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Total body water measurement using the ^{2}H dilution technique for the assessment of body composition of Kuwaiti children

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Abstract

Objective: The ^{2}H dilution technique is the reference method to estimate total body water for body composition assessment. The aims of the present study were to establish the total body water technique at the Kuwait Institute for Scientific Research and assess body composition of Kuwaiti children.
Design: The isotope ratio mass spectrometer was calibrated with defined international reference water standards. A non-random sampling approach was used to recruit a convenience sample of Kuwaiti children. A dose of $^{2}H_2O$, 1–3 g, was consumed after an overnight fast and ^{2}H enrichment in baseline and post-dose urine samples was measured. Total body water was calculated and used to estimate fat-free mass. Fat mass was estimated as body weight minus fat-free mass.
Setting: The total body water study was implemented in primary schools.
Subjects: Seventy-five boys and eighty-three girls (7–9 years).
Results: Measurements of the isotope ratio mass spectrometer were confirmed to be accurate and precise. Children were classified as normal weight, overweight or obese according to the WHO based on BMI-for-age *Z*-scores. Normal-weight and overweight girls had significantly higher percentage body fat (median (range): 32·4 % (24·7–39·3 %) and 38·3 % (29·3–44·2 %), respectively) compared with boys (median (range): 26·5 % (14·2–37·1 %) and 34·6 % (29·9–40·2 %), respectively). No gender difference was found in obese children (median 46·5 % *v.* 45·6 %).
Conclusions: The establishment of a state-of-the-art stable isotope laboratory for assessment of body composition provides an opportunity to explore a wide range of applications to better understand the relationship between body size, body composition and risk of developing non-communicable diseases in Kuwait.

Keywords
Body composition
^{2}H dilution
Childhood obesity

Obesity is a major public health problem globally[1,2], emphasized by the increasing prevalence of overweight and obesity in children[3]. For example, in Kuwait, about a third of schoolchildren aged 5–10 years are either overweight or obese based on BMI-for-age *Z*-score[4] according to the WHO[5,6].

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health[7]. BMI is a simple index based on body weight and height (body weight divided by the square of body height) that is commonly used to classify overweight and obesity. However, as such, it does not provide a measure of body fat. For more detailed information about body composition, other methods are needed to assess the different compartments of the body, i.e. fat-free mass (FFM) and fat mass (FM). Total body water (TBW) assessment by ^{2}H dilution, also known as stable isotope dilution, followed by application of well-established age- and gender-specific hydration factors, is the reference method to estimate FFM. This methodology is most often based on the intake of a dose of $^{2}H_2O$ and collection of biological samples (urine or saliva) at specific time intervals. Guidance on the standardization of all procedures involved are described in detail in a recent document published by the International Atomic Energy Agency (IAEA)[8]. Analysis of the enrichment of biological samples can be made by isotope ratio mass spectrometry (IRMS) or Fourier transform infrared spectroscopy, based on access to equipment, dose of stable isotope administered and other practical considerations[8]. Once FFM has been assessed, FM can be calculated as the difference between body weight and FFM. This methodology is often referred to as a two-compartment model of body composition and is suitable for use in a wide range of settings; it was recently utilized by Liu *et al.*[9] to explore ethnic differences in the relationship between BMI and percentage body fat (%BF) among Asian children aged 8–10 years.

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The aims of the present study were to: (i) establish a stable isotope laboratory for TBW assessment by ^2H dilution by introducing analysis of biological samples by IRMS and testing analytical reproducibility of stable isotope ratios at the Kuwait Institute for Scientific Research (KISR); and (ii) use TBW for body composition assessment, based on a two-compartment model, in a convenience sample of 7–9-year-old Kuwaiti boys and girls.

Experimental methods

Calibration of the isotope ratio mass spectrometer and verification of the KISR water standards

The isotope ratio mass spectrometer (IRMS; Nu Instruments, Wrexham, UK) at KISR was calibrated with defined water standards provided by the Scottish Universities Environmental Research Centre (SUERC), Glasgow, UK. These laboratory standards comprised two standards to calibrate each batch of samples and two additional water standards used as quality controls.

In order to establish a series of locally prepared water standards, referred to as the 'KISR water standards', four standards were prepared gravimetrically using local drinking water (Kadhmah, produced by reverse osmosis at KISR). The water standards were prepared to cover the expected range of ^2H enrichment in biological samples using ^2H-enriched water (99·9 atom% ^2H_2O; Cambridge Isotope Laboratories Inc., Andover, MA, USA). KISR standards were prepared in accordance with the SUERC water standards as follows: (i) Natural Abundance Water (natural abundance of ^2H) and (ii) High Enrichment Water (calculated excess ^2H of 319·4 ppm). These standards were used for calibration. In addition, two standards were prepared for quality control: (iii) Low Quality Control Water (natural abundance of ^2H) and (iv) High Quality Control Water (calculated ^2H excess of 143·8 ppm).

The isotopic composition of the KISR standards was then defined against international reference water standards in a two-stage procedure. The independent international reference water standards were:

1. High enriched reference water with 420·66 ppm ^2H (IsoAnalytical B2190, catalogue no. B2190; Elemental Microanalysis Ltd, Okehampton, UK).
2. Medium enriched reference water with 287·05 ppm ^2H (IsoAnalytical B2191, catalogue no. B2191; Elemental Microanalysis Ltd).
3. Vienna Standard Mean Ocean Water (VSMOW), primary international reference water with 155·74 ppm ^2H (IAEA VSMOW2, no. 346; IAEA, Vienna, Austria).

The first stage of the procedure involved analysing the four KISR standard waters and the international reference waters as unknowns against the SUERC standards. Any small offset between the measured and certified values for the international reference waters was described by linear regression and the offset was used to redefine the composition of the KISR standard waters. All analyses were undertaken with three measurements each of duplicate water samples. The second stage of this process involved using the newly defined KISR water standards during analysis of the three international reference waters.

Assessment of total body water in children

The ^2H dilution technique and protocols used in the present study followed the guidance provided by the IAEA[8,10]. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Joint Committee for the Protection of Human Subjects in Research at the Kuwait Institute of Medical Specialization of the Ministry of Health. Written informed consent was obtained from all participants plus at least one parent per child. During the period March to June 2011, apparently healthy 7–9-year-old Kuwaiti children (males and females) were recruited, using a non-random sampling approach, from public primary schools. This age group was selected in accordance with a previous study of body composition in Asian children and aimed at similar sample size[9]. All children, their parents and teachers were informed about the aims and procedures of the study. On the day of the study, all children were invited to KISR after an overnight fast in groups of five children per day. Body weight and height were measured for all children, who wore light clothing and no shoes. Body weight (to the nearest 0·1 kg) was measured using a digital scale (SECA™, Hamburg, Germany) and height was measured using a portable stadiometer (Holtain, Crymych, UK) to the nearest 0·1 cm. All measurements were made in triplicate.

Doses of ^2H_2O were prepared by dilution with local, bottled drinking water (Kadmah) to 10 atom% ^2H and administered based on body weight; 1 g of 99·8 % ^2H_2O for children weighing less than 35 kg and 3 g of 99·8 % ^2H_2O for children weighing more than 35 kg[10]. Doses were prepared gravimetrically to three decimal points into individual plastic dose bottles and labelled with a unique code for each child. Doses were administered under close supervision and drinking straws were used to avoid potential spillage. Each dose bottle was rinsed twice with Kadmah water (50 ml per rinse) and the rinsing water was consumed to ensure complete intake of the dose. Time and intake of each dose were recorded.

Urine specimens were collected from each child as follows: (i) a pre-dose urine sample (baseline) was collected before intake of the dose of ^2H_2O; and (ii) three post-dose urine samples were collected at approximately 1 h, 2 h and 4 h after intake of the dose. At each urine collection, the child was asked to empty his/her bladder into a plastic jug provided for this purpose. Volume and time of collection were recorded and an aliquot was

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transferred into a labelled cryovial and stored at −20°C until analysis by IRMS. The children were served a standardized, light snack after collection of the first post-dose urine sample and all fluid intake was monitored. Their activities (non-vigorous activities such as reading, watching videos, etc.) were supervised until completion of the study protocol.

IRMS was used to measure ^{2}H enrichment in the pre-dose (baseline) and post-dose urine specimens. The average of the second and third post-dose urine specimens was used to calculate TBW. The calculation included a small correction for the ^2H_2O dose lost in urine prior to reaching equilibration of body water[8,10]. To convert TBW into FFM, Lohman's Hydration Factors for different ages and gender were used[11]. FM was calculated based on the difference between body weight and FFM. Results are presented as TBW (kg), FM (kg) and FFM (kg). In addition, results were used to calculate fat mass index (FMI = FM (kg)/height^2 (m^2)) and fat-free mass index (FFMI = FFM (kg)/height^2 (m^2)). %BF was calculated as [FM (kg)/body weight (kg)] × 100.

Children were classified as normal weight, overweight or obese based on BMI-for-age *Z*-score, according to the WHO growth reference for school-aged children and adolescents[6]. The development of the WHO growth reference 5–19 years is described in detail by de Onis *et al.*[5]. Briefly, cut-offs for overweight and obesity are defined as >+1 SD (equivalent to BMI = 25 kg/m^2 at 19 years) and >+2 SD (equivalent to BMI = 30 kg/m^2 at 19 years), while thinness is defined as <−2 SD (http://www.who.int/growthref/who2007_bmi_for_age/en/index.html).

Statistical methods

Statistical analyses were performed using the SAS statistical software package version 9·1·3. Repeatability of IRMS analyses was tested using the intra-class correlation coefficient. Medians and ranges of information presented in Tables 2 and 3 were determined using PROC MEANS. The PROC UNIVARIATE test was used to test for normal distribution of data. As results were not normally distributed and because the sample size was relatively small, non-parametric tests were used. The PROC NPAR1WAY procedure was used to perform the Wilcoxon score (rank sums) and one-way ANOVA statistics (also called the Kruskal–Wallis test) was used to compare median values presented in Tables 2 and 3. *P* values <0·05 were considered statistically significant.

Results

Calibration of the isotope ratio mass spectrometer and verification of the KISR water standards

Analysis of the international reference water standards showed that the newly installed IRMS at KISR produced values within 0·2 ppm of the certified values (Table 1).

After analysis of KISR water standards, ^{2}H concentration was confirmed as follows: (i) Natural Abundance Water, ^{2}H abundance = 157·2 ppm; (ii) High Enrichment Water, excess ^{2}H = 371·3 ppm; (iii) Low Quality Control Water, ^{2}H abundance = 156·6 ppm; and (iv) High Quality Control Water, excess ^{2}H = 168·1 ppm.

Urine samples were analysed in batches and all batches were calibrated using a two-point calibration with the Natural Abundance Water as the low point and the High Enrichment Water as the high point. Furthermore, each analytical batch included four analyses each of Low Quality Control Water and High Quality Control Water. The repeatability of the quality control samples was recorded throughout the forty-six analytical batches undertaken over 3 months of analysis as follows: mean 324·5 (SD 0·42) ppm and mean 156·1 (SD 0·55) ppm for ^{2}H abundance in Low Quality Control Water and High Quality Control Water, respectively. The mean difference was 168·5 (SD 0·55) ppm between the High Quality Control Water and the Low Quality Control Water.

Total body water assessment

To ensure that the IRMS was operating optimally, analyses of all urine samples from more than a third of the children studied (58/158) were repeated. At the confidence level of 95 %, the intra-class correlation coefficient was 0·99969 (95 % CI −0·017, 0·0399) for the difference between repeated TBW measurements. These results, taken together with the results based on repeatability of the quality control water samples, confirm the analytical capability at KISR.

Table 1 Analysis of certified reference waters by isotope ratio mass spectrometry (IRMS) at Kuwait Institute for Scientific Research (KISR)

	Analysis by IRMS at KISR: measurements of ^{2}H abundance (ppm ^{2}H)	Reported values for certified reference waters of ^{2}H abundance (ppm ^{2}H)
VSMOW2*	155·6	155·7
IsoAB2191†	287·2	287·1
IsoAB2190‡	420·6	420·7

*Vienna Standard Mean Ocean Water (VSMOW), primary international reference water with 155·74 ppm ^{2}H (IAEA VSMOW2, no. 346; International Atomic Energy Agency, Vienna, Austria).
†Medium enriched reference water with 287·05 ppm ^{2}H (IsoAnalytical B2191, catalogue no. B2191; Elemental Microanalysis Ltd, Okehampton, UK).
‡High enriched reference water with 420·66 ppm ^{2}H (IsoAnalytical B2190, catalogue no. B2190; Elemental Microanalysis Ltd).

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Table 2 Age, body weight, body height, BMI and body composition assessment (total body water (TBW), fat-free mass (FFM), fat mass (FM) and percentage body fat (%BF), as well as fat-free mass index (FFMI) and fat mass index (FMI)), of 7–9-year-old Kuwaiti boys and girls participating in the study. *P* values refer to comparisons between genders

	Boys (*n* 75)		Girls (*n* 83)		
	Median	Range	Median	Range	*P* value
Age (years)	8·1	7·0–9·4	8·3	6·5–9·4	0·98
Body weight (kg)	30·0	19·1–65·5	30·9	18·2–76·5	0·86
Body height (cm)	129·5	113·9–148·8	128·2	112·2–149·5	0·4
BMI (kg/m^2)	17·4	13·6–35·2	19·1	13·5–35·7	0·89
TBW (kg)	15·7	11·2–28·1	14·8	10·0–28·3	0·02
FFM (kg)	20·4	14·5–36·5	19·3	13·0–36·9	0·006
FM (kg)	9·7	2·8–34·0	12·2	4·9–39·7	0·15
%BF	33·0	14·2–55·0	38·2	24·7–57·5	0·003
FFMI (kg/m^2)	12·1	9·9–17·5	11·6	9·4–17·1	0·001
FMI (kg/m^2)	5·8	2·1–18·2	7·1	3·3–18·5	0·05

Table 3 Percentage body fat (%BF) in boys and girls (median values and range) classified as normal weight, overweight or obese based on BMI-for-age *Z*-scores according to the WHO[6]. *P* values refer to comparisons between boys and girls within groups of normal-weight, overweight or obese children

	%BF in boys (*n* 75)		%BF in girls (*n* 83)		
	Median	Range	Median	Range	*P* value
Normal weight	26·5	14·2–37·1	32·4	24·7–39·3	$<$0·0001
Overweight	34·6	29·9–40·2	38·8	29·3–44·2	0·048
Obese	45·6	35·4–55·0	46·5	38·4–57·5	0·13

A convenience sample of seventy-five boys (median age 8·1 years) and eighty-three girls (median age 8·3) was recruited, representing a wide range of BMI; 13·5–35·7 kg/m^2 in girls and 13·6–35·2 kg/m^2 in boys. Information about median (range) body weight, height, TBW, FFM, FM, %BF, FFMI and FMI is shown in Table 2. Statistically significant differences were found for TBW, FFM, %BF and FFMI between boys and girls (Table 2).

Among the boys, 51 % were classified as normal weight, 8 % were overweight and 41 % were obese, based on the WHO classification of BMI-for-age *Z* scores[6]. Corresponding values for girls were 43 %, 17 % and 33 %. In Table 3, %BF (median and range) is presented for normal-weight, overweight and obese boys and girls. Normal-weight and overweight girls had significantly higher %BF (median (range): 32·4 % (24·7–39·3 %) and 38·3 % (29·3–44·2 %), respectively) compared with boys (median (range): 26·5 % (14·2–37·1 %) and 34·6 % (29·9–40·2 %), respectively). No gender difference in %BF was found in obese children (median 46·5 % *v.* 45·6 %).

Statistically significant differences were also observed within each gender; Obese girls had significantly higher %BF compared with normal-weight ($P < 0·0001$) and overweight ($P < 0·0001$) girls and obese boys had significantly higher %BF compared with normal-weight ($P < 0·0001$) and overweight ($P = 0·0003$) boys. Overweight boys and girls had significantly higher %BF than their normal-weight counterparts ($P < 0·0001$ for girls and $P = 0·0012$ for boys).

A close correlation between FMI and BMI was observed for both genders ($R^2 = 0·936$ for girls and $R^2 = 0·945$ for boys). These observations are in agreement with data from the UK, reported by Wells[12]; $R^2 = 0·89$ for boys and $R^2 = 0·76$ for girls (mean age 9·88 years). Correlations between %BF and BMI were $R^2 = 0·698$ for girls and $R^2 = 0·7504$ for boys in the present study. These results are in agreement with data presented by Liu *et al.*[9].

Discussion

The first aim of the present study, i.e. to establish a stable isotope laboratory for TBW assessment, was achieved as demonstrated by analysis of the international reference water samples as well as the high repeatability of results based on analysis of quality control water standards.

The second aim of the present study was to use TBW for body composition assessment, based on a two-compartment model, in 7–9-year-old Kuwaiti children. The study demonstrated the use of the ^{2}H dilution technique for body composition assessment under realistic conditions in Kuwait. Access to this technique provides an opportunity for future studies to better define overweight and obesity in different population groups. For example, the stable isotope technique for body composition assessment provides an excellent tool for comparisons between ethnic groups. This approach has been used recently in a study with 8–10-year-old children from different Asian countries, indicating significant differences in body composition between ethnic groups[9]. For example, Filipino boys had lower %BF than Malay and Thai boys, while Thai girls had

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higher %BF than Chinese, Lebanese, Malay and Filipino counterparts.

When compared with the data presented by Liu *et al.*[9], Kuwaiti children in the present study were in a similar range of BMI (13·5–35·7 kg/m^2 *v.* 12·2–34·9 kg/m^2) and %BF (14·2–57·5 % *v.* 5·5–54·5 %) if the extreme value of 5·5 %BF in reference 9 is disregarded. In the Kuwaiti children, a wide range of %BF was observed within normal-weight, overweight and obese boys and girls. For example, boys classified as normal weight had %BF in the range of 14·2–37·1 %; the corresponding range for girls was 24·7–39·3 %. %BF in children classified as overweight varied between 29·3 and 44·2 % in girls and between 29·9 and 40·2 % in boys. In obese children, %BF was in the range of 38·4–57·5 % in girls and 35·4–55·0 % in boys (Table 3).

In the present study, significant gender differences in %BF were observed in normal-weight and overweight children while %BF was not significantly different between obese boys and girls. Normal-weight and overweight girls had significantly higher %BF (median (range): 32·4 % (24·7–39·3 %) and 38·3 % (29·3–44·2 %), respectively) compared with boys (median (range): 26·5 % (14·2–37·1 %) and 34·6 % (29·9–40·2 %), respectively). Of particular interest is the finding that also children with high %BF were classified as normal weight (over 35 % body fat) and overweight (over 40 % body fat) in both boys and girls. These data thus support the discussion by Liu *et al.*[9] regarding the lack of sensitivity of defining overweight and obesity by WHO criteria. Based on these observations, it is clear that more studies are needed to better describe the relationship between body size, body composition and risk of developing non-communicable diseases in children worldwide.

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References

1. Haslam D & James W (2005) Obesity. *Lancet* **366**, 1197–1209.
2. Lobstein T, Baur L & Uauy R (2004) Obesity in children and young people: a crisis in public health. *Obes Rev* **5**, Suppl. 1, 4–104.
3. Wang Y & Lobstein T (2006) Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes* **1**, 11–25.
4. Ministry of Health (2010) *Kuwait Nutrition Surveillance System Report*. Kuwait City: Community Nutrition Promotion Supervisory, Administration of Food and Nutrition, Ministry of Health, State of Kuwait.
5. de Onis M, Onyango AW, Borghi E *et al.* (2007) Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ* **85**, 660–667.
6. World Health Organization (2009) *Software for Assessing Growth of the World's Children and Adolescents*. Geneva: WHO; available at http://www.who.int/growthref/tools/en/
7. World Health Organization (2012) *Obesity and Overweight. Fact sheet* no. 311. Geneva: WHO; available at http://www.who.int/mediacentre/factsheets/fs311/en/
8. International Atomic Energy Agency (2009) *Assessment of Body Composition and Total Energy Expenditure in Humans using Stable Isotope Techniques. IAEA Human Health Series* no. 3. Vienna: IAEA; available at http://www-pub.iaea.org/MTCD/Publications/PDF/Pub1370_web.pdf
9. Liu A, Byrne N, Kagawa M *et al.* (2011) Ethnic differences in the relationship between body mass index and percentage body fat among Asian children from different backgrounds. *Br J Nutr* **106**, 1390–1397.
10. International Atomic Energy Agency (2010) *Introduction to Body Composition Assessment using the Deuterium Dilution Technique with Analysis of Urine Samples by Isotope Ratio Mass Spectrometry. IAEA Human Health Series* no. 13. Vienna: IAEA; available at http://www-pub.iaea.org/MTCD/Publications/PDF/Pub1451_web.pdf
11. Lohman T (1992) Estimating body composition in children and the elderly. In *Advances in Body Composition Assessment*, pp. 65–77 [TG Lohman, editor]. Champaign, IL: Human Kinetics Publishers.
12. Wells JC (2000) A Hattori chart analysis of body mass index in infants and children. *Int J Obese Relat Metab Disord* **24**, 325–329.

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