the amount of human milk consumed by the baby. The model also gives an estimate of the amount of water from sources other than its mother's milk, and therefore whether the baby is exclusively breastfed or not.

### Assessment of Total Energy Expenditure

When determining how much food a person needs, it is important first to deduce how much energy they expend. If water labelled with hydrogen-2 (deuterium oxide) is mixed with water labelled with oxygen-18, the mixture is known as the doubly labelled water (DLW). Researchers can use DLW to get an estimate of total daily energy expenditure (Fig. 4). Total energy expenditure is also used to determine a person's physical activity level.

The participant drinks a dose of DLW, which gets distributed throughout the body water. Every time the person breathes or exercises,

**Fig. 3: Deuterium enrichment in the body water of a mother and her baby**





**Fig. 4: Doubly Labelled Water Method**

After drinking a dose of DLW, the body water is enriched with deuterium and oxygen-18. As time progresses, the deuterium (orange dots) and oxygen-18 (red dots) leave the body and this rate of decline is a direct indication of energy expenditure.

some of the labelled oxygen and hydrogen is lost in their urine, sweat and breath. Deuterium is lost only in water, whereas oxygen-18 is lost in both water and carbon dioxide. The difference in the elimination rates of deuterium and oxygen-18 is a measure of carbon dioxide production rate, from which energy expenditure can be calculated (Fig. 5). Urine samples over a 14 day period reveal the decline in the introduced isotopes. A very slow decline indicates little energy expenditure, while a sharper, faster decline indicates high energy expenditure. The DLW technique is ideal for measuring total daily energy expenditure in normal, daily living conditions, and is being used by the IAEA in projects designed to address childhood obesity and quality of life in the elderly.

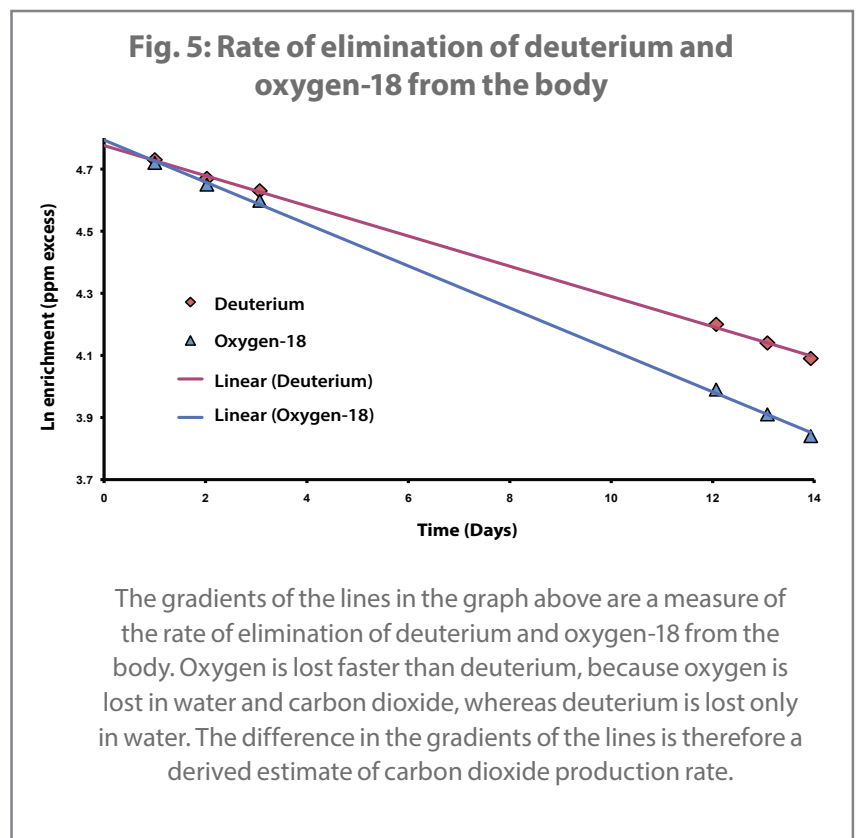
### Assessment of Vitamin A Body Stores

The stable isotope dilution technique is used in studies to determine the change in body vitamin A during an intervention (e.g. vitamin A fortification, supplementation or food-based approaches that encourage consumption of a wide variety of nutritious foods). Stable isotope methods (Fig. 6) are the only non-invasive way to establish that the levels of vitamin A are too high. This can occur when vitamin A supplements and fortification programmes are implemented in the same communities.

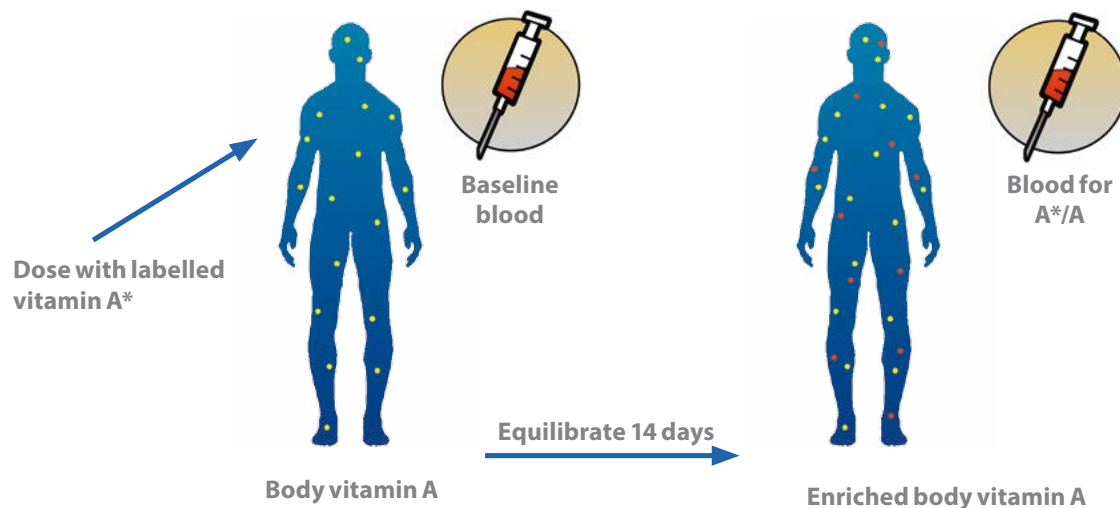
Stable isotopes of hydrogen ( $^2\text{H}$ ) and carbon ( $^{13}\text{C}$ ) can be used to label vitamin A.

### Assessment of the Bioavailability of Iron and Zinc

Assessing the bioavailability (absorption and utilization) of nutrients from food is



important because people usually eat more than one type of food at a time, and some might contain enhancers or inhibitors of absorption. Bioavailability studies of iron and zinc in foods using stable isotopes can reveal large differences in absorption between different food combinations. Iron and zinc stable isotopes are used to determine the bioavailability of the mineral from a test food that has been fortified or biofortified or that is consumed in the same meal as a potential inhibitor (e.g. phytic acid in unrefined grains, nuts, seeds and legumes) or as an enhancer (e.g. vitamin C) of mineral absorption. The stable isotopes of iron and zinc can be added to a test food.



**Fig. 6: Assessment of vitamin A status**

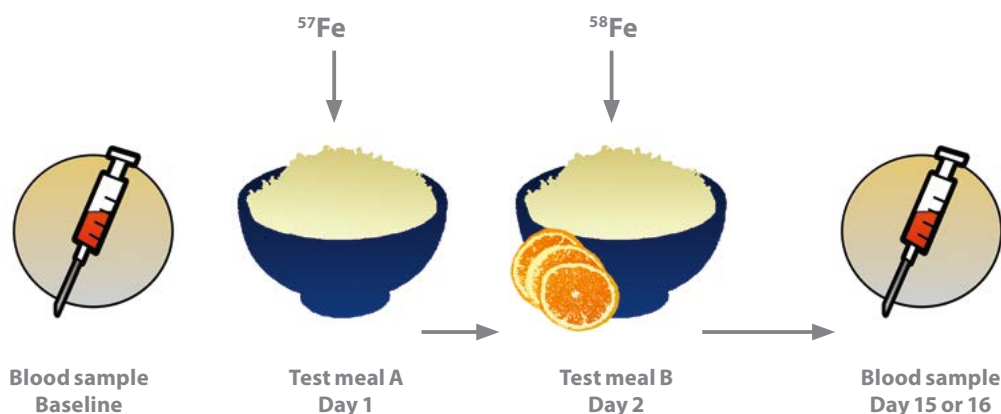
For assessment of vitamin A status, a dose of vitamin A labelled with a stable isotope is administered after a baseline blood sample has been collected. A period of equilibration of the dose with the vitamin A body pool is necessary before the follow-up blood sample is taken for analysis by mass spectrometry. From the dilution of the precisely measured dose of isotope labelled vitamin A, it is possible to calculate the total quantity of exchangeable vitamin A in the body. This is the most sensitive way to non-invasively estimate vitamin A status over the whole range, from deficient to normal to excessive.

Fig. 7 depicts a study design to assess iron incorporation into red blood cells after consumption of a cereal based meal and the same meal with an orange, which contains vitamin C — an enhancer of iron absorption.

A baseline blood sample is collected and a test meal (A), containing a known amount of a stable isotope of iron ( $^{57}\text{Fe}$ ), is consumed. On the following day, a test meal (B) is consumed that contains a known amount of a second stable isotope of iron ( $^{58}\text{Fe}$ ) and a potential enhancer or inhibitor of iron absorption. Half of the study participants receive the test meals in the reverse order.

A second blood sample is collected two weeks later. After processing of the blood samples, the iron isotopes are analysed with an appropriate mass spectrometer. The ratios of stable iron isotopes before and after consumption of the test meals are used to determine the amount of iron absorbed from the meals and incorporated into the red blood cells, thus revealing the effect of enhancers or inhibitors present in the meal.

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**Fig. 7: Assessment of iron absorption**