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No. 13

Introduction to Body Composition Assessment Using the Deuterium Dilution Technique with Analysis of Urine Samples by Isotope Ratio Mass Spectrometry

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INTRODUCTION TO BODY COMPOSITION ASSESSMENT USING THE DEUTERIUM DILUTION TECHNIQUE WITH ANALYSIS OF URINE SAMPLES BY ISOTOPE RATIO MASS SPECTROMETRY

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FOREWORD

The IAEA has fostered the more widespread use of a stable isotope technique to assess body composition in different population groups to address priority areas in public health nutrition in Member States. It has done this by supporting national and regional nutrition projects through its technical cooperation programme and coordinated research projects over many years.

This publication was developed by an international group of experts to provide practical hands-on guidance in the use of this technique in settings where analysis of stable isotope ratios in biological samples is to be made by isotope ratio mass spectrometry. The publication is targeted at new users of this technique, for example nutritionists, analytical chemists and other professionals. More detailed information on the theoretical background and the practical applications of state of the art methodologies to monitor changes in body composition can be found in IAEA Human Health Series No. 3, Assessment of Body Composition and Total Energy Expenditure in Humans by Stable Isotope Techniques.

The IAEA is grateful to the major contributors to this publication (T. Preston, United Kingdom; D. Schoeller, United States of America; C. Slater, United Kingdom; M.E. Valencia Juillerat, Mexico) for sharing their technical expertise and extensive experience in stable isotope techniques in nutrition.

The IAEA officer responsible for this publication was L. Davidsson of the Division of Human Health.

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CONTENTS

1. INTRODUCTION

1.1. BACKGROUND

For many years, the IAEA has fostered the more widespread use of the stable isotope technique to assess body composition in different population groups to address priority areas in public health nutrition in Member States. The objective is to support national and regional nutrition projects through both the IAEA's technical cooperation programme and its coordinated research projects.

1.2. OBJECTIVE

This publication was developed by an international group of experts to provide practical, hands-on guidance in the use of this technique in settings where the analysis of stable isotope ratios in biological samples will be made by isotope ratio mass spectrometry (IRMS).

1.3. SCOPE

This manual is aimed at new users of this technique, for example nutritionists, analytical chemists and other professionals. More detailed information on the theoretical background and the practical application of state of the art methodologies to monitor changes in body composition can be found in the IAEA publication Assessment of Body Composition and Total Energy Expenditure in Humans by Stable Isotope Techniques (IAEA Human Health Series No. 3).

1.4. STRUCTURE

Following this introduction, Section 2 provides a technical overview of parameters related to body composition assessment. Section 3 describes the equilibrium technique of estimating TBW by deuterium dilution. Section 4 details the procedures involved for using the technique, including planning the study, preparation and storage of deuterium oxide doses, measuring TBW and urine sampling. Section 5 explains how to analyse deuterium enrichment, while Section 6 provides detailed information on how to perform the various calculations necessary for applying the deuterium dilution technique for body

composition assessment. Section 7 deals with quality control issues and Section 8 summarizes the critical steps for good quality data. A list of frequently asked questions is given in Section 9. Finally, four appendices provide general information on the safety of deuterium oxide, a sample data sheet for TBW estimation by deuterium oxide dilution, an equipment list and a description of isotopic fractionation, respectively.

2. BACKGROUND

2.1. BODY COMPOSITION

On its own, body weight is a relatively poor indicator of health and nutritional status. A more important indicator is body composition, namely what components comprise an individual's body weight. The human body is often subdivided into two component parts: FM and FFM. This is known as a two compartment model.

2.2. TOTAL BODY WATER

Water is the most abundant constituent of the body. At birth, the body contains 70–75% water, but this proportion decreases as the body matures to 50–60% in lean adults and to less than 40% in obese adults. Water is found exclusively within the FFM, which is approximately 73.2% water in adults. TBW includes both intracellular fluid and extracellular fluid. If there is an estimate of TBW, the amount of FFM can be estimated. Body FM is the difference between body weight and FFM.

In free-living conditions, when adequate food and drink are available, the body water compartment is in a constant state of flux, with water molecules entering and leaving the body. The circulatory system is responsible for providing a regular supply of nutrients to, and removal of waste from, all body cells. Each time we drink fluid, consume food containing moisture or produce a water molecule during energy substrate oxidation, these molecules mix with the body water pool. At the same time, water constantly leaves the body in different forms. This includes insensible water losses as water vapour from the lungs and skin as well as water loss in urine and faeces. In adults, the body water compartment remains relatively constant in size and rarely varies by more than a few per cent

within or between days. During a typical day, the input and output of water is roughly equal, and the pool size remains relatively constant [1].

TBW can be estimated in the field using the deuterium oxide dilution technique. An advantage of this technique is that it can be used to assess longitudinal changes in body composition before and after an intervention. Other field methods to estimate TBW include bioelectrical impedance analysis and predictions from anthropometry (weight, height, sex, age). These are less accurate and require prediction equations to be derived for particular population groups by comparison with a reference method (probably TBW) in a representative sample [2, 3].

2.3. DEUTERIUM

Stable isotope techniques have been used in studies of human nutrition for over 50 years. Deuterium is a stable (non-radioactive) isotope of hydrogen, with the symbol ^{2}H. It is given orally as deuterium oxide ($^{2}H_2O$), and after mixing with body water is eliminated from the body in urine, saliva, sweat and human milk. Deuterium oxide is handled in the body in the same way as water, and is dispersed through the body water within a matter of hours.

More information on deuterium oxide is included in Appendix I.

2.3.1. Analysis of deuterium enrichment in body water

Body water can be sampled in the form of saliva, urine, plasma or human milk and the enrichment of deuterium can be measured by IRMS using duel inlet or continuous flow IRMS [4] or Fourier transform infrared (FTIR) spectrometry [5]. FTIR is not as sensitive as IRMS and, therefore, requires a larger dose of deuterium oxide (approximately ten times as much). FTIR is not suitable for analysis of urine or human milk.

The equilibration of deuterium in body water is faster with saliva than urine: 2–4 h in saliva compared with 3–6 h in urine (in healthy adults). However, small errors are introduced by isotope fractionation in saliva due to evaporative loss, and if no account is taken of the loss of deuterium in urine and insensible water during the equilibration period. Thus, the most accurate estimate of TBW is obtained when urine is sampled, losses are accounted for and the deuterium content is analysed by IRMS. This methodology was originally validated against chemical analysis of cadavers.

2.4. EQUILIBRIUM VERSUS THE BACK-EXTRAPOLATION METHOD OF ESTIMATING TBW

This manual describes the estimation of TBW by deuterium dilution using the equilibrium or plateau technique with urine sampling and analysis of deuterium by IRMS. The technique is appropriate for use in adults and children, but in situations where the participants have high water turnover, such as infants and adults undergoing high physical activity, the back-extrapolation technique gives more accurate results. Body composition is determined by back-extrapolation as part of the doubly labelled water technique of estimating total energy expenditure [6], and in lactating mothers during the 'dose to mother' technique of estimating human milk intake in breastfed babies [7]. The back-extrapolation technique measures water turnover over a two week period in adults and over seven days in infants (3–4 cycles of water turnover). An advantage of the equilibrium technique is that sample collection is completed in a single day.

3. THE EQUILIBRIUM TECHNIQUE FOR ESTIMATING TOTAL BODY WATER BY DEUTERIUM DILUTION

3.1. BRIEF SUMMARY OF THE TECHNIQUE

The technique involves the following steps:

— The body water pool naturally contains a small amount of deuterium (^2H). This represents the natural abundance of ^2H in body water and is usually close to 0.015 at.% ^2H.
— After collection of a baseline sample, a known quantity of deuterium oxide (99.8 or 99.9 at.% ^2H) is ingested (0.05 g/kg body weight for adults). Deuterium oxide is also known as D_2O.
— The ^2H_2O mixes with body water within a few hours (Fig. 1). The amount of deuterium in body water above that naturally present is known as the enrichment of body water. Enrichment reaches a 'plateau' after 2–5 h in body water.
— As the bladder does not empty perfectly, some mixing and delay can occur. It is recommended that two post-dose urine samples are collected at the

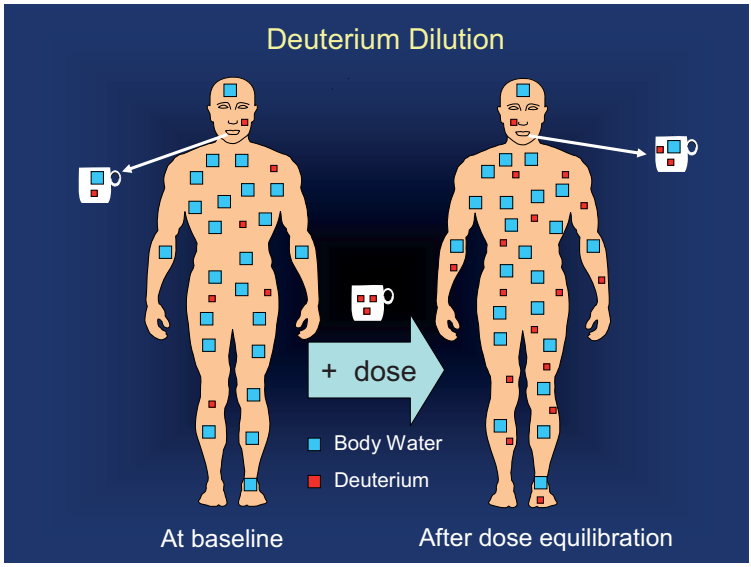

FIG. 1. Estimating TBW by deuterium dilution.

'plateau' enrichment. It is, therefore, necessary to sample urine for 6–8 h after the deuterium oxide was administered.

— The volume of all fluids consumed during the equilibration period should be recorded.

— The enrichment of ^{2}H in urine samples can be measured using continuous flow–isotope ratio mass spectrometry (CF–IRMS).

3.2. ASSUMPTIONS OF THE TECHNIQUE

There are certain assumptions associated with the deuterium dilution technique of estimating TBW [1]. These are:

— The deuterium oxide is distributed only in body water;

— The deuterium oxide is equally distributed in all body water compartments (e.g. saliva, urine, plasma, sweat, human milk);

— The rate of equilibration of deuterium oxide is rapid;

— Neither deuterium oxide nor body water is lost during the equilibration time.

Where the assumptions are not true, a correction factor must be included in the calculation of TBW or precautions taken to minimize the effect. Each of these assumptions is discussed in the following sections.

3.2.1. Assumption 1: The deuterium oxide is distributed only in body water

This is not true. Deuterium in body water enters other pools within the body. This is known as non-aqueous exchange:

— Deuterium exchanges with exchangeable hydrogen atoms in body protein. Exchangeable hydrogen atoms are those on amino ($–NH_2$), hydroxyl (–OH) and carboxyl (–COOH) groups of amino acids.
— Deuterium is also sequestered into fat and protein as these are synthesized.

Thus, the volume of distribution, sometimes known as the dilution space, of deuterium is slightly greater than TBW. The ^2H space (conventionally termed N_D when the dilution space is expressed as moles of H_2O) is 1.041 times that of TBW. N_D is calculated from the dose of deuterium oxide administered and the enrichment of the urine. The calculations are described in detail later (see Section 6).

3.2.2. Assumption 2: The deuterium oxide is equally distributed in all body water compartments

This is true for water in the body but not for water leaving the body as water vapour, which is subject to isotopic fractionation. There is no fractionation in urine, faecal water or sweat. Sweat is excreted from the sweat glands as liquid water and evaporation occurs after it leaves the body water, and, thus, it is not fractionated as it leaves the body. However, water leaving the body as water vapour in breath and transdermal evaporation is subject to fractionation. Transdermal evaporation is insensible water loss from the skin through routes other than the sweat glands. The effect of increased insensible water losses, which contain less deuterium than body water, is to concentrate the deuterium oxide left behind, which leads to an underestimation of TBW and, therefore, an overestimation of body fat. It is important to avoid physical activity during the equilibration period to avoid increasing the rate of breathing and transdermal evaporation.

3.2.3. Assumption 3: The rate of equilibration of deuterium oxide is rapid

This is true in healthy participants but water turnover is slower in the elderly, pregnant women and patients with expanded extracellular water volume

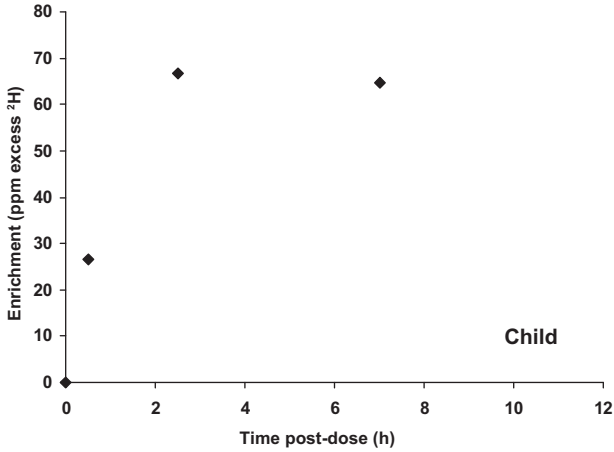

FIG. 2. Equilibration of deuterium oxide in urine in a healthy child.

(such as malnourished children with oedema), and during systemic shock. A longer equilibration time should, therefore, be allowed in these participants:

- Equilibration is the process whereby the deuterium oxide is evenly mixed throughout the body water. After equilibration, all body water compartments will contain the same concentration of deuterium.
- Equilibration between the enriched dose and body water is not instantaneous. Equilibration of body water with saliva is rapid but equilibration with urine, especially in elderly subjects with residual urine post voiding, can take a few hours. The question is how long the delay is before equilibration is complete.
- In healthy adults, equilibration is usually achieved after 2–5 h. Figures 2–4 show typical data from participants of different ages. In general, children have faster water turnover than adults, and elderly adults have slower water turnover than younger adults. Various disease states can also influence water turnover. It is, therefore, important to do a small pilot study to determine the required sampling time before starting the main study. Asking participants to collect three post-dose urine samples usually results in two samples at the plateau enrichment. The first sample is not usually at the plateau enrichment and is, therefore, not used in the calculation of TBW but in the calculation of urinary loss of deuterium.

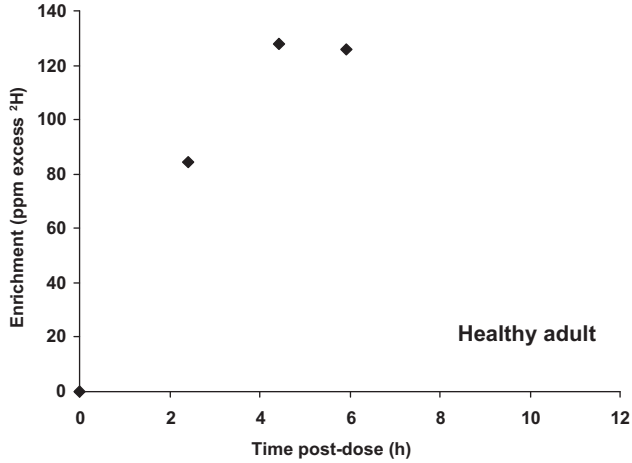

FIG. 3. Equilibration of deuterium oxide in urine in a healthy young adult.

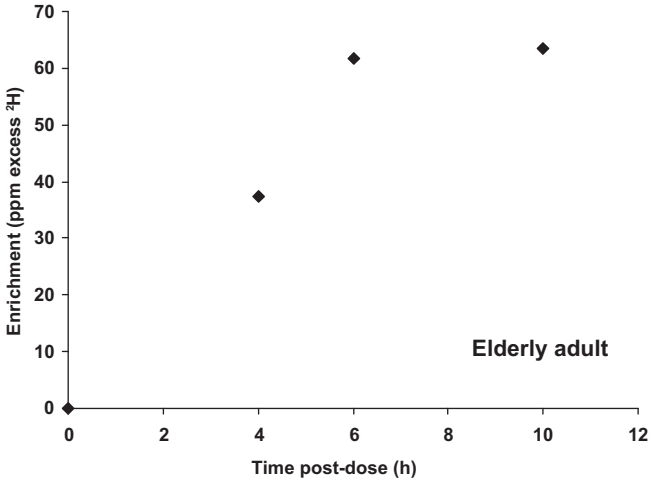

FIG. 4. Equilibration of deuterium oxide in urine in an elderly adult.

3.2.4. Assumption 4: Neither deuterium oxide nor body water is lost during the equilibration time

This is probably not true but precautions should be taken to minimize losses. Body water is not a simple closed system. Body water is a dynamic system with a variety of inputs (drink, food and metabolic water) and outputs (urine,

faeces, sweat, breath, etc.). In temperate climates, approximately 8% of body water is turned over in adults each day. Water turnover is 50–100% greater in tropical climates due to increased insensible water losses in the lungs and from the skin. When TBW is measured using the equilibration technique described here, participants can be asked to avoid physical activity during the equilibration period and, therefore, minimize insensible water loss. Loss of deuterium in urine is measured and the calculated TBW is adjusted to take account of this.

3.2.5. Assessment of TBW in infants

It is preferable to use the back-extrapolation procedure to assess TBW in infants [8]. Furthermore, the help of an expert should be sought if it is planned to assess TBW in infants, as special precautions must be taken to ensure that the dose is consumed correctly, and to avoid fractionation of urine.

3.3. HYDRATION OF FFM

The two compartment model of body composition divides the body into FM and FFM. Hydration of FFM refers to the proportion of water within the FFM. It is assumed that FFM contains 73.2% water in adults (aged 21 years and above).

FFM (kg) = TBW (kg)/0.732

Cellular hydration in all mammals is controlled within strict limits. The classic work of Pace and Rathbun [9] is the source of the commonly used hydration coefficient, 0.732. In vivo studies in adults indicate that there is no effect of ageing on the constant up to age 70 years [10, 11]. Factors that can result in individual variation in the hydration of FFM have been described by Wang et al. [12]. The hydration of FFM can vary by between 2 and 3% (standard deviation) in healthy adults [13, 14]. These values include both measurement errors and physiological variation. The true physiological variation in the hydration of FFM in healthy adults cannot be estimated without knowledge of the measurement errors. Schoeller estimates the average measurement error to be 1%. The within laboratory standard deviation in the measurement of in vivo hydration is estimated to be 1.1% and the physiological variation to be 0.5%, which is quite small [1].

3.3.1. Variation of FFM hydration during infancy and childhood

The adult hydration factor of 0.732 is not appropriate for use in children and infants. The hydration of lean tissue is known to vary as the body develops during infancy. Newborn infants have relatively little muscle mass in proportion to their body weight. As the proportion of muscle mass increases, the hydration of FFM decreases during childhood [15, 16]. Lohman provides hydration factors for children and adolescents (Table 1). In infants, the Fomon hydration factors (Table 2) are often applied to convert TBW to FFM [17]. Data on body composition in infants are also available from Butte et al. [18]. The hydration of FFM in infants was reviewed by Fomon and Nelson [19]. Corresponding data for prematurely born infants are lacking. Thus, until more information becomes available, any assessment of body fat in premature infants should use three or four compartment models of body composition [20].

3.3.2. Variation of FFM hydration during pregnancy and lactation

During pregnancy, the water content of FFM (the hydration coefficient) increases [21]. There is presently no consensus regarding the most appropriate hydration coefficients for different stages of pregnancy. The deuterium dilution technique is, therefore, not recommended for a two compartment model assessment of body composition in women in the second and third trimesters of pregnancy.

TABLE 1. HYDRATION OF FFM (%) IN CHILDREN AND ADOLESCENTS *(reproduced with permission from the author [16])*

Age (years)	Boys	Girls
1	79.0	78.8
1–2	78.6	78.5
3–4	77.8	78.3
5–6	77.0	78.0
7–8	76.8	77.6
9–10	76.2	77.0
11–12	75.4	76.6
13–14	74.7	75.5
15–16	74.2	75.0
17–20	73.8	74.5

TABLE 2. HYDRATION OF FFM (%) IN INFANTS *(reproduced with permission from the author [17])*

Age (months)	Boys	Girls
Birth	80.6	80.6
1	80.5	80.5
2	80.3	80.2
3	80.0	79.9
4	79.9	79.7
5	79.7	79.5
6	79.6	79.4
9	79.3	79.0
12	79.0	78.8
18	78.5	78.4
24	78.1	78.2

The conventional hydration coefficient, 0.732, is generally used in lactating women and women in the first trimester of pregnancy.

4. PROCEDURES

The following sections give detailed descriptions of the steps and procedures involved in the deuterium dilution method of estimating TBW.

These include:

— Planning the study;
— Preparation and storage of deuterium oxide doses;
— Anthropometric measurements of participants;
— Sampling urine and storage of specimens;
— Analysis of deuterium enrichment;
— Calculations.

Details of the analysis of the enrichment of deuterium in urine samples by IRMS are beyond the scope of this manual.

4.1. PLANNING THE STUDY

Careful planning is essential for a successful outcome in any study. The most important part of any study is to determine its purpose. The focus should be on one main issue. What is the hypothesis being tested?

— How many participants are required to address the issue? A sample size calculation should be performed. A biostatistician should be consulted for advice on sample size determination.
— How will the data be handled? What statistical tests will be performed? Expert advice should be sought at the planning stage, not after the data have been collected.
— What is the procedure for obtaining ethical approval?

4.1.1. Ethics

All studies involving human participants must be reviewed and approved by the local ethics committee. Most leading journals will not accept a paper for publication without a statement of its approval by the local ethics committee. The ethics committee is usually composed of medical doctors, scientists and lay people, including religious and community leaders, and an advocate or lawyer. It could be based at the Ministry of Health, Ministry of Science or the local university. The committee should be contacted at an early stage to determine the process for seeking ethical approval and to obtain copies of the required documentation.

Participants must be informed of the purpose of the study in language appropriate to the local situation. Participants must give their voluntary informed consent to take part, and be informed that they are free to withdraw at any time during the study.

The following is an example of the kind of information required by the ethics committee but the details will vary, depending on local circumstances:

— The purpose of the proposed study must be clearly stated;
— A summary of the study design and methodology, including details of the proposed sample size, giving indications of the calculations used to determine the required sample size;
— An outline of the ethical considerations involved in the proposal;

— Details of how consent is to be obtained, including an information sheet written in simple non-technical language;
— Who will have access to the data and what measures will be adopted to maintain confidentiality of the participants;
— Who the investigators are (including assistants) that will conduct the study, and their qualifications and experience;
— Location(s) where the project will be carried out;
— Proposed start date, proposed completion date.

Appendix I contains information about the safety of deuterium oxide, which may be useful in the preparation of applications for ethical approval.

4.1.2. Preparation of participant data sheet

Data from each participant should be recorded in a suitable manner. A low cost way is to keep paper records in the field, which can be transferred to an electronic spreadsheet later. The minimum information required is shown in Table 3, but additional information specific to the study will also be required, such as information on the participant's health. The information sheet should be designed at the planning stage, so that it can be evaluated, and if necessary amended during the pilot study. Information sheets can be designed using a word processor or a spreadsheet and reproduced as many times as necessary. An example of a participant data sheet is included in Appendix II.

4.1.3. Pilot study

If this technique has not been used before, it is advisable to do a pilot study before starting.

A pilot study is important to:

— Practise and test the procedures, including sampling, analysis of samples and data handling;
— Train all people involved;
— Develop routine and team work;
— Develop strategies to overcome practical difficulties.

TABLE 3. MINIMUM INFORMATION REQUIRED FOR A PARTICIPANT DATA SHEET

Project name/code		
Date		
Name/initials of researcher		
Participant's data		
Participant's study ID		
Participant's weight (kg)		
Dose number		
Dose weight (g)		
Time dose taken		
Volume of water consumed (L)		
Specimen data		
	Time	Volume
Baseline urine sample		
1st post-dose urine sample		
2nd post-dose urine sample		
3rd post-dose urine sample		

A pilot study is usually conducted with a relatively small number of participants. It can be used to check the equilibration time in the particular circumstances encountered in the study [22, 23]. Water turnover is affected by the physiological condition of the participants. Age, health status and climate all affect water turnover.

4.1.4. Sample size calculation

In any study, it must be ensured that an appropriate number of participants are included to be able to obtain a reliable answer to the question being asked. Sample size or power calculations are an important component of any study design, and are required by ethical review committees and funding bodies. A power calculation can be used to determine the sample size necessary to obtain a

reliable answer. A biostatistician should be consulted for advice on sample size determination. Power calculations can be performed using statistical software.

To calculate the required sample size, it is necessary to know the standard deviation (SD) of body composition parameters in a population similar to that being studied, and to define the difference between study groups that will be regarded as significant (δ). Before visiting the biostatistician, a literature search should be performed to obtain this information. The number of participants required in a public health situation, where measurements are conducted at field centres, will be more than for measurements conducted under carefully controlled conditions in a research laboratory.

The power of a study is usually expressed as the percentage of time that a study will detect a significant result when there is a true difference. A power of 80% is usually selected, which means that if there is a true difference and a study was performed 100 times, 80 would detect a statistically significant result and 20 would not (these 20 would detect a false negative result). The significance level (α) is fixed at some low value (usually 0.05). This is the probability of a false positive result.

4.1.4.1. Example

If the effect of a nutritional intervention on body composition of adults living with HIV is to be studied, the SD of TBW in the population and the magnitude of change that would be clinically important (δ) need to be known. In a recent study in Africa, the between subject SD (σ) of TBW in 150 HIV sero-positive adults was 5 kg (personal communication). The mean body weight of participants was approximately 60 kg. An increase of FFM equivalent to 5% body weight might be considered clinically important. An FFM of 3 kg is equal to 2.2 kg of TBW (3×0.732). Therefore, if it is assumed that $\sigma = 5$ kg and $\delta = 2.2$ kg of TBW, with a power of 80%, a significance level of 0.05 and two study groups (control and intervention), the required sample size (n) can be calculated using the following equation:

$$n = 2 \times 7.85 \times \left(\frac{\sigma}{\delta} \right)^2$$

where 7.85 is the multiplication factor $f(\alpha, \text{power})$ for a power of 80% and $\alpha = 0.05$ obtained from statistical tables. Therefore:

$$n = 2 \times 7.85 \times \left(\frac{5}{2.2} \right)^2 = 81$$

At least 81 participants in each group are required to achieve statistically significant results. If more power or a higher level of significance is required, then more participants will be required for the study. It is also sensible to add a factor to allow for drop-outs based on local experience. If a 25% attrition rate is assumed, then about 110 participants would need to be recruited in each group.

4.2. PREPARATION AND STORAGE OF DEUTERIUM OXIDE DOSES

The usual dose of deuterium oxide for estimating TBW is 0.05 g of D_2O per kg body weight, when samples are analysed by IRMS. This will result in an enrichment in body water of approximately 100 ppm excess ^2H (0.01 at.% excess ^2H). In large studies, it is easier to give all participants the same dose. A standard dose can be prepared based on the average weight of the participants, or several standardized doses for studies involving participants of different ages and body weight. Suggested doses are given in Table 4. Doses should be prepared in a clean area, e.g. a food preparation area. It is not good practice to prepare the doses for human consumption in a chemistry laboratory because the balance may have previously been used to weigh toxic compounds.

It is preferable to make up doses in batches from a single bottle of deuterium oxide, as it is necessary to analyse a dilution of the dose along with the urine samples. A 1 in 10 dilution of the highly enriched D_2O is prepared in a large glass bottle and mixed. This is then dispensed into leak proof bottles containing the required amount of deuterium oxide, depending on the weight of the participants (see Table 4). Doses must be accurately weighed by laboratory trained personnel.

TABLE 4. RECOMMENDED DOSES FOR PARTICIPANTS OF DIFFERENT BODY WEIGHTS

Category	Body weight range (kg)	Dose D_2O (g)
Adult	>70	6
Adolescent	35–70	3
Child	10–35	1
Infant	<10	0.3

4.2.1. Equipment

All equipment used for preparing doses must be completely dry to avoid contamination with water.

A large glass bottle (such as a 5 L borosilicate glass with a PTFE lined screw cap) is required to make a 1 in 10 dilution of the highly enriched deuterium oxide. The balance used to weigh the stock solution should be able to weigh up to 10 kg with an accuracy of 0.1 g. The dose must be weighed to four significant places at each stage, e.g. 300.1 g, 55.05 g, 6.116 g.

Dose bottles must be screw capped and leak proof (e.g. 120 mL wide mouth, leak proof, autoclavable, polypropylene bottles) to avoid losses during storage and contamination by moisture from the atmosphere. It is not necessary to autoclave the bottles but these bottles will not crack or leak if stored in a freezer.

The balance used to weigh the dose must have a weighing range that is adequate for the amount *and* the container to be weighed. A balance capable of weighing to 0.01 g is recommended.

An equipment list is included in Appendix III.

4.2.2. Procedure

4.2.2.1. Making up the stock dilution

A 1 in 10 dilution of the D_2O is prepared in a large glass bottle using local drinking water. A clean 5 L capacity screw cap bottle is required. A new borosilicate reagent bottle that has a screw cap with a PTFE facing disc would be suitable. A bottle that has previously been used to store chemical reagents should not be used. A balance that can weigh 10 kg to 0.1 g is required.

If the target is to make 50 adult doses each containing 6 g of D_2O, then 300 g of D_2O should be diluted with 2.4 L of local drinking water. Note that the density of deuterium oxide (^2H_2O) is 1.105 g/mL at 25ºC. A quantity of 300 g of ^2H_2O has a volume of 271.5 mL. The density of water (H_2O) at 25ºC is 1.000 g/mL, therefore 2.4 L weigh 2.4 kg.

A record should be kept in a laboratory notebook of the following: the batch number of the stock solution of deuterium oxide used to make the doses, the date the doses were prepared, the dose number, the weight of the bottles, and the weight of the bottle plus water at each stage. This information can be transferred to a spreadsheet later, and the exact amount of D_2O in each dose bottle calculated.

The lid should be kept on the bottle during weighing to avoid loss by evaporation.

If an electronic balance is used:

— The bottle plus the lid should be tared. If not, the bottle plus the lid should be weighed.
— 300 g (approximately 270 mL) of D_2O should be added to the bottle and the lid replaced.
— The weight of deuterium oxide in the bottle should be recorded (A, approximately 300 g).
— 2.4 L of local drinking water should be added to the bottle and the lid replaced. The weight of the deuterium oxide plus drinking water should be recorded (B, approximately 2.7 kg).

Some of the local drinking water should be kept (500 mL) to make a dilution of the dose for analysis with the urine specimens (see Section 4.2.4).

The procedure is summarized in Fig. 5.

4.2.2.2. Dispensing individual doses

If an electronic scale is used:

— The dose bottle with the lid on should be tared (to 0.01 g).
— 55 mL (or the required volume, Table 5) of the stock D_2O dilution should be added to the bottle using a measuring cylinder and the lid replaced.
— The exact weight should be recorded (C) (to 0.01 g).
— The weight of D_2O will not be exactly 55 g, as the density of deuterium oxide is greater than the density of water (the density of D_2O at 25ºC is 1.105 g/mL and the density of H_2O 1.000 g/mL). This does not matter as long as the exact weight is recorded and used in subsequent calculations.
— The amount of D_2O in each dose can be calculated by proportion:

Weight of D_2O in the dose (D) = $C \times A/B$ (g)

The procedure for dispensing individual doses is illustrated in Figs 6 and 7.

Example calculation (adult dose):

Weight of D_2O	= 300.1 g (A)
Weight of D_2O plus drinking water	= 2701.4 g (B)
Weight of diluted D_2O in the dose bottle	= 55.05 g (C)
Weight of D_2O in the dose bottle (D)	= $C \times A/B$
	= $55.05 \times 300.1/2701.4$ g
	= 6.116 g

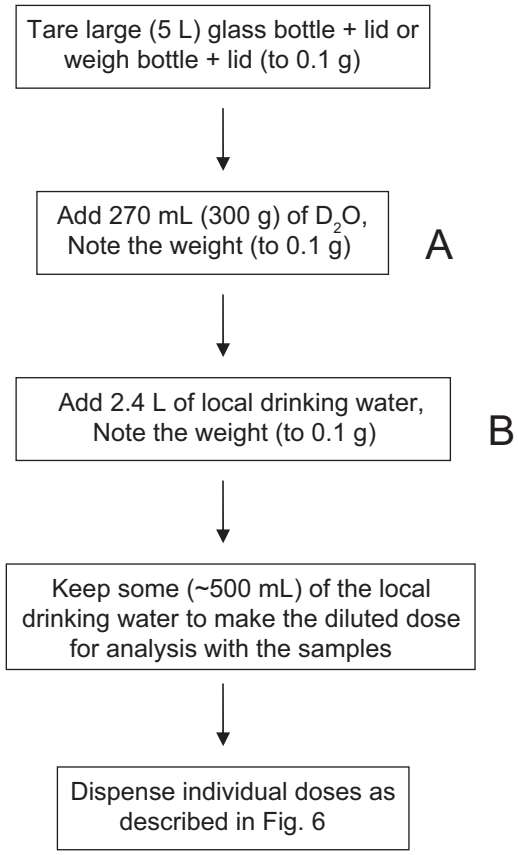

FIG. 5. Preparation of a 1 in 10 dilution of deuterium oxide.

TABLE 5. RECOMMENDED DOSES (1 IN 10 DILUTION) FOR PARTICIPANTS OF DIFFERENT BODY WEIGHTS

Category	Body weight range (kg)	Dose D_2O (g)	Required volume of 1 in 10 dilution (mL)
Adult	>70	6	55
Adolescent	35–70	3	27
Child	10–35	1	9
Infant	<10	0.3	2.7

FIG. 6. Dose preparation — doses should be prepared in a food preparation area, not a laboratory.

A few mL (5 mL) of the dose water (1 in 10 dilution of the D_2O stock solution) should be kept in a small air tight container to make a diluted dose for analysis with the urine specimens (see Section 4.2.4).

4.2.3. Dose storage and transport

The doses can be made in batches and stored in a refrigerator until required.

To ensure good hygiene and avoid cross-contamination, doses should not be stored in the same place as urine specimens. Urine specimens will have a deuterium enrichment of approximately 0.01 at.% excess ^2H (100 ppm excess ^2H), whereas the doses are enriched to approximately 10 at.% excess (100 000 ppm excess ^2H). The dose, therefore, contains 1000 times as much

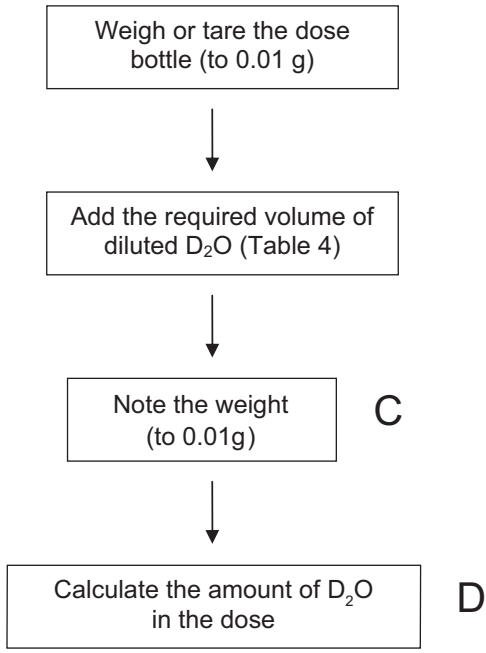

FIG. 7. Preparation of individual doses.

deuterium as the biological specimens. In addition, doses should not be stored with body water specimens to avoid microbial cross-contamination.

When transporting doses to and from the field, separate boxes for doses and specimens should be used.

4.2.4. Making up the diluted dose for analysis

It is important to analyse the enrichment of ^2H in the dose water, as well as in the body water specimens. A small volume (4–5 mL) from each dose batch should be retained for analysis with the body water samples. It is necessary to make a dilution (1:500–1:1000) of this so that the enrichment is similar to that expected in the post-dose body water specimen. Note that if individual doses are prepared for each study participant, then an aliquot (1–2 mL) of each dose must be retained. If the dose is made up in batches, then it is only necessary to analyse an aliquot from each batch. Knowledge of the dose enrichment as well as the dose weight is required for calculation of TBW (see Section 6).

The diluted dose is prepared by blending 0.1 g of the dose (weighed to four decimal places) with 100 g of local drinking water (weighed to four decimal places). To avoid losses by evaporation when weighing small volumes (100 μL), the following procedure is recommended:

(1) A 100 mL volumetric flask with its stopper should be tared or weighed on an analytical balance capable of weighing to four decimal places (0.0001 g) should be used.
(2) Approximately 50 mL of local drinking water should be added. The stopper should be replaced and the weight noted.
(3) 100 μL of the dose water should be added using an automatic pipette. The stopper should be replaced and the weight noted.
(4) The volumetric flask should be filled to the 100 mL mark with local drinking water, the cap replaced and the flask weighed again.
(5) The weight of dose added = 'a' g (i.e. weight at Step 3 minus weight at Step 2).
(6) The total weight of water added = 'W' g (i.e. weight at Step 4 minus weight of dose, 'a' (calculated above) minus the weight of the flask and its stopper, if the balance was not tared to zero).

Example calculation (assuming the balance was tared at Step 1):

Weight of drinking water added to the empty flask = 49.7326 g
Weight of drinking water plus 100 μL of the dose water = 49.8339 g
Weight of dose added (a) = 49.8339 – 49.7326 g = 0.1013 g
Total weight of drinking water plus dose at Step 4 = 99.5187 g
Weight of drinking water (W) = 99.5157 – 0.1013 g = 99.4174 g

This dilution will produce an enrichment similar to the target in body water (0.01 at.% excess ^{2}H or 100 ppm excess ^{2}H).

Aliquots (4–5 mL) of the diluted dose and the drinking water used to make this dilution can be stored in cryovials with screw caps in a freezer (–20°C) with the urine specimens until it is time for them to be analysed.

4.3. PROCEDURE FOR MEASURING TBW

The procedure for measuring TBW by deuterium dilution is summarized in Fig. 8.

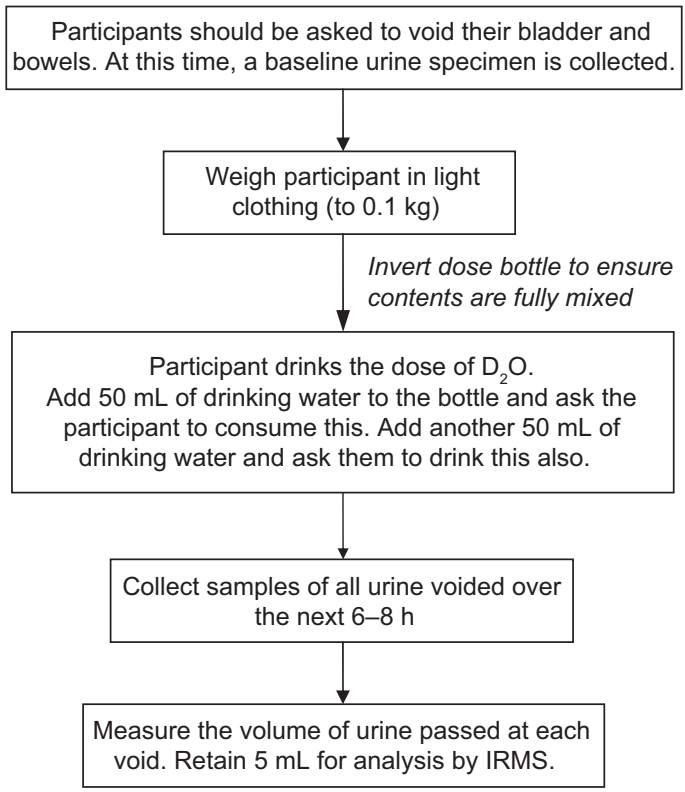

FIG. 8. Flow chart describing the procedure for measuring TBW by deuterium dilution.

The participant should have normal fluid and food intake on the day before the TBW estimation and avoid vigorous exercise after the final meal of the previous day to avoid dehydration and depletion of glycogen stores.

For accurate measurements of TBW, participants should be asked to empty their bladder before starting. This will ensure that body weight is measured under the same conditions each time in longitudinal studies, and that water in urine is not included in TBW.

4.3.1. Anthropometric measurements

An accurate measure of body weight is required because body fat is estimated by the difference of FFM from body weight. Participants should be asked to empty their bladder (and, if possible, bowels) before being weighed, and should be weighed in light clothing. Standardizing conditions in this way is particularly important in longitudinal studies. The accuracy of scales used for

FIG. 9. Measuring weight — weight is measured in light clothing without shoes.

measuring body weight should be checked daily using a calibration weight of known mass. Much care is taken to ensure the accuracy and precision of isotopic data. Body composition results will be compromised if equal care is not taken to ensure the accuracy of anthropometric measurements.

4.3.1.1. Measuring weight and height in adults and children

Measuring weight

— The participant's weight must be measured to the nearest 0.1 kg using electronic scales or any balance with adequate precision.
— The balance must be placed on a level surface. This should be checked using a spirit level, if possible.
— Participants should wear minimal clothing and no shoes (Fig. 9). If they do not wish to wear minimal clothing during the weighing procedure, their clothes could be weighed separately afterwards, and the weight of their clothes subtracted to obtain an accurate measure of body weight.
— The weight should be recorded on the participant's information sheet to 0.1 kg.
— In longitudinal studies measuring changes in body composition over relatively short periods of time, an accurate measurement of body weight is essential. Account must be taken of the weight of any clothing worn during the measurement.

FIG. 10. Measuring height — hair down, eyes forward.

— The accuracy of the scales should be checked daily using a calibration weight of known mass.

Measuring height

— Height must be measured to the nearest 0.1 cm using a stadiometer.
— The stadiometer must be placed on a level surface, which should be checked using a spirit level, if possible. The accuracy of the stadiometer should be checked periodically using measure rods of known length.
— Height is measured without shoes.
— The participant should stand upright with their heels to the wall or touching the vertical post on the stadiometer. Their knees should be straight.
— The participant should be asked to look straight ahead. It should be ensured that their eyes are at the same level as their ears (Fig. 10).
— The beam should be lowered until it just touches the top of the head. Elaborate hair arrangements must be undone. The height should be recorded in centimetres (to the nearest 0.1 cm) on the participant's information sheet. The measurement should be repeated. Both measurements should be recorded and the mean calculated.

In children, height is measured above 85 cm and length is measured below 85 cm (see next section). The World Health Organization has developed detailed instructions on how to measure a child's weight and length/height. The materials can be downloaded from the following web site: http://www.who.int/childgrowth/training/en/index.html**.**

FIG. 11. Measuring the infant's weight.

4.3.1.2. Measuring weight and length in infants

Measuring weight in infants

- Infants must be weighed without clothes using scales accurate to 0.01 kg (Fig. 11).
- A cloth should be left in the weighing pan to prevent chilling the child.
- The scales should be adjusted to zero with the cloth in the pan.
- The naked child should be gently placed on the cloth in the weighing pan.
- It is necessary to wait for the child to settle and the weight to stabilize.
- The weight should be measured (to the nearest 10 g, 0.01 kg) and recorded immediately.

Standardization (levelling) of the scales should be performed weekly or whenever the scales are moved.

Checking the scales

Known weights of 3, 5 and 10 kg, and if appropriate of 20 kg, should be weighed. If calibration weights are not available, sealed bottles containing water can be used. These must have been accurately weighed on a calibrated balance, and the weight should be checked periodically.

To check tared weighing, a 20 kg weight should be weighed, the scale tared and then a 3 kg weight should be added. The scales should indicate the 3 kg weight. If the weights are not accurate, the scales should be calibrated if possible. If it is not possible to recalibrate, the scales need to be replaced.

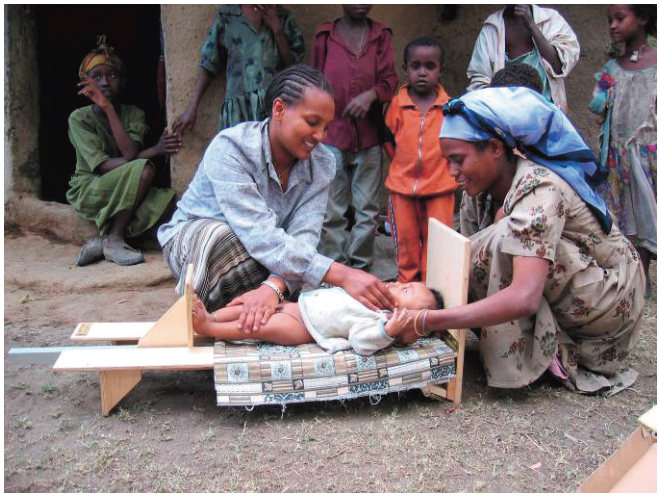

FIG. 12. Measuring the infant's length.

Measuring length in infants

The infant's length is measured using a measuring board (sometimes called an 'infantometer'). Two people are needed to measure the child's length (Fig. 12).

One person should:

— Assist in positioning the child face-up on the measuring board, supporting the head and placing it against the headboard.
— Position the crown of the head against the headboard, compressing the hair. Check that the child lies straight along the centre line of the board and does not change position. Shoulders should touch the board, and the spine should not be arched.

Usually this person stands or kneels behind the headboard.

The second person should:

— Support the trunk as the child is positioned on the board.
— Lay the child flat along the board.
— Place one hand on the shins above the ankles or on the knees and press down firmly. With the other hand, the footplate should be placed firmly against the heels. It should be ensured that the toes do not prevent the footplate coming into contact with the heels.
— Measure the length (to the nearest 0.1 cm) and record it immediately.

The measuring board should be kept clean and stored at normal indoor temperature, protected from humidity. The board should be checked for accuracy every week.

4.3.2. Dose administration

In adults and children, the dose should be consumed at least 2 h after the last meal, preferably after an overnight fast. If this is not possible, a small meal may be given 1 h after the dose was taken. The meal should be simple and less than 1250 kJ (300 kcal). This allows the dose to empty from the stomach before the meal, but water in the meal to equilibrate with body water before the post-dose urine samples are collected. In infants, the dose is usually given with a meal. In breastfed babies, a disposable syringe can be used to administer the dose immediately before feeding. If a disposable syringe is used, the dose should be determined accurately by weighing the syringe while full and again after the dose has been given. In bottle fed babies, the deuterium oxide can be given in the milk. If the dose is not consumed completely, the infant cannot be included in the study. It is recommended that advice is sought from someone with previous experience of measuring TBW in babies:

— Baseline urine specimens must be obtained before the dose is consumed.
— If the dose has been frozen, it should be completely thawed before use.
— Whether stored in a fridge or thawed after freezing, the bottle should be inverted several times to mix any condensation on the cap into the bulk of the liquid. This should be done immediately before the dose is consumed. This is because the condensation is fractionated relative to the bulk liquid (see Appendix IV for more information on fractionation).
— The bottle should not be opened until it is time for the dose to be consumed.

When talking to participants, it is often better to use the term ‘heavy water’ or ‘special water’ rather than ‘deuterium-labelled water’ or ‘stable isotope labelled water’, as there can be confusion over the word ‘isotope’, which is often associated with radioactivity.

There is no radiation hazard associated with the use of deuterium oxide.

(1) The bottle number and the time the dose was taken should be noted on the participant’s data sheet.
(2) The participants should drink the dose through a straw to avoid spillage (Fig. 13).

FIG. 13. Dose administration. The participant should drink the dose through a straw to avoid spillage.

(3) About 50 mL of drinking water should be added to the dose bottle and the participants asked to drink this through the same straw. This should be repeated with another 50 mL of drinking water. This will ensure that no labelled water is left in the bottle.

4.3.3. Food, drink and physical activity during the equilibration period

It is not necessary for participants to fast during the equilibration period. A small meal may be given 1 h after the dose was taken. A note of the volume of all drinks taken during the equilibration period, including the 100 mL used to rinse the dose bottle, should be kept. This volume should be subtracted from the calculated TBW. If no additional fluid is consumed during the equilibration period, the 100 mL of water used to rinse the dose water is usually ignored.

Participants should avoid physical activity during the equilibration period to minimize water loss in breath and evaporation from the skin (insensible water loss). There is less deuterium in water vapour than in body water due to isotopic fractionation. An increase in insensible water loss will, therefore, lead to an error in the calculation of TBW.

4.3.4. Urine sampling

It should be ensured that the following items are available before starting. All equipment must be clean and dry before use.

Urine collection device:

— In male participants, urine can be collected in a dry graduated 1 L polyethylene measuring cylinder. The volume is noted and an aliquot (4–5 mL) retained for analysis. The remainder can be discarded.
— Alternatively, a dry polyethylene jug or plastic toilet hat can be used. The urine is then transferred to a measuring cylinder and the volume noted, as above.
— Cotton wool swabs placed inside a nappy (diaper) can be used to collect urine samples from infants.

Specimen storage vials:

— Must be screw capped with a seal to prevent losses, fractionation and cross-contamination during storage, e.g. 4 mL cryovials. It is a good idea to use different coloured caps for baseline and post-dose samples, e.g. blue caps for baseline samples and red caps for post-dose samples.
— Must be completely dry before use.
— Must not be reused to prevent cross-contamination between enriched (post-dose) samples and unenriched (baseline) samples.
— Should be labelled with the participant's identification number, date and time the sample was taken. Names should not be written on sample bottles to preserve confidentiality.

Gloves:

— New disposable gloves must be worn when handling urine specimens.

Zip-lock bags:

— Two small zip-lock bags are needed for each participant:
  • One for the baseline specimen;
  • One for the post-dose specimens.
— Another zip-lock bag is needed to keep all the specimens from each participant together.
— All bags must be labelled permanently with the participant's identification number.

Labels:

— It should be ensured that labels are of good quality and cannot come off the containers/vials.
— A permanent marker should be used to write on the labels, to avoid the writing being smudged or removed, in particular when the samples are thawed.

Participant data sheets:

— Print outs of data sheets for each participant need to be available before the first sampling (baseline).
— To preserve confidentiality, names should not be written on the data sheets. The names and corresponding participant identification numbers must be recorded separately.
— An example of a participant data sheet is shown in Appendix II.

4.3.4.1. Sampling times

A baseline urine sample must be collected before the dose is consumed.

A specimen from all urine voided for 6–8 h after the dose should be taken and retained. Children have faster water turnover than adults, so 5–6 h of urine sampling will probably be sufficient. The equilibration time will be longer in elderly participants and those with expanded extracellular water volume, such as malnourished children. In these participants, a longer sampling time (up to 8 h) will be required. It is recommended that three post-dose urine samples be collected to confirm that the dose has fully equilibrated with body water and a plateau in body water enrichment has been reached (see Figs 2–4). The optimum equilibration time can be determined in a pilot study in participants of the same age and health status as is planned for the main study. In studies involving elderly participants, the dose can be given in the evening 1 or 2 h before bedtime, with urine sampling the following morning before breakfast, resulting in a 10 h equilibration time. The participant should void between the time of the dose and bedtime to improve equilibration. Urine voided during the night can be discarded to minimize the burden for participants.

4.3.4.2. Sampling procedures

The volume of urine passed at each void should be noted and an aliquot (4–5 mL) retained for analysis. The remainder can be discarded. It is

recommended that two aliquots are stored. One can be sent for analysis and the other kept in case the first one does not reach the analytical laboratory.

Male participants can urinate directly into a dry graduated 1 L polyethylene measuring cylinder. Alternatively, a dry polyethylene jug or plastic toilet hat can be used. The urine is then transferred to a measuring cylinder and the volume noted.

Cotton wool balls that have been stored in a dry low humidity container, placed inside a nappy (cloth diaper), can be used to collect urine samples from infants. The cotton wool ball is placed in the body of a new 20 mL disposable syringe and the urine is squeezed into a storage vial using the plunger. In infants, it is difficult to estimate the volume of urine.

The participant's identification number, date and time the sample was taken should be recorded on each vial. All dates and times of urine collection should be recorded on the participant's data sheet. This information should be copied to a spreadsheet as soon as possible.

Storage vials or syringes should not be reused.

4.3.4.3. Storage and transport of urine samples

A large study will generate hundreds of samples; therefore, careful management and labelling of specimens is essential. The procedure below should be followed:

- Containers must be firmly closed to prevent loss of water by evaporation, and cross-contamination between samples.
- Containers should not be filled beyond 90% of capacity to allow for expansion in case they are frozen at any time before analysis.
- Zip-lock bags can be used to keep all specimens for a single person together and prevent cross-contamination between persons. A small bag should be used for the baseline sample and another small bag for the post-dose samples. The two bags should then be placed in a third larger one, so that the samples from a single participant are kept together.
- The participant's identification number should be written on both the sample vials and the zip-lock bags.
- A log of samples should be kept in a spreadsheet.

Urine specimens should be stored frozen (–20ºC) until analysis. If this is not possible, samples should be stored in a refrigerator at 4°C or in a cool box until they can be transferred to a freezer.

To avoid contamination of samples:

— Samples and doses should never be stored together.
— It should always be ensured that the cap of the specimen vial is tightly closed to avoid losses by evaporation and contamination by moisture from the atmosphere.

4.3.4.4. Transport of specimens to the analytical laboratory

It is not necessary for urine specimens to be kept frozen during transport to the analytical facility. Specimens must be packaged in accordance with the IATA 650 Packaging Instruction. UN3373 Biological Substance Category B. Details can be found at: http://www.iata.org/NR/rdonlyres/C993126E-9AAF-4498-B76E-583B3D774F90/0/DGR_48_PI650.pdf

Briefly, specimens must be in primary containers (e.g. cryovials and zip-lock bags), which are packed inside a secondary container and then in rigid outer packaging. Primary containers must be leak proof and packed inside a secondary container in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary container. The secondary container must also be leak proof. Absorbent material must be placed between the primary and secondary containers. The absorbent material (e.g. cotton wool) must be in sufficient quantity to absorb the entire contents of the primary containers. Either the primary or the secondary container must be capable of withstanding, without leakage, an internal pressure of 95 kPa in the range of –40°C to 55°C (–40°F to 130°F). The outer packaging must not contain more than 4 L. The outer container must be labelled “Biological Substance — Category B packed in accordance with IATA 650.UN3373”.

5. ANALYSIS OF DEUTERIUM ENRICHMENT

Deuterium enrichment is measured using an isotope ratio mass spectrometer. An isotope ratio mass spectrometer is an expensive instrument and requires considerable expertise to operate and maintain, and therefore samples are usually sent to a central location.

A mass spectrometer is an instrument that separates ions in a high vacuum according to their mass to charge ratio (m/z). The major components of a mass spectrometer are the inlet system, high vacuum, ion source, mass analyser and

detector. Modern mass spectrometers are computer controlled and have sophisticated data processing software. A typical isotope ratio mass spectrometer suitable for analysis of deuterium in urine samples is shown in Fig. 14.

The abundance of the stable isotopes of C, H, O and N are measured in simple gases (CO_2, H_2, N_2) using IRMS with individual detectors for each isotope. The mass spectrometer is on the table to the left of the operator in Fig. 14. The operator is loading samples (in Exetainer gas sampling vials) onto the autosampler.

In an isotope ratio mass spectrometer, sample gas is ionized by electron impact from electrons emitted from a hot filament within a high vacuum. The ions are separated in a magnetic field. The current of each ion beam is measured as the charge generated by ions impacting a detector for each isotopic species. The detectors are known as Faraday Cups. Each sample is compared with a reference gas of known composition. IRMS can accurately measure very low enrichments, down to natural abundance.

In hydrogen gas, two species are present: unlabelled hydrogen, $^1H_2$ with m/z = 2 and hydrogen gas containing deuterium (HD or $^1H^2H$) with m/z = 3. The abundance or concentration of ^2H measured by IRMS is often expressed as parts per million (ppm) ^2H [24].

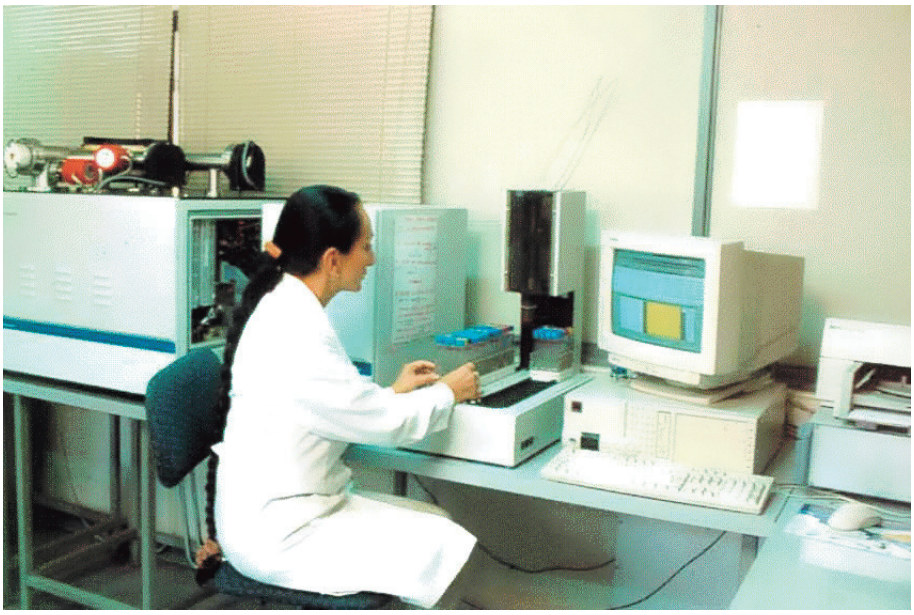

FIG. 14. Continuous flow isotope ratio mass spectrometer.

$$\text{ppm } {}^2\text{H} = \frac{([{}^1\text{H}\,{}^2\text{H}]/2) + [{}^2\text{H}\,{}^2\text{H}]}{[{}^1\text{H}\,{}^1\text{H}] + [{}^1\text{H}\,{}^2\text{H}] + [{}^2\text{H}\,{}^2\text{H}]} \times 10^6$$

This is the ratio of moles of $^1H^2H$ divided by moles of $^1H^2H$ plus moles of $^1H_2$ multiplied by a million. The term $[^2H^2H]$ is usually negligible and can be omitted. Note that these mol/mol ppm are not the same as mg/kg ppm, often used to express concentration. The two units are not equivalent.

Enrichment is the amount of deuterium present above the natural abundance, i.e. the abundance of ^2H in post-dose specimens minus the abundance in the baseline specimen. The unit of enrichment is ppm excess ^2H.

Note that the units of enrichment in IRMS are at.% excess ^2H, also sometimes reported as ppm excess ^2H. These parts per millions are a molar fraction, ppm (mol/mol) and not a weight ratio (mg/kg). It is important not to confuse IRMS ppm excess units with ppm (mg/kg) units reported by FTIR instruments. The two kinds of ppm are not the same and are not interchangeable. This affects the calculation of the pool space. When calculating TBW using IRMS data, the weight of deuterium oxide consumed is converted to moles. TBW will then be in moles and must be converted to kg. It is important to ensure that spreadsheets for calculating TBW contain the correct calculations, depending on the method used to analyse deuterium enrichment.

6. CALCULATIONS

6.1. OUTPUTS FROM BODY COMPOSITION CALCULATIONS

The enrichment of the deuterium oxide in the body water of a participant can be used to calculate the participant's:

— TBW;
— FFM;
— Body fat.

These data can be used to assess the nutritional status of the participant.

6.2. CALCULATION OF TBW

The following information is required to calculate TBW (see also Sections 4.2.2.2 and 4.2.4):

(a) W = Total weight of water added when making the dose dilution (g).
(b) A = Weight of dose taken by the participant (g).
(c) a = Weight of dose in diluted dose (g).
(d) ΔDD = enrichment of ^{2}H in the diluted dose (ppm excess ^{2}H), i.e. the abundance of ^{2}H in the diluted dose (ppm ^{2}H) minus the abundance of ^{2}H in the tap water used to make the dilution (ppm ^{2}H).
(e) ΔBW = enrichment of ^{2}H in body water (ppm excess ^{2}H), i.e. the abundance of ^{2}H in the post-dose urine specimen (ppm ^{2}H) minus the abundance of ^{2}H in the baseline urine specimen (ppm ^{2}H).
(f) Cumulative loss of ^{2}H in urine (this is multiplied by two to estimate the total sensible and insensible loss of body water; if a study specific factor is available, this should be used). Units are either moles (g/18.0153) or kg to match the units of N_D.

In addition, the participant's body weight is required to estimate their body composition from the calculated TBW.

TBW is calculated from the dilution space of ^{2}H (N_D). It is important to remember that the output from the IRMS is a molar ratio. The dilution space is, therefore, in moles of water and must be converted to kg before body composition can be estimated.

$$N_D\,(\text{moles}) = \frac{WA}{18.0153a} \times \frac{\Delta DD}{\Delta BW} - (2 \times \text{cumulative urine loss})$$

$$N_D\,(\text{kg}) = \frac{WA}{18.0153a} \times \frac{\Delta DD}{\Delta BW} \times \frac{18.0153}{1000} - (2 \times \text{cumulative urine loss})$$

which can be simplified to

$$N_D\,(\text{kg}) = \frac{WA}{a} \times \frac{\Delta DD}{\Delta BW} \times \frac{1}{1000} - (2 \times \text{cumulative urine loss})$$

where

W = total weight of water added when making the dose dilution (g);
A = weight of dose taken by the participant (g);
18.0153 = the molecular weight of water
a = weight of dose in diluted dose (g);
ΔDD = enrichment of ^2H in the diluted dose (ppm excess ^2H);
ΔBW = enrichment of ^2H in body water (ppm excess ^2H).

The dilution space of ^2H is 4.1% higher than TBW due to non-aqueous exchange of hydrogen atoms in the body (see Section 3.2.1).

$$\text{TBW (kg)} \frac{\text{WA}}{\text{a}} \times \frac{\Delta\text{DD}}{\Delta\text{BW}} \times \frac{1}{1000 \times 1.041} - (2 \times \text{cumulative urine loss})$$

The following is the above equation in a format suitable for a spreadsheet:

$$\text{TBW (kg)} = ((\text{W} \times \text{A/a}) \times (\Delta\text{DD}/\Delta\text{BW})/(1000 \times 1.041)) - (2 \times \text{cumulative urine loss})$$

6.3. EXAMPLE CALCULATIONS

Table 6 shows example data and intermediate calculations from two adults with a similar body mass index (BMI), but different body composition. An example of data from a child is also shown.

6.3.1. Calculation of cumulative urine loss

Cumulative urine loss is simply the total volume of urine passed from the time the dose was taken up to and including the volume of the current specimen as shown in Table 7.

Loss of water from the body in breath, sweat and faeces is accounted for by multiplying urinary loss by 2.

6.3.2. Calculation of TBW using data from Tables 6 and 7

$$\text{TBW (kg)} = ((\text{W} \times \text{A/a}) \times (\Delta\text{DD}/\Delta\text{BW})/(1000 \times 1.041)) - (2 \times \text{cumulative urine loss})$$

TABLE 6. EXAMPLE DATA SETS

	Adult 1	Adult 2	Child
Study ID	A001	A002	Child1
Study date	15 Aug. 2007	15 Aug. 2007	15 Aug. 2007
Date of birth	9 Jan. 1957	5 Apr. 1945	21 Jul. 1994
Age (years)	50	62	13
Sex	Male	Female	Male
Body weight (kg)	80.0	47.4	50.5
Height (cm)	183.0	141.5	158.7
BMI (kg/m^2)	23.9	23.7	20.1
Weight of dose consumed (g), A	39.9846	27.898	32.039
Time dose was consumed	07:00	10:10	12:26
Time of first post-dose specimen	09:10	12:10	13:35
Volume of first post-dose specimen (mL)	135	50	240
Time of second post-dose specimen	10:05	16:20	16:30
Volume of second post-dose specimen (mL)	60	100	105
Time of third post-dose specimen	11:15	17:30	17:30
Volume of third post-dose specimen (mL)	70	45	100
Weight of dose in diluted dose, DD (g), a	0.1032	0.10130	0.1717
Weight of water in DD (g), W	49.7426	99.41736	54.9684
^2H abundance of water used to make DD (ppm ^2H)	154.3	153.3	150.7
^2H abundance of DD (^2H ppm ^2H)	345.7	255.4	418.1
Enrichment of ^2H in DD (ppm excess ^2H), ΔDD	191.4	102.1	267.4
^2H abundance of baseline specimen (ppm ^2H)	156.6	155.0	156.3
^2H abundance of first post-dose specimen (ppm ^2H)	213.8	271.2	208.6
^2H abundance of second post-dose specimen (ppm ^2H)	240.5	272.6	253.5
^2H abundance of third post-dose specimen (ppm ^2H)	234.1	275.8	252.7

TABLE 6. EXAMPLE DATA SETS (cont.)

	Adult 1	Adult 2	Child
Enrichment of ^2H in first post-dose urine specimen (ppm excess ^2H), ΔBW_1	58.2	116.2	52.3
Enrichment of ^2H in second post-dose urine specimen (ppm excess ^2H), ΔBW_2	84.9	117.6	97.2
Enrichment of ^2H in third post-dose urine specimen (ppm excess ^2H), ΔBW_3	87.5	120.8	94.4

Note: DD = diluted dose.

TABLE 7. CALCULATION OF CUMULATIVE URINE LOSS

	Adult 1	Adult 2	Child
Loss in first post-dose specimen	Volume = 135 mL = 135 g = 0.135 kg	Volume = 50 mL = 50 g = 0.05 kg	Volume = 240 mL = 240 g = 0.24 kg
Cumulative loss in second post-dose specimen	135 + 60 = 195 mL = 195 g = 0.195 kg	50 + 100 = 150 mL = 150 g = 0.15 kg	240 + 105 = 345 mL = 345 g = 0.35 kg
Cumulative loss in third post-dose specimen	195 + 70 = 265 mL = 265 g = 0.265 kg	150 + 45 = 195 mL = 195 g = 0.195 kg	345 + 100 = 445 mL = 445 g = 0.445 kg

6.3.2.1. Adult 1

1(a) TBW using first post-dose specimen without adjustment for urinary loss:

$$TBW\ (kg) = (49.7426 \times 39.9846/0.1032) \times (191.4/58.2)/(1000 \times 1.041) = 60.94$$

With adjustment for urinary loss:

$$TBW\ (kg) = 60.94 - (2 \times 0.135) = 60.94 - 0.27 = 60.67$$

1(b) TBW using second post-dose specimen without adjustment for urinary loss:

$$TBW\ (kg) = (49.7426 \times 39.9846/0.1032) \times (191.4/84.9)/(1000 \times 1.041) = 41.78$$

With adjustment for urinary loss:

TBW (kg) = 41.78 – (2 × 0.195) = 41.78 – 0.39 = 41.39

1(c) TBW using third post-dose specimen without adjustment for urinary loss:

TBW (kg) = (49.7426 × 39.9846/0.1032) × (191.4/87.5)/(1000 × 1.041)
= 40.54

With adjustment for urinary loss:

TBW (kg) = 40.54 – (2 × 265) = 40.54 – 0.53 = 40.01

Mean TBW (kg) calculated from second and third post-dose specimens = 41.16 before correction for urinary water loss and 40.70 after correction for urinary water loss.

The deuterium oxide dose had not fully equilibrated with body water at the time of the first specimen (2 h and 10 min after the dose was consumed), resulting in an overestimation of TBW.

6.3.2.2. Adult 2

2(a) TBW using first post-dose specimen without adjustment for urinary loss:

TBW (kg) = (99.41736 × 27.898/0.10130) × (102.1/116.2)/(1000 × 1.041)
= 21.30

With adjustment for urinary loss:

TBW (kg) = 21.30 – (2 × 0.05) = 21.30 – 0.10 = 21.20

2(b) TBW using second post-dose specimen without adjustment for urinary loss:

TBW (kg) = (99.41736 × 27.898/0.10130) × (102.1/117.6)/(1000 × 1.041)
= 21.04

With adjustment for urinary loss:

TBW (kg) = 21.04 – (2 × 0.15) = 21.04 – 0.30 = 20.74

2(c) TBW using third post-dose specimen without adjustment for urinary loss:

$$\text{TBW (kg)} = (99.41736 \times 27.898/0.10130) \times (102.1/120.8)/(1000 \times 1.041)$$
$$= 20.49$$

With adjustment for urinary loss:

$$\text{TBW (kg)} = 20.49 - (2 \times 0.195) = 20.49 - 0.39 = 20.10$$

Mean TBW (kg) calculated from second and third post-dose specimens = 20.94 before correction for urinary water loss and 20.42 after correction for urinary water loss.

There is great inter-individual variation in equilibration time. In this participant, the deuterium oxide dose was almost equilibrated with body water at the time of the first specimen (2 h after the dose was consumed). It can often take 5–6 h for the dose to fully equilibrate. Having three post-dose specimens gives confidence that the plateau enrichment can be identified.

6.3.2.3. Child

3(a) TBW using first post-dose specimen without adjustment for urinary loss:

$$\text{TBW (kg)} = (54.9684 \times 32.039/0.1717) \times (267.4/52.3)/(1000 \times 1.041)$$
$$= 54.34$$

With adjustment for urinary loss:

$$\text{TBW (kg)} = 54.34 - (2 \times 0.24) = 54.34 - 0.48 = 53.86$$

3(b) TBW using second post-dose specimen without adjustment for urinary loss:

$$\text{TBW (kg)} = (54.9684 \times 32.039/0.1717) \times (267.4/97.2)/(1000 \times 1.041)$$
$$= 29.24$$

With adjustment for urinary loss:

$$\text{TBW (kg)} = 29.24 - (2 \times 0.35) = 29.24 - 0.70 = 28.54$$

3(c) TBW using third post-dose specimen without adjustment for urinary loss:

$$\text{TBW (kg)} = (54.9684 \times 32.039/0.1717) \times (267.4/94.4)/(1000 \times 1.041)$$
$$= 29.48$$

With adjustment for urinary loss:

$$\text{TBW (kg)} = 29.48 - (2 \times 0.445) = 29.48 - 0.89 = 28.59$$

Mean TBW (kg) calculated from second and third post-dose specimens = 29.36 before correction for urinary water loss and 28.57 after correction for urinary water loss.

Comment: Although children generally have faster water turnover than adults, the dose was not fully equilibrated with body water at the time of the first specimen (3 h after the dose was consumed), but it was by 4 h, when the second specimen was collected.

6.4. ESTIMATION OF BODY COMPOSITION FROM TBW

TBW is used to estimate FFM, assuming that the hydration of FFM is 73.2% in adults:

$$\text{FFM (kg)} = \text{TBW (kg)}/0.732$$

In infants and children, the hydration of FFM is calculated according to the age of the child (see Tables 1 and 2, Section 3.3.1).

FM is calculated by the difference between body weight and FFM:

$$\text{FM (kg)} = \text{body weight (kg)} - \text{FFM (kg)}$$

Results are often expressed as % body weight:

$$\text{FM (\%)} = \text{FM (kg)}/\text{body weight (kg)} \times 100$$

Table 8 shows the calculation of body composition from TBW.

TABLE 8. ESTIMATION OF BODY COMPOSITION FROM TBW

	Adult 1	Adult 2	Child
TBW (kg)	41.70	20.42	28.57
Hydration factor (adults 0.732; children, see Table 1)	0.732	0.732	0.747
FFM (kg) = TBW (kg)/hydration factor	55.6	27.9	38.2
FM (kg) = body weight (kg) – FFM (kg)	24.4	19.5	12.3
FM (% body weight) = FM (kg)/body weight (kg) × 100	30.5	41.2	24.3
FFM (% body weight) = FFM (kg)/body weight (kg) × 100	69.5	58.9	75.7
TBW (% body weight) = TBW (kg)/body weight (kg) × 100	50.9	43.1	56.6

6.5. EFFECT OF URINE LOSS CORRECTION

The difference between TBW calculated with and without adjustment for urinary loss is relatively small (1–3% of TBW in the examples shown here, Table 9); therefore, some laboratories choose to ignore urinary loss to simplify the procedure. However, this small difference could be significant in longitudinal studies looking for relatively small changes in body composition. If it is not possible to measure urine output, then a note should be kept of fluid intake, and cumulative fluid intake should be subtracted from the calculated TBW. Whichever study design is chosen, the same approach should be followed for all participants.

TABLE 9. EFFECT OF URINE LOSS CORRECTION ON CALCULATED BODY FAT %

	Adult 1	Adult 2	Child
TBW (kg) without urinary loss correction	41.2	20.8	29.4
TBW (kg) with urinary loss correction (true TBW)	40.7	20.4	28.6
Difference % true TBW	1.1	1.7	2.8
% Body fat without urinary loss correction	29.7	39.6	22.2
% Body fat with urinary loss correction	30.5	41.2	24.3
Body fat (kg) without urinary loss correction	23.8	19.0	11.2
Body fat (kg) with urinary loss correction	24.4	19.5	12.3
Difference body fat (kg) as % body weight	0.8	1.0	2.1

7. QUALITY CONTROL ISSUES

7.1. INSTRUMENT CALIBRATION

All analyses are compared with natural abundance and enriched water standards of known deuterium content. Standards should be analysed at the beginning and end of each batch of samples, and at intervals during the run to check for instrumental drift.

7.2. ANALYTICAL PRECISION

Analysis of replicate samples can be used to estimate analytical precision. When using IRMS, a precision (SD) of 1 ppm ^{2}H excess should be attainable.

7.3. MEASUREMENT OR ASSAY VARIATION

Comparison of TBW calculated from each post-dose sample can be used to estimate measurement or assay variation, which includes equilibration, sampling, handling and analytical precision. The first post-dose urine sample is often omitted as urine may not have equilibrated with TBW by this time (that is, TBW is obviously overestimated, as seen in Adult 1 and the child examples in Section 6.3.2). The second and third post-dose urine samples give values that are within 2% of their mean.

7.4. IDENTIFYING OUTLIERS

Measured TBW can be compared with a predicted value using the method of Bland and Altman [25], and the data flagged for checking or re-analysis if outside a normal range. If no other prediction is available, the relationship with height^3 can be used: TBW = 7.4 × height^3 (m^3), validated in children and adults [26]. If the measurements fall outside the 95% confidence intervals of this relationship, the data and calculation should be checked and the samples re-analysed if necessary. However, it is expected that 2.5% of measurements are more than +2SD from the mean difference (in obese participants with a high BMI) and 2.5% less than the mean difference –2SD (in participants with a low BMI).

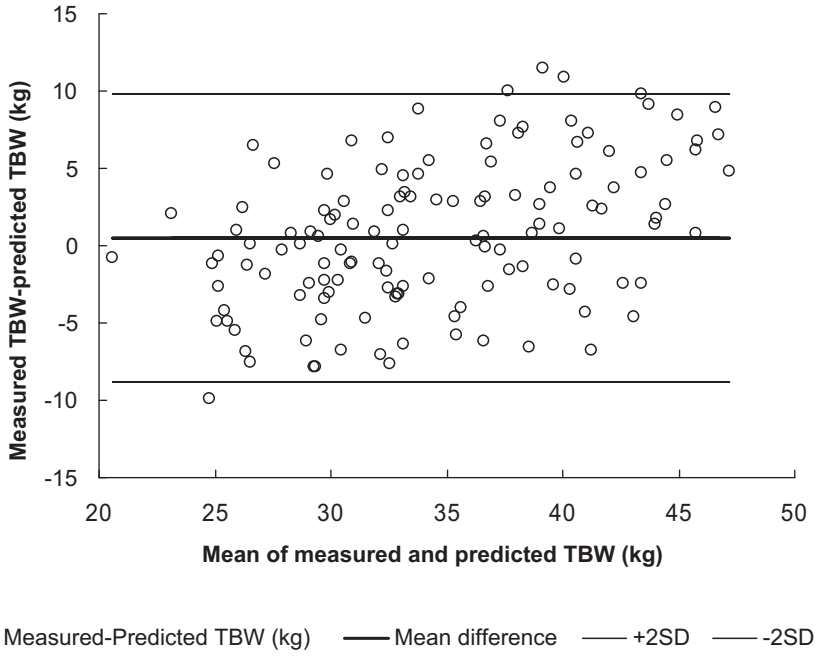

FIG. 15. Bland–Altman (residual) plot of TBW measured by deuterium dilution, and TBW estimated from height [26].

An example is shown in Fig. 15. TBW was assessed by deuterium dilution in 131 adults with various disease states, including pancreatic cancer, lung cancer, mild hypertension and type-2 diabetes. The mean difference between TBW calculated from deuterium enrichment and TBW estimated from height was 0.5 kg. The 95% confidence interval for the difference was –8.8 to +9.8 kg.

8. SUMMARY OF CRITICAL STEPS FOR GOOD QUALITY DATA

Dose preparation:

— The doses should be weighed to correct precision (at least 0.001 g). This should preferably be undertaken by trained scientists in an analytical laboratory.

In the field:

— Training of field workers: Well trained field workers can help with anthropometry and collection of urine samples, but it is important that they appreciate the importance of care in making measurements of height and weight, and accurate record keeping.
— Participants should have normal food and fluid intake the day before the measurement and should not take part in strenuous activities after the final meal of the previous day to avoid dehydration and depletion of glycogen stores.
— Participants should be weighed in minimal clothing (to 0.1 kg); the weight of any clothing worn during the measurement must be subtracted from the measured weight to give body weight.
— Before the dose bottle is opened, it should be inverted a few times to mix in condensation on the lid.
— The bottle should not be opened until it is time for the dose to be consumed.
— It should be ensured that 100% of the dose is consumed by adding water to the bottle and asking participants to drink this also.
— Sampling time: enough time should be allowed for tracer equilibration (4–6 h). In elderly and sick participants, 6–8 h should be allowed. Collecting three post-dose samples helps to identify the plateau enrichment.
— Sample vials should be labelled with the participant's identification number, time and date.
— All data should be recorded on the patient information sheet.
— The data should be transferred to a spreadsheet, e.g. Microsoft Excel, as soon as possible.
— Paper records should be kept as a backup.

9. FREQUENTLY ASKED QUESTIONS

Q. Why did I get a negative value for % body fat?

A. Negative values occur when the deuterium dose has not had sufficient time to fully equilibrate with body water or the dose was not completely consumed. This is seen as low deuterium enrichment, resulting in an overestimation of the size of the body water pool and, hence, a high FFM and low % body fat.

Q. How soon can I repeat a measurement?

A. For adults, it takes about 5 weeks for the deuterium oxide dose to wash out of the body water and the concentration of deuterium to return to baseline levels in tropical regions and 10 weeks in temperate regions. However, since body water is calculated from the difference in deuterium concentration between the pre-dose and post-dose specimens, it is not necessary to wait for this length of time before repeating a measurement using the equilibration method, which takes only a few hours. A second baseline specimen should be collected on the day of the repeat measurement. When time between measurements is short, water intake between the time of the dose and final urine specimen on the day of the repeat measurement should be minimized. Water intake at this time will have a larger dilution effect during the repeat measurement than during the first measurement.

Q. Why is it necessary to collect three post-dose specimens?

A. It is necessary to collect three post-dose specimens to ensure that the plateau enrichment has been reached. Two samples with the same enrichment (within 2%) confirm that the dose has fully equilibrated with body water. Occasionally, in participants with slow water turnover, the dose will not have fully equilibrated with body water at 5–6 h. Do not try to save time by stopping urine sampling after 6 h in elderly participants. Do a pilot study to confirm the equilibration time under your local conditions before commencing the main study. Consult with more experienced people to evaluate the results of the pilot study.

Q. Is it necessary to fast during the protocol?

A. Fasting during the protocol gives a more accurate estimate of TBW. Keep a record of the volume of fluids taken and subtract this from the calculated TBW if urinary losses are not measured.

Appendix I

GENERAL INFORMATION ON THE SAFETY OF DEUTERIUM OXIDE

I.1. ISOTOPES OF HYDROGEN

An atom consists of a central nucleus composed of neutrons and protons, which is surrounded by electrons that orbit around the nucleus. Protons carry a positive charge of 1 and have a mass of about 1 atomic mass unit (amu). Neutrons are electrically neutral and have a mass of about 1 amu. Electrons carry a negative charge of 1 and have a mass of 0.000 55 amu.

Atoms with different numbers of protons are called elements. For example, hydrogen has one proton, carbon has six protons and oxygen has eight. Isotopes of an element have the same number of protons and a different number of neutrons. Stable isotopes are not radioactive and are present naturally in the environment, including in the human body, in proportions known as the 'natural abundance' of the isotope. Most elements are a mixture of various stable isotopes. All atoms of an element have the same number of protons in their nucleus, while the number of neutrons may differ if more than one stable combination is possible. Stable isotopes of several elements (carbon, hydrogen, oxygen and nitrogen) have been used extensively in biomedical research.

Hydrogen consists of a nucleus with one proton (which is positively charged) and one electron (which is negatively charged). One proton represents a mass of 1 and, thus, the mass for hydrogen is 1; this stable isotope is also called protium. In deuterium, a heavier stable isotope of hydrogen, the nucleus contains one proton and one neutron (which has no charge and a mass of 1). Thus, the mass of deuterium is 2. The mass of an element is often shown at the top left of the letter indicating the element. Thus, hydrogen is ^{1}H and deuterium ^{2}H. Deuterium is also commonly indicated as D. Deuterium was discovered in 1932.

Hydrogen has one proton in the nucleus	^{1}H (stable isotope)
If one neutron is present in the nucleus, this is **deuterium**	^{2}H (stable isotope)
If two neutrons are present in the nucleus, this is **tritium**	^{3}H (radioactive)

The natural abundance of deuterium is 0.015%. This means that an adult woman weighing 55 kg with 30 kg of body water contains about 4.5 g of deuterium in her body water.

Deuterium oxide is water in which 99.8 or 99.9% of the hydrogen atoms are in the form of deuterium. This is referred to as 99.8 (or 99.9) at.% $^{2}H_{2}O$ or 99.8 (or 99.9) at.% $D_{2}O$. Deuterium oxide can be used to measure the size of the body water pool (TBW) by isotope dilution.

I.2. DEUTERIUM OXIDE SAFETY

Stable isotopes have been used in human metabolic studies for over half a century. Although stable isotopes of hydrogen emit no potentially harmful radiation, the mass of deuterium is 2 (^{2}H) and the mass of hydrogen is 1 (^{1}H). The mass difference between deuterium and hydrogen (a factor of two) is greater than for any other stable isotopes of the same element. This mass difference may cause significant 'isotope effects' at very high concentrations (>15%) of deuterium oxide in the tissues. Isotope effects are caused by the fact that the presence of deuterium in a molecule shortens covalent bonds, making them stronger and more resistant to breakage. Molecules containing deuterium, therefore, display slightly different reaction rates than those containing only hydrogen. The difference in rate constants between a reaction involving a molecule containing only hydrogen and that involving a molecule containing deuterium is known as the 'kinetic isotope effect', and can occur during enzymatically catalysed reactions in the body. Animal studies have shown that tissues containing more than 15% of deuterium labelled water exhibit a multitude of effects, including impaired protein and nucleic acid synthesis, altered conformation and stability of biopolymers, altered rates of enzymatic reactions, impaired cell division and morphological changes [27]. The overall effect of deuterium labelling appears to be a depression of tissue metabolism due to lower reaction rates of deuterium labelled compounds in vivo. While some toxic effects of deuterium labelling are reversible, very high concentrations may prove lethal. In mammals, concentrations of deuterium below 15% have not been associated with harmful effects. Levels of deuterium labelling of 15% must be maintained by continual dosage before adverse effects become evident [27]. However, lesser effects, such as transitory episodes of vertigo, have been reported in human adults consuming an amount of deuterium oxide sufficient to enrich body water to 0.35–0.65% [27]. It has been suggested that a threshold for noticeable transitory side effects exists when body water is enriched above 0.2%. The threshold of deuterium toxicity has been defined as 15% and is far in excess of concentrations conceivable for use in human studies [27]. The amount of deuterium consumed in studies of human milk output and body composition enriches body water to a maximum in the region of 0.1% in the mother and less than half of this in her baby. At this level, no adverse side effects have been reported.

Appendix II

SAMPLE DATA SHEET FOR TBW ESTIMATION BY DEUTERIUM OXIDE DILUTION

Person performing the test:_____ Date: _____/_____/_____
Day Month Year

I. Participant

Name:_____ Code/ID:_____
Weight: _____ . __ __ kg Height/Length: _____ . __ cm BMI _____ kg/m^2
Date of Birth: _____/_____/_____ Age:_____ years Gender: M ☐ F ☐
Healthy: YES ☐ NO ☐
Notes (health):_____
_____

II. Dose

Dose bottle number: _____
Dose weight: ___ ___ . __ __ __ g
Did the participant fast overnight? YES ☐ NO ☐
If no, how long was the fast before the dose? _____
Was the container opened just before the dosage? YES ☐ NO ☐
Was the dose consumed correctly? YES ☐ NO ☐
If not, what was the weight of the dose not consumed? ___ . ___ ___ ___
Was the container rinsed with 2 × 50 mL of water? YES ☐ NO ☐
Was the same straw used? YES ☐ NO ☐
Notes: _____

III. Specimen times and volumes

Time of baseline urine sample: ____:_____
Time dose was taken: ____:_____
Post-dose urine samples:

1st post-dose	Time: _____:_____	Volume: _____ mL
2nd post-dose	Time: _____:_____	Volume: _____ mL
3rd post-dose	Time: _____:_____	Volume: _____ mL

Appendix III

EQUIPMENT LIST

III.1. DOSE PREPARATION AND PREPARATION OF DILUTED DOSE

Deuterium oxide (99.8 or 99.9 at.% ^{2}H).
Drinking water.
Large screw cap bottles for preparing doses (e.g. 5 L borosilicate glass bottles with PTFE lined screw caps).
Dose bottles (screw cap, leak proof, e.g. 120 mL wide mouth, polypropylene leak proof autoclavable bottles).
Glass measuring cylinder to transfer doses to dose bottles.
Glass or plastic funnel.
Volumetric flask (100 mL) for making dose dilution.
Automatic pipette (200 μL) plus tips.
Large capacity balance weighing up to 10 kg with an accuracy of 0.1 g.
Electronic balance weighing to 0.001 g for weighing doses.
Electronic balance weighing to 0.0001 g for making dose dilution.
Refrigerator or freezer (–20ºC) for storing doses.
Freezer (–20ºC) for storing urine specimens.
Voltage stabilizers for all electronic equipment.

III.2. IN THE FIELD

Doses (prepared in the laboratory).
Drinking water.
Drinking straws.
Balance weighing to 0.1 kg for weighing participants.
Stadiometer for measuring participants' height.
1 L polyethylene measuring cylinder to measure urine volume.
Urine collection device (measuring cylinder, polyethylene jug or toilet hat).
Urine storage vials with screw cap (e.g. 4 mL internal thread self-standing cryovials).
Labels for sample vials.
Permanent ink pens for writing on labels.
Zip-lock bags for sample vials.
Disposable gloves.
Plastic bags/boxes for storing/transporting urine specimens.
Watch (to note time of urine sampling).

Refrigerator for storing doses if working in the field for several days without returning to base.

Cool box with ice pack (for storing samples in the field until they can be frozen).

Appendix IV

ISOTOPIC FRACTIONATION

Deuterium oxide (^2H_2O) is not identical to water with respect to its physical properties.

When deuterium oxide mixes with body water, three isotopic forms are found (Fig. 16). For example, in a water sample containing 1000 mg/kg (ppm) of deuterium oxide, the probability of any particular H being ^2H is 0.001, and the probability of it being ^1H is 0.999.

For any molecule of water, the probability of both H being ^1H ($^1H–O–^1H$) is:

$$P\ (^1H–O–^1H) = 0.999 \times 0.999 = 0.998001 \text{ or } 99.8001\%$$

The probability of both H being ^2H ($^2H–O–^2H$) is:

$$P\ (^2H–O–^2H) = 0.001 \times 0.001 = 0.000001 \text{ or } 0.0001\%$$

The probability of any particular water molecule containing one ^1H and one ^2H is:

$$P\ (^1H^2HO) = 2 \times 0.999 \times 0.001 = 0.001998 \text{ or } 0.1998\%$$

The factor of two arises because there are two possible arrangements, $^1H–O–^2H$ and $^2H–O–^1H$, which are equivalent.

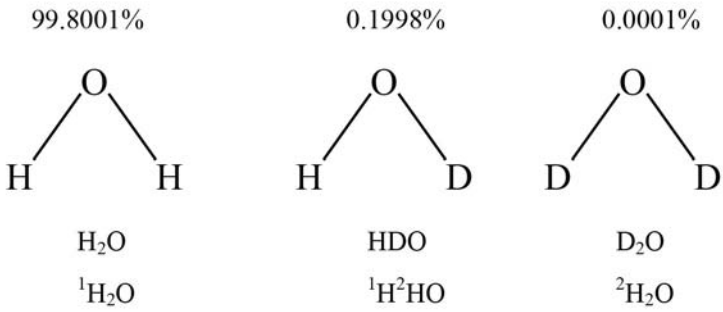

FIG. 16. Abundance of different molecules in a water sample containing 0.1 at.% (1000 ppm) ^2H.

The energy of the bond between deuterium (^{2}H or D) and oxygen (O) is slightly greater than the energy of the bond between hydrogen (^{1}H) and O. This can lead to isotopic fractionation when water undergoes a chemical or physical change. Isotopic fractionation of water occurs when water liquid becomes water vapour (gas).

There is less deuterium in water vapour than in the main volume of liquid water from which the vapour evaporated. The fractionation factor (f) for deuterium between water vapour (a gas) and liquid water is 0.941 at 25ºC.

There is very little isotopic fractionation of water within the body. Plasma, urine, human milk and sweat show little fractionation. However, water leaving the body as water vapour in breath and transdermal evaporation contains less deuterium than body water. Transdermal evaporation is insensible water loss from the skin through routes other than the sweat glands. The effect of increased insensible water losses, which contain less deuterium than body water, is to concentrate the deuterium oxide left behind, which could lead to an underestimation of TBW and, therefore, an overestimation of body fat. For this reason, participants should not take part in excessive physical activity during the urine sampling period.

Similarly, condensed water vapour on the caps of bottles used for storing doses, samples and standards contains less deuterium than the bulk of the liquid and, therefore, bottles should be inverted or centrifuged to mix the contents before opening, and should not be left open to the atmosphere.

The following example (Fig. 17) shows the effect of fractionation if 100 µL of condensation is clinging to the lid of a sample vial containing 4 mL of saliva, which originally contained 0.1 at.% (1000 ppm) ^{2}H.

The effect of fractionation is more pronounced when the volume of saliva is small. For example, if a vial containing 1 mL of saliva is left open to the atmosphere and 100 µL evaporate, there will be only 900 µL (0.9 mL) left behind, and this will contain 1006 ppm ^{2}H (Fig. 18).

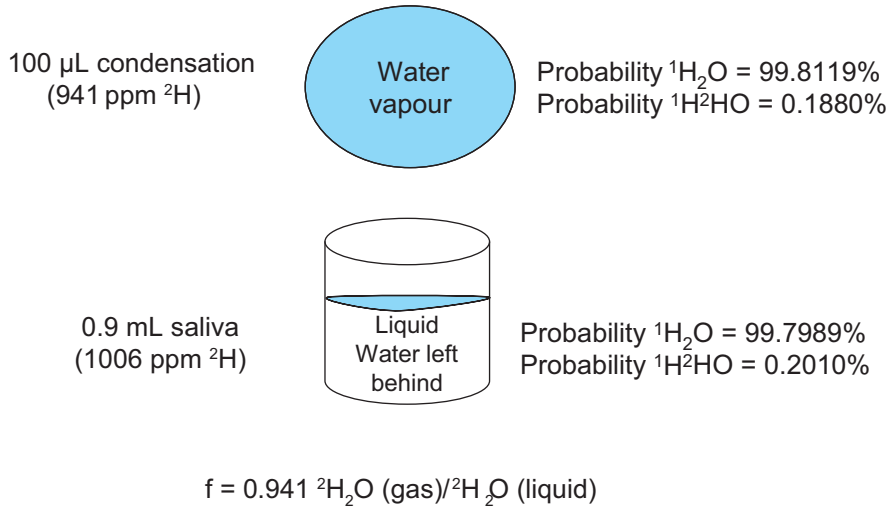

FIG. 17. Effect of isotopic fractionation in a 4 mL saliva sample originally containing 0.1 at.% (1000 ppm) ^{2}H.

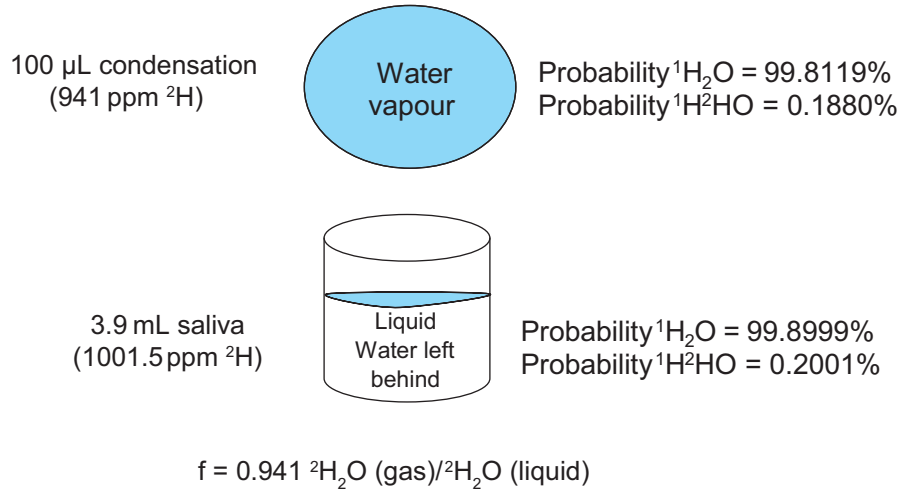

FIG. 18. Effect of isotopic fractionation in a 1 mL saliva sample originally containing 0.1 at.% (1000 ppm) ^{2}H.

GLOSSARY

atom per cent (at.%). The number of atoms of the stable isotope of interest expressed as a proportion of the total number of atoms of that element, e.g.

$$\text{at.\% } {}^2\text{H} = \frac{[{}^2\text{H}]}{[{}^1\text{H}]+[{}^2\text{H}]+[{}^3\text{H}]} \times 100$$

In practice, the number of ^{3}H atoms is negligible and is, therefore, ignored.

deuterium. The stable isotope of hydrogen with the symbol ^{2}H, sometimes abbreviated to D.

deuterium oxide. Water in which 99.8 or 99.9% of the hydrogen atoms are in the form of deuterium (^2H_2O or D_2O).

deuterium oxide dilution method of measuring total body water. A well established technique to measure total body water (TBW) from which body composition is estimated using a two compartment model, assuming the body is composed of fat and fat free mass (FFM). FFM is 73.2% water in healthy adults. TBW (kg)/0.732 = FFM (kg). Fat mass (FM) is calculated as the difference between FFM and body weight.

enrichment. As stable isotopes are naturally present, baseline samples must be taken before administration of the labelled compound. Enrichment is the concentration of the isotope above the baseline level. The concentration of deuterium in body water (above the baseline level) can be measured by Fourier transform infrared (FTIR) spectrometry or isotope ratio mass spectrometry (IRMS).

equilibration. The hydrogen atoms on water molecules in the body are not permanently attached to the oxygen atoms, but are constantly exchanging. They are in a state of constant flux. When a person drinks a dose of deuterium oxide, it is not a simple matter of the deuterium oxide (^2H_2O) mixing with water in the body. The deuterium atoms in ^2H_2O exchange with hydrogen atoms in water molecules, so that after a few hours, the probability of finding a molecule of ^2H_2O is very low. Most water molecules are still in the form of ^1H_2O, but a few are in the form of $^1H^2HO$ after exchange of ^{1}H with ^{2}H. This is the process of equilibration.

fat free mass. The term used in body composition studies to refer to the part of the body that is not fat. FFM includes water, protein, bone minerals and non-bone minerals. FFM contains 73.2% water in healthy adults [9], but the hydration of FFM is higher in children, the latter stages of pregnancy and certain clinical conditions.

Fourier transform infrared spectrometry. A technique that can be used to measure deuterium enrichment concentration in saliva samples from studies of body composition and human milk intake. FTIR is not as sensitive as isotope ratio mass spectrometry and cannot be used for the analysis of urine samples.

fractionation. Isotopic fractionation is the term used to describe the fact that molecules containing different isotopes display slightly different reaction rates. This can occur during physical changes such as evaporation. Water leaving the body as water vapour in breath contains less deuterium than body water. Similarly, condensed water vapour on the caps of bottles used for storing doses, samples and standards contains less deuterium than the bulk of the liquid. Bottles should, therefore, be inverted to mix the contents before opening.

insensible water loss. Insensible water loss refers to water lost from the body in breath and transdermal evaporation, which is water lost from the skin by routes other than the sweat glands. Water leaving the body as water vapour contains less deuterium than liquid body water, due to fractionation. A correction is made for insensible water losses when water intake from sources other than human milk is estimated in breastfed infants using the deuterium oxide dose-to-mother technique.

isotope. An element with the same number of protons and a different number of neutrons.

Hydrogen has one proton in the nucleus ^{1}H (stable isotope)
If one neutron is present in the nucleus, this is **deuterium** ^{2}H (stable isotope)
If two neutrons are present in the nucleus, this is **tritium**
^{3}H (radioactive isotope)

isotope dilution. A known amount of a labelled compound is added to a biological system and mixes fully with that pool. The dilution of the labelled compound by endogenous unlabelled compound will give a

measure of the size of the pool. This principle is the basis of the deuterium dilution method of measuring TBW.

isotope exchange. Deuterium (^2H) can exchange with hydrogen (^1H) atoms in water molecules and in other compounds. This is known as isotope exchange.

isotope ratio mass spectrometer (IRMS). A low resolution magnetic sector mass spectrometer. The sample must be introduced to the mass spectrometer ion source in the form of a pure gas (CO_2, N_2 or H_2). The gas is ionized by electron impact from a hot filament. The ions are separated in a magnetic field. The detectors are Farady Cups. IRMS can measure very low enrichments, down to natural abundance, very accurately.

mass spectrometer. an instrument which separates ions in a vacuum according to their mass to charge ratio (m/z). The major components of a mass spectrometer are an inlet system, ion source, mass analyser, detector and a vacuum system.

non-aqueous exchange. The process whereby isotopes in body water enter components of the body, other than water, is known as non-aqueous exchange. For example, deuterium exchanges with exchangeable hydrogen atoms (mainly –NH and –OH) in body protein. Hydrogen isotopes are also sequestered into fat and protein as these are synthesized. The volume of distribution, also known as the dilution space or pool space, of the tracer is, therefore, slightly greater than TBW. The ^2H dilution space (N_D) is 1.041 times that of TBW. This is accounted for by dividing the calculated volume of distribution (N_D, moles) by 1.041 to achieve TBW.

radioactive isotope. Radioactive isotopes have unstable nuclei which emit ionizing radiation in the form of particles or waves. Radioactive decay is the process by which a nucleus releases energy and transforms to a lower energy state. Tritium is the radioactive nuclide of hydrogen. Tritium has a half-life of 12.35 years.

stable isotope. Stable isotopes are not radioactive, are present naturally in the environment, including the human body, in concentrations known as the 'natural abundance' of the isotope. Hydrogen has two stable isotopes: ^1H or protium, the major stable isotope of hydrogen, and ^2H or deuterium, the minor stable isotope of hydrogen. Approximately 0.015% of hydrogen atoms in natural water are in the form of deuterium (^2H).

total body water. The term used to refer to the total water content of the body, which makes up 70–75% of body weight at birth, but decreases to 50–60% of body weight in lean adults and less than 40% in obese adults. FFM is approximately 73.2% water in adults. Measuring TBW establishes the amount of FFM. FM is calculated as the difference between FFM and body weight. TBW includes both intracellular fluid (ICF) and extracellular fluid (ECF).

volume of distribution. The volume through which the isotope is distributed, also known as the pool space or dilution space (N). In studies of TBW by deuterium dilution, the volume of distribution (N_D) is larger than TBW due to non-aqueous exchange.

REFERENCES

[1] SCHOELLER, D.A., "Hydrometry", in Human Body Composition, 2nd edn (HEYMSFIELD, S.B., LOHMAN, T.G., WANG, Z.M., GOING, S.B., Eds), Human Kinetics, Champaign, IL (2005) 35–49.

[2] PARKER, L., REILLY, J.J., SLATER, C., WELLS, J.C.K., PITSILADIS, Y., Validity of six field and laboratory methods for measurement of body composition in boys, Obes. Res. **11** (2003) 852–858.

[3] WELLS, J.C.K., FEWTRELL, M.S., Measuring body composition, Arch. Dis. Child. **91** (2006) 612–617.

[4] PROSSER, S.J., SCRIMGEOUR, C.M., High precision determination of $^{2}H/^{1}H$ in H_2 and H_2O by continuous-flow isotope ratio mass spectrometry, Anal. Chem. **67** (1995) 1992–1997.

[5] JENNINGS, G., BLUCK, L., WRIGHT, A., ELIA, M., The use of infrared spectrophotometry for measuring body water spaces, Clin. Chem. **45** 7 (1999) 1077–1081.

[6] INTERNATIONAL ATOMIC ENERGY AGENCY, Assessment of Body Composition and Total Energy Expenditure in Humans by Stable Isotope Techniques, IAEA Human Health Series No. 3, IAEA, Vienna (2009).

[7] COWARD, W.A., et al., Breast-milk intake measurement in mixed-fed infants by administration of deuterium oxide to their mothers, Hum. Nutr. Clin. Nutr. **36C** (1982) 141–148.

[8] DAVIES, P.S.W., WELLS, J.C.K., Calculation of total body water in infancy, Eur. J. Clin. Nutr. **48** (1994) 490–495.

[9] PACE, N., RATHBUN, E.N., Studies on body composition, III. The body water and chemically combined nitrogen content in relation to fat content, J. Biol. Chem. **158** (1945) 685–691.

[10] RITZ, P., Body water spaces and cellular hydration during healthy aging, Ann. N. Y. Acad. Sci. **904** (2000) 474–483.

[11] SCHOELLER, D.A., Changes in total body water with age, Am. J. Clin. Nutr. **50** (1989) 1176–1181.

[12] WANG, Z.M., et al., Hydration of fat-free body mass: review and critique of a classic body-composition constant, Am J. Clin. Nutr. **69** (1999) 833–841.

[13] HEWITT, M.J., GOING, S.B., WILLIAMS, D.P., LOHMAN, T.G., Hydration of fat-free mass in children and adults: Implications for body composition assessment, Am. J. Physiol. **265** (1993) E88–E95.

[14] LOHMAN, T.G., HARRIS, M., TEIXEIRA, P.J., WEISS, L., Assessing body composition and changes in body composition: another look at dual-energy X-ray absorptiometry, Ann. N. Y. Acad. Sci. **904** (2000) 45–54.

[15] LOHMAN, T.G., "Applicability of body composition techniques and constants for children and youths", Exercise and Sports Science Reviews (PANDOLF, K.B., Ed.), MacMillan, New York (1986) 325–357.

[16] LOHMAN, T.G., "Estimating body composition in children and the elderly", Advances in Body Composition Assessment (Current Issues in Exercise Science, Monograph 3) (LOHMAN, T.G., Ed.), Human Kinetics, Champaign, IL (1992) 65–77.

[17] FOMON, S.J., HASCHKE, F., ZIEGLER, E.E., NELSON, S.E., Body composition of reference children from birth to age 10 years, Am. J. Clin. Nutr. **35** (1982) 1169–1175.
[18] BUTTE, N.F., HOPKINSON, J.M., WONG, W.W., SMITH, E.O., ELLIS, K.J., Body composition during the first 2 years of life: An updated reference, Pediatr. Res. **47** (2000) 578–585.
[19] FOMON, S.J., NELSON, S.E., Body composition of the male and female reference infant, Annu. Rev. Nutr. **22** (2002) 1–17.
[20] WELLS, J.C., et al., Four compartment model of body composition in children: density and hydration of fat-free mass and comparison with simpler models, Am. J. Clin. Nutr. **69** (1999) 904–912.
[21] LOF, M., FORSUM, E., Hydration of fat-free mass in healthy women with special reference to the effect of pregnancy, Am. J. Clin. Nutr. **80** (2004) 960–965.
[22] COLLEY, R.C., BYRNE, N.M., HILLS, A.P., Implications of the variability in time to isotopic equilibrium in the deuterium dilution technique, Eur. J. Clin. Nutr. **61** (2007) 1250–1255.
[23] VAN MARKEN LICHTENBELT, W.D., WESTERTERP, K.R., WOUTERS, L., Deuterium dilution as a method to determine total body water: effect of test protocol and sampling time, Br. J. Nutr. **72** (1994) 491–497.
[24] SLATER, C., PRESTON, T., WEAVER, L.T., Stable isotopes and the international system of units, Rapid Commun. Mass Spectrom. **15** 15 (2001) 1270–1273.
[25] BLAND, J.M., ALTMAN, D.G., Statistical methods for assessing agreement between two methods of clinical measurement, Lancet **1** (1986) 307–310.
[26] SLATER, C., PRESTON, T., A simple prediction of total body water to aid quality control in isotope dilution studies in subjects from 3-87 years of age, Isot. Environ. Health Stud. **41** 2 (2005) 99–107.
[27] JONES, P.J., LEATHERDALE, S.T., Stable isotopes in clinical research: safety reaffirmed, Clin. Sci. **80** (1991) 277–280.

ADDITIONAL READING

COPLEN, T.B., New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data, Geochim. Cosmochim. Acta **60** 9 (1996) 3359–3360.

FULLER, N.J., JEBB, S.A., LASKEY, M.A., COWARD, W.A., ELIA, M., Four compartment model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass, Clin. Sci. **82** 6 (1992) 687–693.

GIBSON, R.S., Principles of Nutrition Assessment, 2nd edn, Oxford University Press, New York (2005).

HALLIDAY, D., MILLER, A.G., Precise measurement of total body water using trace quantities of deuterium oxide, Biomed. Mass Spectrom. **4** 2 (1977) 82–87.

HEYMSFIELD, S.B., LOHMAN, T.G., WANG, Z.M., GOING, S.B. (Eds), Human Body Composition, 2nd edn, Human Kinetics, Champaign, IL (2005).

KOLETZKO, B., SAUERWALD, T., DEMMELMAIR, H., Safety of stable isotope use, Eur. J. Pediatr. **156** Suppl. 1 (1997) S12–S17.

RAMÍREZ, E., et al., Four compartment model and validation of deuterium dilution techniques to estimate fat-free mass in Mexican youth, Nutrition **25** (2009) 194–199.

SCHOELLER, D.A., et al., Total body water measurement in humans with ^{18}O and ^{2}H labeled water, Am. J. Clin. Nutr. **33** (1980) 2686–2693.

SCHOELLER, D.A., JONES, P.J.H., "Measurement of total body water by isotope dilution: a unified approach to calculations", In Vivo Body Composition Studies (ELLIS, K.J., YASUMURA, S., MORGAN, W.D., Eds), Institute of Physical Sciences in Medicine, London (1987) 131–137.

SCRIMGEOUR, C.M., ROLLO, M.M., MUDAMBO, S.M.K.T., HANDLEY, L.L., PROSSER, S.J., A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin, Biol. Mass Spectrom. **22** 7 (1993) 383–387.

SNYDER, W.S., et al., Report of the task group on Reference Man, Pergamon Press, Oxford (1984).

VAN RAAIJ, J.M., et al., New equations for estimating body fat mass in pregnancy from body density or total body water, Am. J. Clin. Nutr. **48** (1998) 24–29.

WELLS, J.C., et al., Prediction of total body water in infants and children, Arch. Dis. Child. **90** (2005) 965–971.

WELLS, J.C.K., STRICKLAND, S.S., Measurement of nutritional status using conventional anthropometry and D_2O in Sarawak, Malaysia, Eur. J. Clin. Nutr. **50** (1996) 668–671.

WESTERTERP, K.R., Body composition, water turnover and energy turnover assessment with labelled water, Proc. Nutr. Soc. **58** (1999) 945–951.

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