USE OF DEUTERIUM OXIDE TO MEASURE BREAST-MILK INTAKE IN CHILDREN AGED 7 TO 12 MONTHS RECEIVING COMPLEMENTARY FOODS

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Abstract

The present study is being conducted to pilot the use of the deuterium oxide method for the measurement of breast-milk intake in children 7 - 12 months of age receiving complementary foods. This will be applied to a community efficacy study to determine the effects on total energy and nutrient intake and on breast-milk consumption of an intensive education intervention using locally available, culturally acceptable complementary foods. In order to apply the methodology to this evaluation the washout period of deuterium from the mother and the child after the administration of a dose to the mother is being determined and the comparison of this methodology with the test weighing technique for breast-milk intake. The measurement of deuterium oxide using the infrared spectrometer of the Instituto de Investigacion Nutricional [IIN] is being compared with the IR Mass Spectrometer of INTA Chile.

During the present period we have conducted a pilot study to measure breast-milk intake using deuterium oxide in 9 mother-child pairs of children aged 7 - 11 months of age; samples of saliva have been taken for analyses. One child has completed the 28 days of the study and 8 children are in process. Test weighing for 48 hours has been conducted on 5 children; unadjusted breast-milk intake ranges from 589 to 682 g per 24 hours. The samples are awaiting analysis for deuterium oxide.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Childhood malnutrition, characterized by linear growth retardation, affects approximately 25% of the world's children under five years of age. These levels have barely changed during the past 20 years despite numerous attempts to intervene. This stunting is associated with diarrhoea, where it is related to longer duration and increased severity and mortality, particularly during the 6 - 24 months period [1-5], impaired cognitive function and school performance as well as reduced work capacity [6,7], and obstetric performance. Mild to moderate malnutrition has been shown to be related with increased risk of mortality [8]. The critical time for post-natal growth retardation is during the weaning period when foods other than breast-milk are introduced to the diet, principally between 6 and 12 months of age. It appears that this stunting is generally not reversible later in life unless improved nutritional intake occurs at a very young age [9]. Thus, improvement in child nutrition needs to occur during this critical period to prevent growth retardation and its consequences.

A number of studies indicate that the most important factor influencing growth at this stage is food intake even in the presence of a high prevalence of diarrhoea [10,11]. A combination of energy and multiple nutrient deficiencies is the main reason for poor growth in Peru, as in other parts of the world, although in some areas certain nutrients, and low bioavailability, may be specific limiting factor [12-16].

Previous studies in many parts of the world, including peri-urban areas of Lima, have shown that the median growth rate of children is the same as the NCHS standards from birth to 4 - 5 months of age. Thereafter, both length and weight fall away from the reference curves, with the result that 20% of children have a weight-for-age of <-2 SD by the end of the first year [17] a trend which continues until 18-20 months of life [18,19]. When compared with predominantly breast-fed privileged children [20], the growth curves coincide until around 8 months of age [21], when that of the Peruvian population begins to fall behind, indicating that the nutritional intake of these infants in Lima becomes deficient during the second 6 months of life.

Studies in this population by the investigators, have demonstrated a progressive decrease in dietary intake in relation to recommended amounts during the course of the first year [22]. Mean energy intakes of infants in this population was near recommended intakes during the first trimester of life, but decreased to a mean of 82% in the last trimester, according to recently published energy requirements [23]. Similarly the intake of several micronutrients decreases in relation to requirements during this period. Added to this, during diarrhoea or fever there is a reduction of around 10% of energy intake from the normal, "healthy" day intake, due entirely to a decrease in the consumption of non-breast-milk foods [24]. These reductions coincide with the introduction of low energy and nutrient density weaning foods, on average that similar to milk [0.6 - 0.7 kcals/g], thus providing inadequate complementation of breast-milk.

There are a number of approaches to improving food and nutrient intake of small children. These include the distribution of a pre-prepared, multi-fortified food to provide a major part of the energy and nutrient requirements of the child, fortification of widely used foods or specific nutrient supplementation. However, in populations where there is access to suitable food, the ideal intervention is the promotion of local food sources to provide a varied diet of adequate nutritional content, and thus obtain increased intake for several nutrients as well as contribute to optimum nutritional practices. In several parts of Peru, including Lima, a variety of foods are available and economically accessible to the population, yet are mostly given in preparations of inadequate energy and nutrient density, and in reduced quantity and frequency, according to local cultural practices and perceptions of the child's needs.

There have been a number of projects in Peru to improve food and nutrient intake during weaning through behavioural change interventions and which have shown the increased use of the promoted concepts, recipes or food [25-27]. Yet most of these studies were not evaluated for improved nutritional outcomes.

A community-targeted communications campaign in the proposed population demonstrated an improvement in weaning practices [26]. As a result of the campaign there was a significant, positive, delay in the inappropriately early introduction of foods: at 4 months 47% of children had received weaning foods prior to the intervention as compared to 26% afterwards. There was a significant increase, from 15% to 28% of children 6 - 12 months of age, consuming 2 servings per day of energy-dense food preparations, evaluated by 24-hour recall; savoury purees of appropriate food combinations being the preparations more commonly adopted. The non-breast-milk energy density of prepared foods increased significantly from an estimated mean of 0.63 to 0.82 kcal/g., also evaluated by 24-hour recall. However, there was apparently little change in the total amount of food consumed with the intervention and no change in the feeding frequency; the latter was not one of the messages of the campaign as it was not considered critical at the time.

In a separate study the preparation and serving of appropriate weaning foods from a community kitchen in Lima led to a slight but not significant increase in dietary intake on the days that these foods were consumed and with no effect on nutritional status between the intervention and control groups. It appears that the promoted complementary foods replaced some of the more liquid foods [soups]; the effect on breast-milk intake was not clear, although some substitution appears to have occurred [28].

The object of changing weaning behaviour is to achieve a positive effect on the nutritional and health status of the population. However, although targeted to improve nutrition and health, the promotion of weaning practices has not always translated into improved dietary intake or nutritional status [25,29,30]. Nevertheless such strategies are an important component of all child survival programmes including WHO and UNICEF. Hence it's important to document that this approach can work.

A major concern is whether the promotion of weaning foods with the possible substitution or replacement that may occur, might lead to a negative effect on the child's diet, and not only the absence of a beneficial effect. This is particularly critical in relation to the premature or excessive replacement of breast-milk during the weaning period, when breast-milk remains important both nutritionally and for protection against disease. Recent studies from Honduras indicate that substitution of breast-milk occurs with no nutritional benefit when complementary foods are added to infants' diets between 4 and 6 months [31]. Similar observations have been made in children under 1 year in rural Thailand [32]. Consequently it is important to study whether it is possible to increase total dietary intake through the adoption of appropriate weaning practices under carefully controlled conditions in the community and investigate the effects on the different components of the diet.

The present study is a pilot study to explore the use of deuterium oxide for the measurement of breast-milk intake. Once the technique has been perfected locally and validated, and if the budget permits, it will be applied to a community based controlled efficacy trial, the objective of which is to measure whether weaning practices using locally available, culturally acceptable foods, can indeed lead to a beneficial effect and increase total daily energy and nutrient intake, and to what extent there may be substitution of other foods, including breast-milk, during the critical weaning period.

Children between the ages of 7 and 12 months, living in an urban shanty town of Lima where growth retardation is prevalent, will be enrolled in the intervention and control groups: 80 in each group. The intervention group will receive individualized nutrition advice. Mothers will be asked to implement the recommendations during 3 - 4 weeks. Dietary intake will be evaluated before and after the intervention by individually weighing food intake, breast-milk intake by test weighing [2 consecutive 12-hour periods] with and in selected cases using deuterium oxide. Test weighing will be conducted for 48 hours on a sub-sample to develop regression equations to extrapolate 12 to 24-hour breast-milk intakes.

We have previously used the test weighing technique extensively in these communities [22]. We have found it very useful and consistent results have been achieved, nevertheless it has certain disadvantages and limitations. In this population test-weighing needs to be conducted by an outside trained field worker rather than the mother and thus involves a considerable degree of invasiveness in the home. The presence of an outside observer and the weighing of the child may well change breast-feeding patterns; for example: the mother may be reluctant to breast-feed due to this procedure, the child may fall asleep at the breast while feeding and wake up when placed on the balance, and require more milk afterwards. It is impossible to conduct this procedure for more than 2 successive days and the field worker needs to remain in the house at night to measure breast-milk intake around the clock.

The deuterium oxide methodology is an attractive alternative which permits the overcoming of several of these difficulties and limitations, also giving the breast-milk intake over a longer period of 14 days, thus taking into account daily variation [33,34]. However this has been mostly used to date to measure breast-milk intake in small infants; it has not yet been used to explore breast-milk production in relation to complementary feeding practices for children over 6 months of life.

The use of Isotope Ratio Mass Spectrometry has been used for determination of breast-milk intake. However as many of these studies need to be conducted in the field in countries where stunting occurs, the application of these techniques using the IR spectrometer is an advantage. The method has recently been validated for measurement of breast-milk intake with Indonesian mothers [35].

In order to apply this technique to the described protocol for the measurement of breast-milk intake before and after an intensive educational intervention of 3 - 4 weeks it is necessary to know the washout time of the deuterium from the bodies of the mother and infant so that the timing of the baseline evaluation can be correctly programmed so as not to interfere with the intervention, and to calculate the time for initiating the final evaluation, assuring that there is no deuterium from the first dose still remains and which could interfere with the final breast-milk measurement.

The specific objectives of this phase of the study are:

a) Measure the time required to return to base-line values of deuterium oxide in the mother and the infant, after a dose to the mother for the measurement of breast-milk intake in children of this age group who are receiving complementary foods.

b) Validate the infrared spectroscopy methodology for the evaluation of breast-milk intake in the field using the equipment of the IIN in comparison with the isotope ratio mass spectrometry in collaboration with Dr. Gabriela Salazar at INTA, Chile.

The community efficacy trial has just been financed by Thrasher Research Fund and WHO-CHD. Dr. Gabriela Salazar has been collaborating closely with the deuterium oxide measurements.

2. METHODS

2.1. Administration of deuterium oxide and collection of samples

Breast-milk intake is measured using the dose to the mother methodology [33,34,36]. A base-line sample of 2 ml of saliva is collected from the mother and the child, day 0, the exact time of the sample noted, ensuring that neither the mother of child has consumed any food within the previous 1/2 hour which could dilute the deuterium oxide in the mouth. Salivettes or small pieces of cotton wool attached by a thread is placed in the mouth for the time required to collect sufficient sample. Both the mother and the child are weighed and height or length measured.

Thirty grams of deuterium oxide [1 g per kg total body water [approx.] of the mother] are weighed in a bottle and given to the mother. The bottle is then re-weighed so that the exact amount of the deuterium oxide taken by the mother is calculated. The mother then drinks some water to ensure that no deuterium oxide remains in the mouth. The exact time of giving the sample is noted.

During the successive days saliva samples are collected and the exact time of each collection noted. In the case of the infant samples are collected on days 1, 2, 5, 6, 13, 14, 21, 28. In the case of the mother 1,6,14,21,28. Although breast-milk intake will be measured on days 1 - 14, days 21 and 28 are included to see whether there is still residual deuterium oxide in the body. The mother and child's weights are measured on days 14 and 28.

Urine samples have been collected on one child [AB1] for comparison with saliva.

2.2. Sample preparation

The saliva samples are stored at -20^{0}C for purification and analyses. The aqueous portion of the saliva or urine is extracted by vacuum sublimation. The liquid sample is frozen along the inside walls of a test tube to maximize its surface area. The test tube is connected to a condensing tube submerged in a -50^{0}C bath. The top of the condenser tube is attached to a vacuum. Water vapour is drawn by the vacuum from the test tube containing the sample and trapped and frozen in the condenser.

2.3. Deuterium determination

Samples/standards are pumped continuously from the autosampler through the cell of the Infra Red Spectrometer with a distilled water wash between them. The change in absorbance is recorded as a chromatogram by an integrator connected directly to the IRS, the peak height corresponding to each sample is related to its D_2O concentration.

2.4. Test-weighing

The test-weighing method, which we have used extensively in previous studies, is being used for comparison with the deuterium oxide methodology. We are conducting the test-weighing procedure during 48 hours, to coincide with days 5 and 6 of the deuterium study, and assuming that these 48 hours are representative of the normal intake. The infant is weighed, fully-clothed, before and after each breast-feed, using calibrated Ohaus mechanical balances that weigh to a precision of 1 g., the difference in weight being the amount of breast-milk consumed. A factor of 3% is applied to take into account insensible water losses during the feed. The time at the breast is also noted.

Other foods consumed during the 48-hour period are also being observed and noted in order to get an idea of complementary food and liquid intake.

One field worker remains with the mother for the first 24 hours and is replaced by a second field worker for the second 24-hour period.

3. RESULTS

The number of children enrolled to date are shown in Table I. Child AB-1 has been completed, the others are in process. Both male and female children in the age range of 7 - 11 months are included.

3.1. Problems encountered

In a few cases we have not found it easy to collect the samples on the allotted day due to absence of the mother and/or child, including travel. In these cases the nearest day has been taken for the sample collection. The studies are programmed so that sample collection days do not coincide with weekends.

We have also tried the collection of urine instead of the saliva as the collection of sufficient saliva from one infant was difficult. However, we have found that the collection of saliva is easier and the exact time of obtaining the sample is more precise. In the case of the urine the mother collects the sample at the time that she changes the nappy, but the actual time of urine excretion is unknown. However with most of the children it has not been difficult to collect the saliva although with some infants it has been difficult. However with a small group of children it has become increasingly difficult to collect saliva, we are continuing to do so rather than change to urine, but wonder about this as an alternative.

We are currently programming the test weighing on days 5 and 6 of the deuterium collection days, thus coinciding with the middle days of the deuterium measurement. We wonder whether this is the most appropriate or whether we could have more variation in this. This is important when applying the methodology to the full protocol.

Interestingly [and not surprisingly] the acceptance rate on the part of the mothers is much higher for the deuterium oxide method than the test weighing method as it is less invasive and uncomfortable for the mother. Nevertheless we have achieved a high acceptance rate. We have had an acceptance rate of 70% for the deuterium and of these 63% accepted the test weighing for 48 hours.

Almost all the children had "colds" at the time of the study, although none had diarrhoea or any other symptom.

After a series of problems during the past year the IR spec. of the IIN is currently undertaking final adjustments to improve its sensitivity, particularly for readings of lower concentrations with the running of validations. It will be in working order to initiate the analyses of the present samples during the course of the next week.

4. PLANS FOR FUTURE WORK

By the end of this period [late Jan.] we will complete 12 - 14 mother-infant pairs for deuterium and 9 test weighing if the acceptance continues as it is.

We plan to do some evaluations of total body water with the next group of 6 children in January, using ^{18}O.

The community-based efficacy complementary feeding study has now been financed and will commence in early 1998. We need to await the results of the present study but to date several advantages of the deuterium methodology have been perceived. We are considering the possibility of readjusting the budget to include deuterium oxide on all of the children in the study. Yet at US$12 per sample this would be 80 [children per group]*2[interval & control]*2 [pre and post]*11 [samples per mother-child pair] = US$42,240.

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TABLE I. CHARACTERISTICS OF CHILDREN IN DEUTERIUM OXIDE BREAST-MILK MEASUREMENT STUDY

	Age of child months	Sex	Age of Mother years	NE Samples Collected child	mother
AB-1	7.1	F	26	7*	6**
AB-2	7.1	F	31	7	5
AB-3	9.5	F	34	7	5
AB-4	9.2	M	20	7	5
AB-5	9.2	M	24	7	5
AB-6	6.6	F	21	5	4
AB-7	10.0	M	21	5	4
AB-8	7.0	M	40	5	4
AB-9	10.1	M	15	5	4

[*] Days: 0,1,2,7,8,11,28
[**] Days: 0,1,2,8,11,28

The unadjusted amount of breast-milk consumed during the 2 day period is presented in table II. In fact the amount of breast-milk consumed by the children is very similar. Varying amounts of other foods have been eaten by the children.

TABLE II. BREAST-MILK INTAKE BY TEST WEIGHING

Child	Age [months]	Study day	Breast Milk Intake [g]			Time at Breast [mins]		
			Day 1	Day 2	Mean	Day 1	Day 2	Mean
AB-2	7.3	5-6	666	698	682	81	99	90
AB-3	9.7	5-6	537	783	660	138	80	109
AB-4	9.5	7-8	-	661	661	-	70	70
AB-6	6.8	5-6	568	609	589	138	71	105
AB-9	10.2	5-6	597	722	660	73	67	70

The proportion of breast-milk consumed during the nighttime period [6pm to 6am] is around 50% [table III], some slightly more during the night, others slightly less.

TABLE III. BREAST-MILK INTAKE: DAY AND NIGHT

			6 am to 6 pm			6 pm to 6 am		
			Day 1	Day 2	Mean	Day 1	Day 2	Mean
AB- 2	Intake	g.	341	338	340	325	360	343
		(%)	(51)	(48)	(50)	(49)	(52)	(50)
AB- 3	Intake	g.	242	357	300	295	426	361
		(%)	(45)	(46)	(45)	(55)	(54)	(55)
AB- 4	Intake	g.	-	337	337	-	268	268
		(%)	-	(56)	(56)	-	(44)	(44)
AB- 6	Intake	g.	321	381	351	247	228	238
		(%)	(57)	(63)	(60)	(43)	(37)	(40)
AB- 9	Intake	g.	303	307	305	294	415	355
		(%)	(51)	(43)	(46)	(49)	(57)	(54)