Doubly labelled water technique to assess total daily energy expenditure

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In adults:

Total Energy Expenditure = Total Energy Intake

In children:

TEE = Total Energy Intake – Energy used for growth

- TEE can be measured using the DLW technique
- DLW can also be used to validate methods of assessing dietary intake
- DLW is a mixture of $\text{D}_2\text{O}$ and $\text{H}_2^{18}\text{O}$ (10 at.%)

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Recommendations:

• DLW is the best method to determine TEE of individuals undertaking normal daily living activities

• Other methods of energy expenditure measurement should be validated against DLW
Energy Requirements for Cuban Children

- PAL of normal Cuban children (4-6 y) is nearly 1.8; much higher than that reported in studies of children from industrialized countries using the DLW technique. (Hernandez et al. 2002)
- This information was incorporated into the Dietary Reference Intakes for the Cuban Population, 2008
Energy requirements vary depending on lifestyle.

More information is required from developing world settings.

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Free living daily energy expenditure by the doubly labelled water method ($^{2}$H$_{2}^{18}$O)
Difference in elimination rates is a measure of CO$_2$ production rate

**CO$_2$ production rate is used to estimate TEE**
Brief History

• The method was invented and validated in animals in the 1950s by Nathan Lifson and his research group at the University of Minneapolis, USA.
• In 1982 the first application of the method in humans was published by Dale Schoeller.
• This was followed by an intense period of technical development of the method and vigorous debate over the correct methodologies to be employed and the correct equations to use to convert the estimated isotope turnovers into energy expenditure.
• In 1987 the International Dietary Energy consultancy group of the IAEA convened a meeting in Cambridge UK. 24 users of the method were invited to discuss the methodology.
• The IDECG Report was published in 1990.
• This summarised many of the technical and calculation issues being debated at the time. The report is available online at http://archive.unu.edu/unupress/food2/UID05E/UID05E00.HTM
Use of the method continued to grow exponentially through the late 1980s and into the early 1990s (see plot). By the mid 1990s the technique was being published on about 90-115 times annually.

Doubly Labelled Water Theory and Practice
JR Speakman, 1997
Cited 559 times

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http://www.abdn.ac.uk/energetics-research/doubly-labelled-water/
Procedure

- Subject drinks a dose of DLW
- Sample saliva or urine over 1-2 weeks
- $^2$H lost as water (urine, breath)
- $^{18}$O lost as water and CO$_2$
- Measure the rate constants for disappearance
- Difference = CO$_2$ production
- $r$CO$_2$ is used to calculate energy expenditure
Preparing Doses 1

• The dose given to each child depends on the analytical precision of the IRMS used for the analysis. **Contact the analytical lab for advice.**
• A typical dose includes $0.9 \text{ g} 10 \text{ at.}\% \text{ H}_2^{18}\text{O/kg body weight}$ and $1.2 \text{ g} 99.8 \text{ at.}\% \text{ D}_2\text{O}$.  
• Prepare sufficient for the whole study in a large (3 L) glass bottle with screw cap.
Preparing Doses 2

- Prepare individual doses in 120 mL leak-proof Nalgene bottles; either based on the weight of the child or with all bottles containing the same amount based on the average expected weight.
- Clearly label dose bottles with a dose ID and the participant ID, if the dose is prepared for a particular child.
- **VERY IMPORTANT!** Keep some of the dose for analysis with the samples.
Preparing doses 3

- Use an analytical balance weighing to 0.0001 g.
- Weigh the empty bottle with its lid.
- Add the required amount of DLW. Replace the lid.
- Weigh again and calculate the weight of DLW in the bottle.
Preparation of dose dilution

• A 1 in 500 dilution of the dose should be prepared, and stored with the samples (not the doses) for characterisation in the analytical lab.

• This will determine the enrichment of the dose consumed. This analysis is critical, because the results will be used in the calculation of the dilution space.
Delivering the dose

- The dose is consumed through a straw
- Make sure all the water is consumed
- Add 50 mL drinking water to the bottle and ask the child to drink the water through the straw. Repeat.
Sampling the body water pool

- Urine is the most commonly collected matrix for DLW, but saliva can also be sampled.
- Store at least two aliquots in 4-5 mL screw-cap cryovials.
- Samples can be stored in a freezer at -18 to -20°C until analysis.
Sampling duration

- Samples should be collected for 2-3 times the water turnover time.
- Children have faster water turnover than adults.
- Very active people will have faster water turnover than sedentary people.
- The sampling time in infants and young children is usually 7 days.
- In children aged 7-10, sample for 10 days.
- In sedentary adults sample for 14 days.
- Check the water turnover during in the pilot study.
Sampling times

- Urine should be sampled at the same time each day, avoiding the first void in the morning.
- There are two commonly used protocols:
  - Two-sample protocol
  - Multi-sample protocols

Each have advantages and disadvantages
Two-sample protocol for an infant

Actually at least 3 samples!

Variations:
Baseline; 1 post dose after 24 h; 1 post dose after 7 d

Two point protocols originate from the time when sample preparation was extremely labour intensive, but with modern continuous flow IRMS instrumentation this is no longer an issue.
Multipoint protocols

- Many variations – the most complex samples every day for 14 days. It is not necessary to analyse all the samples.

- Some protocols collect a baseline sample on more than one day.
• Consult with the analytical lab at the project design phase
1 Measure the isotope enrichment
2 Plot the elimination curve

- The elimination rates ($k_D$ and $k_O$) are the gradients of the plot of the natural logarithm (ln) of the enrichment in body water versus time since the dose was consumed.
- $k_D = -0.0533$, $k_O = -0.074$
- $k_O / k_D = 1.2155$

QC check! $k_O / k_D$ should lie between 1.1 and 1.7
3 Calculate the isotope dilution space

The isotope dilution space (Nx) is calculated from the y-intercept, the weight of DLW consumed and the analysis of a 1:500 dilution of the dose.

**QC Check!** The pool space ratio (N_D/N_O) should be between 1.000 and 1.070 (mean 1.034).
Water turnover time

- Water turnover time can be calculated from $k_D$
- $k_D = -0.053$ per day
- Therefore water turnover = 5.3% per day
- $3 \times 5.3 = 15.9$ days = OK!
4 Body Composition from DLW

- TBW is calculated from the dilution space by correcting for non-aqueous exchange
  - $\text{TBW}_O = \frac{N_O}{1.007}$
  - $\text{TBW}_H = \frac{N_H}{1.041}$
  - Average = $\frac{(\text{TBW}_O + \text{TBW}_H)}{2}$
- FFM = TBW/hydration factor
- FM = BW - FFM
5 Calculate CO₂ production rate (rCO₂)

- Calculate CO₂ production rate (rCO₂) L/day
- Calculate TEE using the modified Weir equation
- TEE (kcal/d) = rCO₂ (L/d) x (1.10 + 3.90/R)

R is the respiratory exchange ratio, sometimes called RQ (respiratory quotient)

- In people consuming Western diets that provide 30-35% energy from fat, RQ is usually assumed to be 0.85 or 0.86
- It is recommended that a value representing the local diet is used
Respiratory Quotient (RQ) = \frac{\text{CO}_2 \text{ produced}}{\text{O}_2 \text{ consumed}}

RQ = \text{Food Quotient (FQ)}

\[ \text{O}_2 = \frac{\text{CO}_2}{\text{FQ}} \]

Diet data 24 h recall
Weighed food intake
Evaluation of food intake to calculate the food quotient (FQ)
FQ = (P \times 0.81) + (F \times 0.71) + (C \times 1.0) + (A \times 0.67)

Where:
P, F, C & A = Represent the energy contribution as a fraction of metabolizable energy

Constants = are the classical RQ values for these individual fuels

(Lusk, 1928)
Calculation of FQ

CA = (Px0.81) + (Fx0.71) + (Cx1.0) + (Ax0.67)

Assume a diet where % of energy intake is

15% from protein
45% from fat
40% from carbohydrate

FQ = (0.15x0.81) + (0.45x0.71) + (0.40x1.0)
FQ = 0.841
Energy expenditure final calculation

\[ EE^* \text{ kcal/d} = VCO_2 \text{ (L/d)} \times 4.598 + VO_2 \text{ (L/d)} \times 16.302 \]

From DLW

\[ FQ = \frac{VCO_2}{VO_2} = RQ \]

\[ VO_2 = \frac{VCO_2}{0.841} \]

*(Weir, 1949)*
Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques