1. INTRODUCTION

- Stable isotope techniques have been used in studies of human nutrition for over 50 years.
- Deuterium oxide (D₂O or 2H₂O) is given orally and after mixing with body water is eliminated from the body in urine, saliva, sweat and human milk.
- D₂O is metabolised in the body in the same way as water, and is dispersed through the body water within a matter of hours.
- Body water can be sampled in the form of saliva, urine, plasma or human milk and the enrichment of deuterium can be measured by isotope ratio mass spectrometry (IRMS) or Fourier transform infrared spectrometry (FTIR).

2. DEUTERIUM OXIDE DOSE PREPARATION

- Weigh the labelled dose bottle plus lid. Record the weight.
- Add D₂O to the dose bottle using a measuring cylinder, replace lid.
- Weigh the bottle plus D₂O. Record the exact weight of D₂O in each dose.
- Doses should be prepared in a food preparation area, not a laboratory.

3. SALIVA SAMPLING AND DOSING

- Ask participant to empty bladder.
- Weigh participant in light clothing (to 0.1 kg).
- Collect baseline saliva sample.
- Invert dose bottle to ensure contents are fully mixed.
- Participant drinks the dose of D₂O. Add 50 ml drinking water to the bottle and ask the participant to consume this. Add another 50 ml drinking water and ask them to drink this also.
- Collect first post dose saliva sample. Wait for the dose to equilibrate with body water (3-4 hours).
- Collect second post dose saliva sample. Wait for a further 1 hour.

4. TOTAL BODY WATER (TBW) ANALYSIS

- Analyse saliva samples using FTIR.
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5. CALCULATIONS

- TBW (kg) = V_D / 1.041
- FFM (kg) = TBW (kg) / Hydration factor
- FM (kg) = body weight (kg) – FFM (kg)

   FM=Fat mass
   FFM=Fat-free mass
   V_D=Volume of distribution

6. REFERENCES