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ORIGINAL COMMUNICATION

^2H_2O turnover method as a means to detect bias in estimations of intake of nonbreast milk liquids in breast-fed infants

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Objective: Firstly, to compare food, and macronutrient intake as obtained from a single 24-h recall and a frequency questionnaire (FQ) covering a 14-day period in breast-fed infants aged 4 months of age. Secondly, nonbreast milk water intake (NB-WI, ml/day) was used as an estimation of energy and macronutrient intake, and NB-WI as calculated from FQ ($NB\text{-}WI_{FQ}$) was compared with NB-WI as measured using the dose-to-the-mother ^2H_2O turnover method ($NB\text{-}WI_{DO}$) covering the same 14-day period.
Design: Cross-sectional.
Setting: Community-based study in urban Pelotas, Southern Brazil.
Subjects: In all, 67 breast-fed infants aged 4 months of age recruited at birth.
Main outcome measures: (1) Bias in estimations of food and macronutrient intake of the 24-h recall relative to FQ; (2) Bias in $NB\text{-}WI_{FQ}$ relative to $NB\text{-}WI_{DO}$.
Results: In infants with an energy $intake_{FQ}$ from complementary foods above the 50th percentile (1.03 kcal/day), estimations of water, tea, juice, and milk intake were not different between 24-h recall and FQ ($n = 34$). Nor were estimations of energy and macronutrient intake (protein, fat, and carbohydrates) different between the two methods, and bias was nonsignificant. $NB\text{-}WI_{DO}$ was divided into quintiles and compared with $NB\text{-}WI_{FQ}$. The first two quintiles included negative values for $NB\text{-}WI_{DO}$ as a result of random errors of the ^2H_2O turnover method. Subsequently, bias of $NB\text{-}WI_{FQ}$ relative to $NB\text{-}WI_{DO}$ was positive in the 1st ($P = 0.001$) and 2nd quintile ($P = 0.638$), respectively. Bias was negative for the three highest quintiles, and within this group, underestimation by FQ was significant for the 3rd and 4th quintile (−57.4%, $P = 0.019$; −43.7%, $P = 0.019$).
Conclusions: Firstly, at the age of 4 months FQ covering a 14-day period provides similar results on food and macronutrient intake as compared to a single 24-h recall for estimations of complementary liquid foods. Secondly, $NB\text{-}WI_{FQ}$ appeared to be a good proxy for macronutrient and energy intake in breast-fed infants receiving other liquids. In infants with $NB\text{-}WI_{DO} > 0$, the method provides a useful tool for the detection of bias from FQ, and results indicate an underestimation from FQ relative to the ^2H_2O turnover method. This exercise could be applied wherever the ^2H_2O turnover method is used in combination with conventional food consumption techniques for measuring intake of nonbreast milk liquids of breast-fed infants in whom solid foods have not yet been introduced. It would help interpreting estimations of macronutrient intake, and could be relevant to studies of dietary intake of infants and its relationship with growth and health.
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Introduction

Assessment of dietary intake is essential for defining relationships between diet and health. For breast-fed infants, this means that the intakes of both breast milk and complementary foods need to be accurately measured. Breast milk intake can be measured by test-weighing or calculated from water turnover measured using stable isotopically labelled water (deuterium oxide, ^2H_2O). In the latter case, isotope doses may be given either to the infant (Coward *et al*, 1979; Butte *et al*, 1983, 1991; Lucas *et al*, 1987) or to the mother (Coward *et al*, 1982; Coward, 1984; Orr-Ewing *et al*, 1986; Butte *et al*, 1988; Haisma *et al*, 2003). The latter method is referred to as the dose-to-the-mother ^2H_2O turnover method, and yields estimates for (1) the water flux from mother to baby (ie water from milk, including metabolic water from milk oxidation); (2) the water flux from nonbreast milk foods or liquids (ie nonbreast milk water, including metabolic water from oxidation of foods and liquids (NB-WI_{DO})). A comprehensive review of comparisons between the isotope methods and test-weighing (Scanlon *et al*, 2002) indicates that they give comparable results but in the case of the dose to the infant method appropriate corrections need to be made for environmental water influx and insensible water losses.

Assessment of complementary feeding is a challenging task for several reasons: (1) Infants eat small amounts of foods at frequent intervals; (2) They often spend time under the care of several different persons; (3) They are unable to complete questionnaires on their own. A number of methods have been used to estimate the intake of complementary foods, including direct weighing of foods consumed (de Kanashiro *et al*, 1990; Piwoz *et al*, 1994; de Bruin *et al*, 1998), or estimations from food diaries (Lanigan *et al*, 2001), frequency questionnaires (Nielsen *et al*, 1998), and 24-h dietary recall (Ferguson *et al*, 1994; Olinto *et al*, 1995). Most widely used is the 24-h recall because of its practicability in field studies. However, this method may be affected by recall bias, and several authors have evaluated their validity against a reference method. The latter studies have compared the 24-h recall against food-weighing or the doubly labelled water method, demonstrating that systematic bias towards underestimation frequently occurs (Klesges *et al*, 1987; Ferguson *et al*, 1994; Livingstone & Robson, 2000), although overestimation is also found (Horst *et al*, 1988; Olinto *et al*, 1995). Since between-day variation can be quite large in adults (Balogh *et al*, 1971; Beaton *et al*, 1979), it is recommended that repeated recalls are obtained for assessment of usual intake. This may be less important in infants, as day-to-day variation of infants' food intake is likely to be smaller (Kylberg, 1986). Day-to-day variation was found to increase with the introduction of solids in infants 2–4 months of age (Black *et al*, 1983), and was also found to be larger in Indo-Asian (Harbottle & Duggan, 1994) as compared to Caucasian infants (Black *et al*, 1983).

In the present study, we compared a single 24-h recall and an FQ covering a 14-day period for the estimation of intake of macronutrients from complementary liquid foods in breast-fed infants aged 4 months of age. In addition, NB-WI_{DO} as measured during a 14-day period using the dose-to-the-mother ^2H_2O turnover method was compared against NB-WI_{FQ} calculated from an FQ covering the same 14-days. NB-WI was used as a proxy for macronutrient intake, and a bias in NB-WI_{FQ} relative to NB-WI_{DO} was interpreted as a bias in estimation of macronutrient intake from FQ. This work was an ancillary part of a study on breast-milk and energy intake (Haisma *et al*, 2003) and was embedded in a lactation counselling intervention (Albernaz *et al*, 2003).

Methods

Subjects

Mother–infant pairs were recruited at birth from three main hospitals in Pelotas, a city in the south of Brazil. Eligibility criteria were (de Onis *et al*, 2001): (1) the mothers were living in the urban area of Pelotas, were nonsmokers and were willing to breast-feed; (2) the babies were single births, gestational age was between 37 and 42 weeks and the postnatal stay at the intensive care unit was <24 h; (3) family income was more than 800 reais per month (equivalent to about USD 500/month). Mothers who introduced formula or cows' milk during the first 14 days after birth, and who started smoking during this period were also excluded from participation.

NB-WI_{DO} was measured in 70 infants. From two infants no data were available on the volume of liquids consumed using the FQ, and from one infant a 24-h recall was not obtained. A complete data set was available from 67 infants, and analyses were based on that sample.

Food and macronutrient intake

Food and macronutrient intake was assessed using (1) an FQ (reference method); (2) a 24-h recall.

FQ. At the last day of the deuterium study (see below), when the infants were on average 122 days old (range 115–134 days) an FQ was applied. The FQ could have been administered before, after or on the same day as the 24-h recall, and the effect of order of application was studied (see Statistics section). Mothers were asked to recall the number of days water, tea, juice, fruits, and solids had been given during the entire 14-day study. In addition, mothers estimated the volume of nonbreast milk liquids, and reported the intake of fruits (whole, half-, one-third, etc) and solid foods (in table spoons). The weight of a fruit or solid was calculated from these units using standardised Brazilian tables. No information was obtained on the type of fruit or composition of the vegetable stew consumed, and weights of intake of solids are presented only to show that intake was negligible in these infants.

Infants were classified by energy intake as measured using FQ. A comparison with 24-h recall was made only for those infants with an energy intake from nonbreast milk sources above the 50th percentile (1.03 kcal/day), as below that infants would be receiving no other liquids or foods other than breast milk. In all, 33 infants had an energy intake lower than 1.03 kcal/day, and 34 had an energy intake higher than the cut-off.

24-h recall. When the infants were on average 122 days of age (range 115–129 days), a 24-h recall was used to assess the frequency of intake of complementary foods. For liquid foods the volume of intake was also estimated using household measures. No quantitative information was obtained on the intake of fruits and solids. Macronutrient intake from liquid foods was calculated using a Brazilian food composition table (IBGE, 1981).

Nonbreast milk water intake (NB-WI)
NB-WI (g/day) was assessed in the same children using two methods: (1) the dose-to-the-mother ^2H_2O turnover method (reference method); (2) an FQ.

Dose-to-the-mother ^2H_2O turnover method. Breast milk intake was measured over a 14 days' period and was initiated when the infants were on average 108 days of age (range 101–120 days). Details of the method are described elsewhere (Coward *et al*, 1982; Orr-Ewing *et al*, 1986; Butte *et al*, 1988; Haisma *et al*, 2003), but in short, the method included the administration of about 10 g of deuterium to the mother (exact quantity determined to the nearest 0.01 g using an analytical Sartorius scale) and sampling of saliva samples from the mother on day 0 (baseline), 1,4,14, and of urine from the baby on day 0 (baseline), 1,3,4,13,14. Samples were sent to MRC Human Nutrition Research, Cambridge, UK for analysis and ^2H enrichment was measured using isotope ratio mass spectrometry after equilibration with H_2 gas as described by Hoffman *et al* (2000). Precision of the measurements was 0.26 ppm. Curve fitting was performed by using the 'Solver' function in Excel® to minimise the sum of the squares of the differences between observed and fitted enrichment values for mother and baby data combined. This procedure yields estimates for (1) the water flux from mother to baby (ie water from milk, including metabolic water from milk oxidation); (2) the water flux from nonbreast milk foods or liquids (ie nonbreast milk water, including metabolic water from oxidation of foods and liquids ($NB\text{-}WI_{DO}$)).

Infants were divided into quintiles of $NB\text{-}WI_{DO}$ to allow discriminating between those who were not receiving any liquids or foods other than breast milk and those who were. The ^2H_2O turnover method allows estimates of $NB\text{-}WI_{DO}$ to be negative, and the first two quintiles included infants with negative $NB\text{-}WI_{DO}$ values. The first cut-off was — 9.8 ml/day ($n=13$), the second 15.8 ml/day ($n=14$), the third 63.4 ml/day ($n=13$), the fourth 287.6 ml/day ($n=14$), and the last quintile included infants with a $NB\text{-}WI_{DO}>287.6$ ml/day ($n=13$).

FQ. $NB\text{-}WI_{FQ}$ (ml/day) over the 14 days' period was calculated as follows:

$NB\text{-}WI_{FQ}$ (ml/day) = the water content of foods + metabolic water from oxidation of food nutrients.

The water content of foods was calculated using Brazilian food composition tables (IBGE, 1981). As mentioned above, no information was obtained on the type of fruit or vegetable stew consumed, but in the calculations of water intake we used the average food composition data of most commonly consumed fruits (ie banana, apple, papaya), and for vegetable stew we used the average composition of pumpkin, potato, carrot. Metabolic water from the oxidation of foods was calculated knowing that 1 g of fat, protein and carbohydrate yield 1.07, 0.41, and 0.55 g metabolic water, respectively (Bergmann *et al*, 1974). Deposition of nutrients in growth rather than oxidations was ignored as corrections would have been trivial. Changes in total body water during the 14-day period are accounted for in the model used.

Statistical analysis
A possible confounding effect of the lactation counselling intervention (Albernaz *et al*, 2003) on the differences between $NB\text{-}WI_{DO}$ and $NB\text{-}WI_{FQ}$ was studied using criteria as described by Rothman and Greenland (1998). As such an effect was not found, there was no need to adjust for this variable in subsequent analyses.

Food and macronutrient intakes were not normally distributed, and differences between methods were therefore tested using a Wilcoxon signed-rank nonparametric test for paired samples (SPSS software package). The effect of order of application of 24-h recall and FQ on energy and macronutrient intake was studied using a Kruskal–Wallis nonparametric test for comparison of multiple independent samples.

The Bland and Altman method was used to compare food and macronutrient intakes as measured with FQ (reference) and 24-h recall (Bland & Altman, 1986). This method plots the difference of 24-h recall and FQ against the average of the two methods. Mean differences and limits of agreement (mean differences $\pm$2s.d.) are presented in the tables. Bias of the 24-h recall relative to the average of FQ and 24-h recall was calculated from:

$$\text{Bias 24-h recall (\%)} = \frac{(\text{macronutrient}_{24h} - \text{macronutrient}_{FQ})}{(\text{macronutrient}_{24h} + \text{macronutrient}_{FQ})/2} \times 100\%$$

Bias of the FQ relative to the ^2H_2O deuterium method for estimation of NB-WI was plotted and calculated using the technique of Bland and Altman (Bland & Altman, 1986). The ^2H_2O turnover method measures rather than estimates NB-WI, and was considered superior to the FQ. The Bland and Altman method was therefore slightly adapted in that the difference in $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$ was plotted against $NB\text{-}WI_{DO}$ rather than against the average of $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$. Bias of the FQ relative to the ^2H_2O turnover method was calculated as follows:

$$\text{Bias FQ (\%)} = \frac{(NB\text{-}WI_{FQ} - NB\text{-}WI_{DO})}{NB\text{-}WI_{DO}} \times 100\%$$

Ethics

The study was approved by the ethical committee of the Universidade Federal de Pelotas, affiliated with the National Commission on Research Ethics or the Brazilian Ministry of Health, and an informed consent was signed by the parents.

Results

Sample

Table 1 presents characteristics of mother–infant pairs.

Comparison of 24-h recall *vs* FQ for measurement of food, and macronutrient intake

Of the 67 infants, 34 had an energy intake from complementary foods above the 50th percentile (1 kcal/day), and comparisons between FQ and 24-h recall will be restricted to those infants. Energy $intake_{FQ}$ of infants below the 50th percentile was 0.06 (s.d. 0.15) kcal/day; above the 50th percentile energy $intake_{FQ}$ was 118 (s.d. 162) kcal/day.

Figure 1 shows a scatter plot of energy $intake_{FQ}$ *vs* energy $intake_{24\,h}$. Spearman's rho was 0.855 ($P=0.000$). FQ and 24-h recall did not differ in their estimations of food (Table 2) or macronutrient intakes (Tables 3 and 4), and bias was not significant.

Table 2 shows that intake of fruits and solids was very low in these infants.

Table 1 Characteristics of mother–infant pairs ($n=67$)

	Mean (s.d.)
Age of the mother (y)	28.4 (5.9)
Parity	2.0 (1.0)
Maternal education (y)	11.2 (3.1)
Family income (reais per month)	1535 (1069)
Family income (no. of minimum salaries per month)	11.3 (7.9)
Mother's height (cm)	159.2 (5.9)
Mother's weight at 4 months (kg)	62.3 (9.6)
Mother's body mass index (kg/m^2)	24.7 (3.9)
Baby's birth weight (kg)	3.2 (0.3)
Baby's length at birth (cm)	48.4 (1.7)
Baby's weight at 4 months (kg)	6.7 (0.8)
Baby's length at 4 months (cm)	63.1 (2.1)
Sex ratio (male/female)	36/31

The infants were evenly distributed between those in whom the 24-h recall was applied before ($n=11$), on the same day ($n=9$), or after the FQ ($n=14$), and there was a tendency for 24-h recall to give higher values of energy and macronutrient intake as compared to FQ in the first two categories, and lower values in the latter. This trend was significant only for carbohydrates ($P=0.038$).

$NB\text{-}WI_{FQ}$ as a proxy of macronutrient intake

Figure 2 shows the association between $NB\text{-}WI_{FQ}$ and energy intake from nonbreast milk liquids as measured with FQ. Spearman's rho was 0.973 ($P=0.000$). The association with protein, carbohydrate was of the same magnitude ($r=0.968$, $P=0.000$; $r=0.979$, $P=0.000$). The association with fat intake was slightly lower ($r=0.748$, $P=0.000$).

Comparison of $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$

Figure 3 shows a scatter plot of $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$. Spearman's rho was 0.735 ($P=0.000$). Means, standard

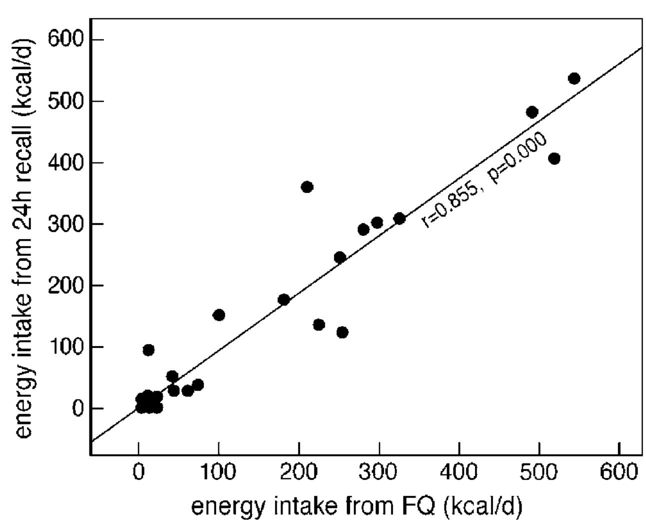

Figure 1 Scatterplot of energy intake (kcal/day) as measured with FQ and 24-h recall. The line represents the relationship between FQ and 24-h recall.

Table 2 Comparisons of sample means of intakes of food as measured with a 24-h recall and FQ ($n=34$)

	24-h recall			*FQ*				
Food	*Mean*	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*	*Bias (% mean)*	*P-value*[a]
Water (ml/day)	7.9	23.3	0–100	3.8	11.6	0–60	71.0	0.507
Tea (ml/day)	31.0	54.6	0–240	34.4	51.6	0–240	−10.3	0.768
Juice (ml/day)	25.2	51.9	0–230	29.4	49.4	0–180	−15.4	0.501
Milk (ml/day)	147	230	0–800	149	235	0–800	−1.1	0.556
Fruits (unit/day)	—	—	—	0.15	0.38	0–2	—	—
Solids (spoons/day)	—	—	—	0.06	0.30	0–1.7	—	—

[a]Wilcoxon signed-rank nonparametric test for paired samples.

Table 3 Comparison of sample means for intake of energy and energy-yielding nutrients from nonbreast milk liquids ($n=34$)

	24-h recall			*FQ*			
Nutrient	*Mean*	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*	P-*value*[a]
Energy (kJ/day)	27.2	37.5	0–128	28.2	38.7	0.2–130	0.235
Energy (kcal/day)	114	157	0–536	118	162	1–542	
Protein (g/day)	4.1	5.3	0–17.6	4.0	5.3	0–18.5	0.908
Fat (g/day)	4.4	6.9	0–24.0	4.5	7.0	0–24.0	0.556
Carbohydrate (g/day)	16.3	19.4	0–63.3	17.5	20.3	0.34–67.3	0.448

[a]Wilcoxon signed-rank nonparametric test for paired samples.

Table 4 Absolute and relative bias and limits of agreement of 24-h recall *vs* FQ (reference) ($n=34$)

Nutrient	*Bias*	*Limits of agreement*	*Bias (% mean)*
Energy (kJ/day)	−1.00	22.6	−3.6
Energy (kcal/day)	−4.28	94.4	
Protein (g/day)	0.06	3.7	1.5
Fat (g/day)	−0.04	3.7	1.0
Carbohydrate (g/day)	−1.22	14.8	7.2

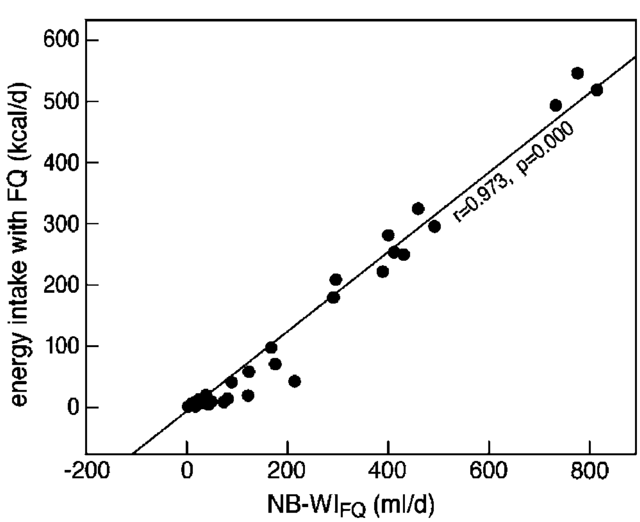

Figure 2 Scatterplot of the association between NB-WI_{FQ} and energy intake as measured with FQ.

deviations, and ranges are presented in Table 5; and Table 6 shows absolute and relative biases, and limits of agreement. The first two quintiles included negative values for NB-WI_{DO}, and as NB-WI_{FQ} can only be positive, the bias of NB-WI_{FQ} relative to NB-WI_{DO} was positive by 110 and 44.6% in the 1st and 2nd quintile, respectively. Bias was negative for the three highest quintiles, and within this group, underestimation by FQ was significant for the 3rd and 4th quintile (−57.4%, $P=0.019$; −43.7%, $P=0.019$). Figure 4 illustrates that underestimation was systematic in the lower range of NB-WI_{DO}, and that with higher NB-WI_{DO} bias was more randomly distributed.

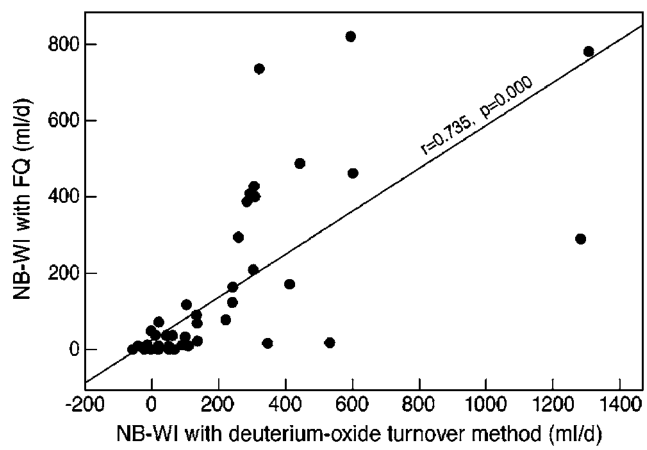

Figure 3 Scatterplot of NBWI as measured with deuterium-oxide and FQ. The line represents the association between deuterium-oxide turnover method and FQ.

Discussion

Measurement of food intake of infants has been recognised to be a difficult task. In studies of food intake of breast-fed infants, both breast milk intake and intake of complementary foods need to be accurately assessed. In adults, repeated measurements are needed to obtain accurate estimates of macronutrient and energy intake, but in young infants this may be different due to smaller day-to-day variation in food intake (Kylberg, 1986). We found very similar results for a 14-day FQ and a single day 24-h recall. The implication of this is that in young infants who are receiving negligible amounts of solids, there is no need for multiple 24-h recalls even if the interest is in usual intake. A single 24-h recall or alternatively a 14-day FQ could be applied.

The dose-to-the-mother ^2H_2O turnover method provides information not only on breast milk intake but also on NB-WI_{DO}. The latter was used as a reference value against which the FQ was tested. NB-WI_{FQ} was used as a proxy of energy and macronutrient intake. Essential to the relevance of comparing NB-WI_{DO} and NB-WI as calculated from a conventional food intake method, is the appropriateness of using NB-WI as a proxy of energy and macronutrient intakes, and this again depends on the accuracy with which water intake from foods can be estimated. In infants not yet

Table 5 Comparisons of $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$ (ml/day)

	FQ (ml/day)			*^2H_2O turnover method (ml/day)*			
Quintile	*Mean*	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*	P*-value*[a]
<−9.8 ml/day	2.6	4.0	0–10.8	−25.5	15.5	−58 to −10	0.001
<15.8 ml/day	6.8	16.1	0–48.6	4.7	8.7	−10–15	0.638
<63.4 ml/day	9.8	21.9	0–74.2	35.7	14.7	18–60	0.019
<288 ml/day	105	113	0–389	156	76.0	64–284	0.019
≥288 ml/day	404	263	18.5–817	542	352	294–1306	0.279
All infants	104	197	0–817	141	259	−58–1306	0.135

[a]Wilcoxon signed-rank nonparametric test for paired samples.

Table 6 Absolute, and relative bias and limits of agreement of $NB\text{-}WI_{FQ}$ *vs* $NB\text{-}WI_{DO}$

$NB\text{-}WI_{DO}$	*Bias (ml/day)*	*Limits of agreement (ml/day)*	*Bias (%)*
<−9.8 ml/day (*n* = 13)	28.1	31.7	110
<15.8 ml/day (*n* = 14)	2.1	38.4	44.6
<63.4 ml/day (*n* = 13)	−25.8	58.8	−72.3
<288 ml/day (*n* = 14)	−51.1	133	−32.8
≥288 ml/day (*n* = 13)	−138	758	−25.5
All infants	−36.7	349	−26.0

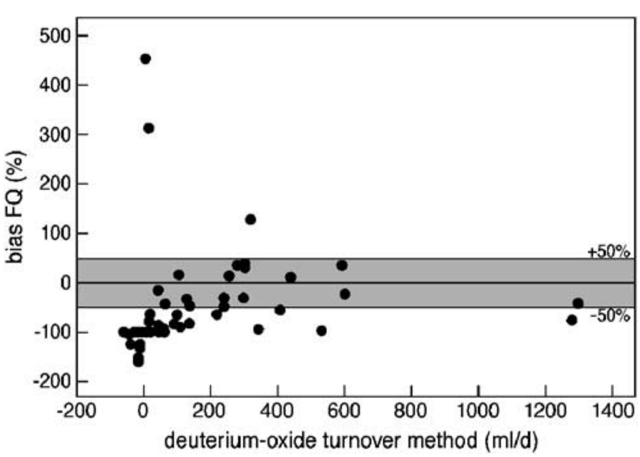

Figure 4 Bias of the FQ method (%) relative to the deuterium-oxide turnover method for measurement of NB-WI (ml/day).

receiving solid foods, $NB\text{-}WI_{FQ}$ appeared to be an excellent proxy of energy and macronutrient intakes, and the dose-to-the-mother ^2H_2O turnover method proved to be a useful tool in the detection of bias from FQ in these infants. Food composition tables include data on the water content of foods, and to allow comparison with $NB\text{-}WI_{DO}$, metabolic water from oxidation of foods was added to constitute $NB\text{-}WI_{FQ}$.

As a consequence of the error of the ^2H_2O turnover method for estimation of $NB\text{-}WI_{DO}$, negative values were obtained in infants with zero or low nonbreast milk water intake. In those infants, the ^2H_2O turnover method underestimates true intake. In infants with a $NB\text{-}WI_{DO} > 0$, FQ appears to underestimate intake, but significantly so only in the 3rd and 4th quintile. Although a comparison was presented only for $NB\text{-}WI_{FQ}$ and $NB\text{-}WI_{DO}$, it was also done for $NB\text{-}WI_{24\,h}$ and $NB\text{-}WI_{DO}$, with very similar results.

A strength of the ^2H_2O turnover method (both for measurement of breast milk intake and NB-WI) is that it does not interfere with daily routine and activities, and that (for NB-WI) it covers consumption of foods at times when the infant was not under the direct supervision of the parent (or the person interviewed). This is difficult to achieve with any recall method. It is therefore not surprising that the ^2H_2O turnover method gives higher values for nonbreast milk water intake than FQ.

The validity of the dose-to-the-mother ^2H_2O turnover method for the assessment of NB-WI has been studied by Infante *et al* (1991). They used formula milk labelled with exponentially decreasing doses of ^2H_2O which represented milk from a 'pseudo mother' of exclusively bottle-fed infants. Unlabelled formula represented milk from other sources. Good agreement existed between mean NB-WI data obtained using weighted bottles, and ^2H_2O. At an individual level however, the ^2H_2O turnover method may either over- or underestimate NB-WI, and the negative values found in our study are an obvious example of underestimation.

Validation studies of food consumption have used food-weighing as the gold standard (Dop *et al*, 1994; Lanigan *et al*, 2001). Or alternatively, the DLW method has been used to compare with energy intake (Perks *et al*, 2000; Lanigan *et al*, 2001). But to our knowledge, the ^2H_2O turnover method has not been used for this purpose before. The results indicate that although the 24-h recall and the 14-day FQ show good agreement, both methods may underestimate true intake.

In studies where breast milk intake is measured using the ^2H_2O turnover method, data on intake of other foods are usually obtained from conventional food intake data. A comparison of NB-WI between the methods then comes for free, and does not include any extra work or costs. Still, for interpretation of macronutrient intake including both breast milk and nonbreast milk liquids, it can make an important contribution.

Unfortunately however, the use of ^2H_2O for comparison of NB-WI cannot be extended to infants already receiving complementary solid foods. Although in theory, the method should also work in older infants, in another study (Haisma, unpublished results), we found that the association between energy intake from complementary foods and NB-WI measured with 1-day food weighing was poor, due to difficulties in estimating the water content of solids and prepared foods.

A last comment concerns the external validity of the study. The mothers participating in this study were all from high socioeconomic class, and it is possibly that different results would be obtained in infants from lower socioeconomic class. For example, from the same research centre overestimation of dietary intake using a 24-h recall was reported in infants from low socioeconomic class (Olinto *et al*, 1995), and the estimation of breast-feeding duration also appeared to depend on socioeconomic class (Huttly *et al*, 1990).

Summarizing, the ^2H_2O turnover method not only provides data on breast milk intake, but also on water intake from nonbreast milk foods. We suggest that in breast-fed infants in whom solid foods have not yet been introduced the latter component be used for a comparison with data obtained from conventional methods that usually complement the isotope work. This will help interpreting estimations of macronutrient intake in these infants, which are known to be difficult to obtain accurately. This in turn, will have direct relevance to studies of dietary intake of infants and its relationship with growth and health.

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