Dilution kinetics of H_2^{18}O for the measurement of total body water in preterm babies in the first week after birth

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Abstract

The aim of this study was to determine the kinetics of H_2^{18}O equilibration and elimination in preterm babies for the estimation of total body water. Thirteen, clinically stable, preterm babies of less than 32 weeks' gestation were studied in the first week after birth. Blood and urine samples were obtained for baseline measurement of $^{18}\text{O}:^{16}\text{O}$ ratio and 1 ml/kg of 10% H_2^{18}O (0.1 g/kg isotope) administered orally. Eleven blood samples were obtained over the next six hours and between one and four over the next 18 hours. During the same 24 hour period between three and eight urine samples were also obtained. The dilution space at zero time (volume of distribution or total body water) was estimated using double exponential curve fitting using all available points, from single samples and from linear regression on the log data using two or three samples.

Equilibration time was variable and showed a significant correlation with percentage change in body weight from birth. For blood samples, the median time to equilibrium was 81 minutes (range 2 to 191). A plateau phase was not detected, with H_2^{18}O enrichment declining after the point of maximum enrichment. The median volume of distribution at time 0, based on double exponential curve fit analysis, was 859 ml/kg (range 755 to 995). The volume of distribution, estimated from linear regression on the log data using two serum samples obtained at three and six hours, approximated most closely to that based on exponential curve fit analysis with a median difference of -4 ml/kg (range -41 to 73). It was concluded that in most situations blood sampling at three and six hours may be acceptable. However, as equilibration time is variable and influenced by the state of expansion or depletion of body water compartments, when studying overhydration states, multiple sampling is advised in order to be certain that the elimination phase has been reached.

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There are many influences on body water distribution in the perinatal period. Hydration in utero varies from baby to baby. At birth, weight is reported to correlate well with total body water but poorly with extracellular

water.¹ After birth, there is a reduction in total body water and body water distribution changes. Extracellular water is lost and this is marked clinically by the well recognised post-natal natriuresis/diuresis; the trigger to this diuresis is not known but there is evidence that relates it to cardiopulmonary adaptation.² Fluid balance is further influenced by organ immaturity and by the difficulties of clinical management.

The ability to repeatedly and non-invasively measure body water compartments in immature infants would be invaluable. As part of a larger study to develop non-invasive methods of estimating body water distribution, we wished to optimise standard methods of measuring total body water as applied to preterm babies. The dilution of isotopic water, H_2^{18}O , is a standard method in older subjects.³ This method is based on Fick's principle, which states that the volume (V) of a fluid space may be calculated after the administration of a marker into that space if the exact amount of the marker injected (Q) and its concentration (C) in the fluid are known. The marker must diffuse evenly within a predictable period of time throughout its volume of distribution, it must have no influence on fluxes of fluids across membranes, and must neither be metabolised nor cleared over the duration of the study. As this last condition is rarely met, extrapolation of the line of clearance, or elimination, to time 0, or y intercept, allows a volume of distribution to be calculated, as $V=Q/C_0$, where C_0 is concentration at time 0. Accurate extrapolation of the line of clearance to time 0 requires an understanding of equilibration and clearance of the marker in the subject studied. The kinetics of H_2^{18}O have not been well defined, particularly in the extremely immature neonate. The limited data available is conflicting^{4,5} and mainly derived from animal studies⁴ and studies in older populations.⁵ Trowbridge *et al* describe studies in premature infants after the first eight days after birth but provide no details of birth weight, gestational age, or postnatal age at the time of study.⁵ There have been no previous studies of human infants in the first week after birth. The aim of this study was to determine the kinetics of H_2^{18}O in premature babies in order to enable optimal timing of a minimal number of blood samples as frequent sampling is undesirable.

Subjects and methods

Thirteen preterm babies admitted to the neonatal unit at Queen Charlotte's and Chelsea

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Hospital were studied. The babies were clinically stable and intra-arterial access was in place for clinical indications. None of the babies had clinical or laboratory evidence of renal, gastrointestinal, cardiac, or hepatic disease. Each baby was studied once, commencing within the first seven days of birth. Two blood samples (0.1 ml) and one urine sample were collected for determination of basal ^{18}O : ^{16}O ratio before a dose of 0.1 g/kg of $H_2^{18}O$ was administered orally. Blood samples after the dose of $H_2^{18}O$ were obtained at 10, 20, 30, 45, 60, and 90 minutes, at hourly intervals for the next five hours, and on between one and four occasions during the next 18 hours. During the same 24 hour period between three and eight urine samples were also obtained. Blood samples were immediately centrifuged and the serum separated. Both serum and urine samples were then kept at $-20^\circ C$ until assayed. The study was approved by the Hammersmith and Queen Charlotte's research ethics committee and written parental consent was obtained.

$H_2^{18}O$ ANALYSIS

Serum and urine aliquots of 50 μl were equilibrated with 5% carbon dioxide in 20 ml Vacutainers (Becton Dickinson) for three days before analysis of the carbon dioxide for ^{18}O : ^{16}O ratio using a Finnigan MAT Delta D gas isotope ratio mass spectrometer fitted with an automated breath gas analysis system as previously described.⁶ Standard corrections for fractionation and dilution of the label by the added carbon dioxide were applied. Total body water was calculated by the method of Schoeller *et al*³ after standardising the enrichment of the given tracer by analysis of suitable dilutions with tap water. The analytical error is about 6 ppm (SD). Precision was 1.2%.

KINETICS AND STATISTICAL ANALYSIS

Assessment of the agreement between the various methods for estimating volume of distribution was based on the method of Bland and Altman.⁷ Results are quoted as median and range as we wish to make no assumptions about the distribution of the results. Concentration at time 0 (C_0) was initially estimated by fitting a double exponential curve to concentration/time data using BMDP program 3R for non-linear regression. The form of the curve was

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_t is concentration at time t . $Ae^{-\alpha t}$ refers to the fast distribution or mixing phase and $Be^{-\beta t}$ to the slower elimination or clearance phase. The constraint $A = -B$ was used to ensure that the fitted curve passed through the point (0,0). The kinetic parameters extracted from this curve were $C_0 = B$, elimination rate coefficient $= \beta$, equilibration time (defined as the time of the peak of the curve) $= \log_e(\alpha/\beta)/(\beta - \alpha)$, elimination half life $= \log_e(2)/\beta$ and volume of distribution, derived from C_0 as above. C_0 could also be

estimated from linear regression on the log data from the elimination phase,

$$C_t = Be^{-\beta t}$$

$$\text{so} \quad \log_e(C_t) = V + Wt$$

$$\text{where} \quad V = \log_e(B) \text{ and } W = -\beta$$

$$\text{giving} \quad C_0 = e^V$$

When only two time points are used C_0 could also be estimated by fitting a straight line to the log_e data and extrapolating the line back to time 0. However this technique requires a knowledge of equilibration time, to be certain that the elimination phase has been entered. As we wished to make no assumptions about equilibration time, we used the double exponential curve method, using all available data, to obtain our 'reference' estimate. We then compared estimates based on double exponential curve fit analysis with estimates derived by linear regression, on the log_e data, using serum samples obtained at three and six hours, four and six hours and three, six, and 12 hours. Similarly, comparison was also made with estimates based on single serum samples taken at three, four, five, and six hours. For urine samples we compared the reference estimate (double exponential curve fitting of all serum points) with estimates based on single urine samples taken as close as possible to six and 12 hours and with estimates based on double exponential curve fitting using all available urine samples. As urine obtained at a particular time would contain ^{18}O eliminated at all times from the previous urine sample, the time of urine sampling would not be truly comparable with the time relating to the serum O^{18} concentration. Therefore the urine based estimates were repeated having adjusted the time of the urine sample to be the mid point between the time of the previous and the current sample.

Results

Thirteen infants were studied, at a median postnatal age of 3 days (range 1 to 6). Three were girls and one was small for gestational age. The median gestational age was 29 weeks (range 24 to 33) and median birth weight 1050 g (range 680–2650).

Equilibration time, defined as the point of maximum concentration of ^{18}O , was variable and showed a significant correlation with percentage change in body weight from birth ($r = 0.72$, $p = 0.006$) (fig 1). A plateau phase did not occur, with $H_2^{18}O$ enrichment declining after the point of maximum enrichment.

The concentration-time curves of ^{18}O in serum and urine for each baby are shown in figs 2 and 3. The kinetic results are summarised in table 1.

The median volume of distribution at zero time, based on double exponential curve fit analysis of serum samples, was 859 ml/kg (range 755 to 995). Table 2 shows the estimated volume of distribution for all 13 babies using the different methods. Table 3 shows a

Table 1 Kinetic results

	Median	Minimum	Maximum
Concentration at time 0 (ppm)	125	98	144
Elimination rate coefficient (/hour)	0.00882	0.00429	0.0163
Elimination half life (min)	79	43	162
Volume of distribution (ml/kg)	859	755	995
Equilibration time (min)	81	1.7	191

Figure 1 Equilibration time (hours) by % change in body weight from birth.

Figure 2 Serum concentration-time curves for each baby.

Figure 3 Urine concentration-time curves for each baby.

comparison of each method of estimating volume of distribution with the estimate based on double exponential curve fit analysis. Table 4 shows the mean differences and limits of

Table 2 Volume of distribution (ml/kg) for each method

Method	Baby No												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Serum													
Double exponential	878	939	755	808	800	806	915	995	872	860	846	859	822
3	889	936	760	837	815	824	928	968	904	935	*	909	860
4	895	959	767	862	801	803	939	987	880	*	*	928	*
5	861	962	784	838	*	830	934	962	903	913	893	948	*
6	861	973	788	845	813	835	985	956	962	937	917	976	840
3 and 6	918	904	734	829	816	813	873	979	850	933	*	841	880
4 and 6	967	936	727	898	777	741	852	1052	736	*	*	838	*
3, 6, and 12	859	912	723	824	770	798	910	905	890	933	744	849	827
Urine													
Double exponential	810	837	716	614	395	647	759	774	750	785	661	928	771
Double exponential (adjusted times)	821	869	730	677	734	727	812	812	779	822	758	941	798
6	863	892	761	812	816	793	1018	945	826	871	992	1031	826
12	924	1022	852	883	915	886	1011	1092	1016	*	1170	1013	901

* Indicates no estimate available due to missing data.
Sampling times in hours.

agreement⁷ for the three methods providing the closest approximation to our reference estimate. The volume of distribution estimated from the regression line of the log_e data using two blood samples obtained at three and six hours, approximated most closely to that based on double exponential curve fit analysis with a median difference of -4 ml/kg (range -41 to 73).

Discussion

There have been few studies of total body water in extremely immature infants. In other age groups, water soluble tracers such as antipyrine or urea have been used in dilution studies, but they give rise to errors because the tracers are either rapidly cleared, bound to proteins, or slowly and incompletely distributed to all body water compartments.⁸ Frequently used tracers have been water labelled with either tritium or deuterium. Unfortunately, a level of deuterated water in excess of 10% is harmful,³ and therefore the isotope can be administered only in small amounts. The deuterium enrichments are therefore low and must be measured by 2H/H isotope ratio mass spectrometry that involves the time consuming process of first converting the water sample to gaseous hydrogen. Although tritium is easily assayed by scintillation counting, the radiation hazard makes it unsuitable for children. Furthermore, the hydrogen dilution volume is greater than the water space because of the exchange with labile protein hydrogen.³

¹⁸O, as a tracer for body water measurements, has the advantages of deuterium and tritium without their disadvantages. It is stable, non-radioactive, non-toxic, and a naturally occurring isotope that is normally present in the body. The replacement of 60% of total body water in mice by H₂¹⁸O, over three generations, resulted in no change in mortality or reproductive performance nor in histological changes.⁹ The dose used in dilution studies will only increase the existing concentration of ¹⁸O in the body by 4% or equivalent to 0.05% of total body water.

¹⁸O can be measured non-invasively as breath carbon dioxide because the oxygen in water is in rapid equilibrium with the oxygen in blood bicarbonate. However, this analysis requires rapid transport of breath samples to

Table 3 Comparison of each method of estimating volume of distribution (ml/kg) with double exponential curve fit estimate; difference between the two methods

	Difference from double exponential estimate			
	No	Median	Minimum	Maximum
Serum				
3	12	16.5	-27.2	74.8
4	10	14.6	-7.7	69.0
5	11	29.5	-32.8	89.3
6	13	34.1	-38.6	117.4
3 and 6	12	-4.1	-41.2	72.8
4 and 6	10	-22.0	-136.3	90.3
3, 6, and 12	13	-10.0	-102.4	72.8
Urine				
Double exponential	13	-122.8	-405.1	69.2
Double exponential (adjusted times)	13	-69.6	-182.4	82.9
6	13	4.6	-49.7	172.8
12	12	97.0	46.4	323.5

Sampling times in hours.

Table 4 Comparison of best three methods with double exponential curve fit estimate; mean difference and limits of agreement

Serum sampling time (hours)	Mean difference	Limits of agreement	Average of methods
3	21.4	-30.0, 72.8	870
4	19.4	-29.0, 67.8	872
3 and 6	5.3	-67.4, 77.9	862

the laboratory. When samples are collected at a remote site, other biological fluids, such as serum or urine are necessary. After a small dose of isotope ^{18}O is administered, total body water may be estimated from the subsequent concentration of the isotope in blood samples. The accuracy of this technique depends on appropriately timed sampling based on a knowledge of the kinetics of the isotope in the population studied. It is obviously desirable to minimise or avoid blood sampling, and the concentration of ^{18}O in urine has also been used in older age groups.

We have shown that, in this population and in contrast to other reports, time to equilibration was variable. Trowbridge *et al.*, studying infants between 6–36 months, suggested that equilibration was complete by four hours and then remained stable for at least two hours.⁵ The same workers, studying preterm infants after the first week, showed good agreement between estimates of total body water based on urine samples with blood samples taken at six hours. However these studies were undertaken on preterm infants of varying postnatal age and, in addition, equilibration at six hours was assumed. Brans *et al.*, reporting a study of four full term baboon neonates between 2–6 days, found virtually instantaneous equilibration, with the ^{18}O : ^{16}O ratio beginning to return to normal within minutes of enrichment.⁴ The speed of equilibration in our study was closely related to the change in weight from birth, with rapid equilibration seen in those infants who were below their birth weight (fig 1). As weight loss is to be expected in such a population during the first week after birth, the inference must be that water flux, and therefore ^{18}O equilibration, is slower in babies who are volume replete, as shown by inappropriate weight gain.

We did not find a stable 'plateau' phase, as ^{18}O concentration began to decline almost immediately after reaching maximal concentration. Therefore, unlike Trowbridge *et al.*,⁵

we cannot recommend single sampling between four and six hours. A problem with our study population was that fluid balance could not be held absolutely stable during the period over which the observations were made as all infants had steady rate intravenous infusions running and, in addition, body water compartments are known to be changing during the first days after birth. Nevertheless, as the majority of samples were taken over a short period of a few hours we feel that any error introduced must be relatively small.

As volume of distribution is inversely proportional to concentration of tracer, measurements based on a single blood or urine sample should overestimate total body water. Linear regression on the $\log_e$ data from the exponential elimination phase with extrapolation to time 0 is acceptable but can only be applied if one is certain that the elimination phase has been entered. We have shown that volume replete subjects have a longer equilibration time. On balance, taking into account the size of the bias, the range of differences from the reference standard and the distribution of differences in relation to the average of the alternative method and the reference standard (assessed using plots of difference *v* average⁷), the methods performing best in a statistical sense were those based on single serum samples taken at three or four hours and on paired sampling at three and six hours. Estimates based on urine samples were unsatisfactory, although a closer approximation was derived by adjusting the sample times to the mean time between samples.

In conclusion, when using the $H_2^{18}O$ dilution technique to assess total body water in preterm neonates, estimates based on paired blood samples taken at three and six hours may be acceptable. However when studying overhydration states, we advocate multiple sampling in order to be certain that the elimination phase has been reached.

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