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

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Abstract

In the present study we performed a pilot study using deuterium oxide method to determine the breast-milk intake in children 7-12 months of age receiving complementary food. This is 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. We determined the washout period for the deuterium finding a value of 21 days for the mother and child. This measurement was performed using the infrared spectrometer of the Instituto de Investigación Nutricional and compared with the values obtained with the IR Mass Spectrometer of INTA Chile. The test weighing was conduced on 14 children and compared with the values obtained using the deuterium methodology. Our result suggest that the breast milk intake determined by the weighing test was lower with regard to the value obtained with the deuterium methodology.

1. SCIENTIFIC BACKGROUND AND SCOPE OF WORK

Childhood malnutrition, characterised 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, contributing to longer duration and increased severity and mortality, particularly during the 6-24 months period [1,2,3,4,5], 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, at which time nutritional intake becomes deficient. This has been demonstrated in Peruvian and other Latin American populations. It appears that this stunting is generally not reversible later in life [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, namely giving inadequate weaning foods to complement breast milk [10,11]. Thus improving weaning practices will prevent malnutrition.

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 [12,13,14]. Nevertheless such strategies are an important component of all child survival programmes. Hence it's important to document that this approach can work.

A major concern is whether the promotion of weaning foods might lead to substitution or replacement of other foods, which could result in no increase or even a reduction in overall energy or nutrient intake. 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 nutritional and for protection against disease. Consequently it is important to study whether it is possible to increase total dietary intake through the adoption of appropriate weaning practices in the community and investigate the effects on the different components of the diet.

The measurement of total food and breast milk intake is a necessary component of the evaluation of programmes during this critical period. To date the test weighing method has been used for the determination of breast milk intake [15] for evaluation in developing countries. However the application of the techniques using deuterium oxide for this purpose will greatly facilitate these evaluations [16,17,18].

A protocol for the determination of the change of energy and nutrient intake from both breast milk and complementary foods with adoption of currently recommended complementary feeding practices has been funded by the Thrasher Fund and WHO. The present project is a complementary component to evaluate the application of he use of the deuterium oxide methodology for the measurement of breast milk. The information obtained will permit the evaluation of complementary feeding programmes and their effect on intake of breast milk and other dietary components. There is very little information available to date on the effects of complementary feeding programmes on breast milk intake and on total energy and nutrient intake.

It is necessary to evaluate the effect of programmes on dietary intake over relatively short periods as increase in age has a marked effect on breast milk and other food intake during this transitional time of changing feeding patterns. Thus it is necessary to explore the possibility of successive measurements of breast milk intake using D_2O over short periods.

There has been little work documenting the effect of introduction of complementary foods in breast milk intake and total energy and nutrient intake during the weaning period. Recent studies from Honduras indicate that substitution of breast milk occurs with no nutritional benefit when complementary foods are added to infant's diet between 4 and 6 months [19]. Similar observations have been made in children under 1 year in rural Thailand [20] and no beneficial effect was demonstrated with the promotion of a nutritionally adequate complementary food in Nigeria [13].

The use of deuterium oxide for the measurement of breast milk intake has been used in different centres to document breast milk intake in different situations, such as with smoking mothers [21] but mostly during the first few months of life. It has not yet been employed during the weaning period to elucidate issues such as a lack of nutritional impact of nutritional intervention, etc.

The use of Isotope Ration Mass Spectrometry (IRMS) has been used for determination of breast milk intake. However, as many of these studies need to be conducted in the field in the countries where stunting occurs, the application of these techniques using the IR spectrometer is an advantage. The method has been recently validated for measurement of breast milk intake with Indonesian mothers [22].

To date breast milk intake has been measured by test weighing in the field projects of IIN. We have had considerable experience in this technique. It is necessary to continue to use this technique due to the sample size required for the study design of measuring changes in dietary intake. We measure breast milk intake during 12-hour periods and, on a sub sample, for 24-hour periods to be able to extrapolate the 12 to 24 hour breast milk intake. We have considered the possibility of using D_2O determination for the 24 hour intake, thus facilitating the calculation of the 12 to 24 hour intake.

1.1.Objectives

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, aged 7 – 12 months.

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

c) Use the deuterium oxide methodology to measure the change in breast milk intake with the adoption of recommended complementary feeding practices in a group of mother-children pairs aged 7 – 12 months.

In addition we included a fourth objective:

d) Comparison of the breast milk intake to the test weighing methodology in a group of children 7 – 12 months of age.

In this project we have been co-ordinating with INTA, Chile, with Dr. Gabriela Salazar who helped us to start the project with the training of the project team in the procedures, and in the analysis of the samples using IRMS at INTA. We have also been co-ordinating with Johns Hopkins University, Dr. Benjamin Caballero, who advised us in the development of the study protocol and in the discussion of the initial results.

2. METHODS

2.1. Field procedures

Prior to the initiation of the protocol each mother participating in the project was visited, the project explained to her and written consent obtained. For each of the mother-child pairs participating in the study the following procedures were conducted:

On day 0 a base-line sample of 2ml. of saliva was collected from the mother and the child and exact time noted. Neither the mother nor the child had taken food or drink or breast milk for at least 1 hour prior to the taking of the sample. The sample was collected using Salivets and in the case of the children, using a small piece of cotton wool secured with thread, as this was found to be more comfortable for the children. Both the mother and the child were weighed and measured.

A dosis of 30g of deuterium oxide was given to the mother. The bottle in which the sample was weighed with the deuterium oxide was weighed again after administration, so that the exact amount of deuterium oxide given to the mother was calculated.

The house was subsequently visited at a similar time on the days when samples were collected from the mother and the child. Samples of saliva were taken from the infant on days 1,2,5,6,13,14,21,28, and in the case of the mother on days 1,6,14,21, and 28. The samples on days 21 and 28 were collected in order to examine the presence of residual deuterium on these days, to determine the washout period for purposes of the phase 2 part of the study, where breast milk intake needs to be

measured successively after a period of nutrition education. The samples of saliva were kept at –20C for future purification and analysis.

2.2. Sample preparation

The aqueous portion of the saliva or urine was extracted by vacuum sublimation. The liquid sample was frozen along the inside walls of a test tube to maximise its surface area. The test tube is connected to a condensing tube submerged in a -50C bath. The top of the condenser tube is attached to a vacuum. Water vapour is drawn by the vacuum from the test tube containing sample and trapped and frozen in the condenser.

2.4. Deuterium determination (D_2O quantification)

Samples/standards are pumped continuously from the autosampler through the cell of the infrared spectrometer (IRS), 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.

2.5. Calculation of breast milk intake

The calculation of the volume of breast milk intake from the results of the analyses of deuterium oxide were made using the formulae of the spreadsheets provided by Dr. Andy Coward. (Include formulae) Two formulae were provided for the calculations, the second one adjusted for a better fit of the chi-squared to the curve.

2.6. Test weighing

Test weighing for 48 hours was conducted on a sub-group of the children for comparison with the deuterium oxide method. Four field workers were trained in the methodology. One field worker was present in the house for the first 24 hours followed by a second field worker who replaced her for the subsequent 24 hours. During the observation period the child was weighed on Ohaus mechanical balances, weighing to a precision of 1 g., before and after each breast-feed during the 48 hours. The total 24-hour intake was corrected for insensible water losses during the weighing period using the factor of 3%. The test weighing was conducted mostly on days 5 and 6 of the deuterium study. There was good acceptance on the part of the mothers. The intake of foods during the observation period was noted, although this was not measured quantitatively.

2.7. Comparison of deuterium oxide between infrared and mass spectrometry

Duplicate samples from a selected group of mother-infant pairs were sent to INTA for analysis using mass spectrometry. These analyses are underway, we already have some preliminary results.

To date we have completed objectives 1 and 4. Analysis of the deuterium oxide by mass spectrometry in Chile for comparison with the infrared of the IIN is currently being completed.

The evaluation of the change in food and breast milk intake as a result of an intensive period of nutrition education is currently being initiated. The community efficacy study is supported by the Thrasher Research Fund and WHO. The protocol

involves a 2 day weighed dietary intake study. The intervention period is followed by a period of 3 – 4 weeks of intensive education with the selected complementary feeding messages developed as a result of previous studies and of formative research for this study, followed by a repeat of the 2 day dietary intake evaluation to explore changes in total dietary intake, feeding patterns, intake of the different foods and breast milk as a result of the education.. As the sample size for this study is 90 per group (intervention and control) it is not possible to measure breast milk intake with the deuterium oxide method on all the children. Thus test weighing is being used and D_2O on a sub-sample of 8 mother-child pairs per group (total 16, each with 2 determinations).

This study commenced with the formative research in 1998 and the second part, the efficacy study, commenced in the middle of this year. Consequently we are just commencing the evaluation of breast milk intake using deuterium oxide in a sub-sample of these children. We shall complete sample collection in January and expect to have the analysis and final results shortly afterwards.

3. RESULTS AND DISCUSSION

3.1. Number of participants

A total of 30 mother-child pairs participated in the deuterium oxide study, we surpassed our originally proposed sample. The distribution by age group, between 6 and 11 months is shown in Table I. Of these children 14 participated in the test weighing comparison study, as shown in Table II.

3.2. Breast milk intake volume and comparison between methods

The breast milk volume was calculated using the formula 2 in the Excel spreadsheet. In figure 1 the kinetics of the exchange of deuterium oxide on one mother-child pair is shown as an example. It can be seen that by day 21 there is minimal content of deuterium, as read by the infrared, in the saliva of both the mother and the child, indicating that this is sufficient washout period at this level of dose of deuterium oxide.

In Table III the individual data used for the calculation and the final result of breast milk volume from the excel spreadsheet for the same mother-child pair is shown.

The results of the breast milk intake volume for both the deuterium oxide and the test weighing methods are shown in Table IV. The percentage difference between the 2 methods, calculated for formula 1 and formula 2 and the test weighing results are presented. The percentage difference varies between –6.21% to 36.47% for the individual children, with a mean difference between the 2 methods of 17.25% when formula 2 is used, and a mean of 18.93% for formula 1. For all but one child, test weighing gives a lower volume of breast milk intake than the D_2O method. The percent variation per child between the methods and for each of the formula is presented graphically in Figure 2.

Figures 3, 4 and 5 show the linear regressions of the infrared against the test weighing method results using each of the formulae and the mean of F1 and F2. It can be seen that there is a better correlation between the 2 methods when formula 2 is used, with a R2 = 0.6839.These results indicate that there is considerable variability between the 2 methods and the test weighing gives mostly lower intakes than the deuterium. On the whole the differences are greater than those reported by other authors [17].

3.3. Comparison of the deuterium oxide results using mass spectrometry and infrared analyses

We have the initial results of the concentrations of deuterium oxide from the 2 methods for 4 children, shown in table V. The mean difference between the analyses is 2.96%. Further analyses will provide more light on these results, and the comparison of the breast milk intake calculated by the 2 methods will be interesting.

3.4. Other foods consumed by the children

The frequency of consumption of the complementary weaning foods consumed by the children during the 48-hour observation period is shown in table VI. In fact these children were eating little; although their complementary food intake was not measured quantitatively it can be seen that it is inadequate in terms of the frequency of foods consumed in these cases. Bread and biscuits were the most common foods consumed, followed by stews or purees, milk preparations and soups and broths. On averaging the foods by child/day the mean frequency of consuming bread is 0.74 times per day and stews or purees, 0.6 times per child per day.

4. CONCLUSIONS

From the results of the kinetics of the exchange of the deuterium oxide there was little residual deuterium oxide in the body of the mother or the child at 21 days with the dosis of 30g given to the mother. This means that the methodology can be applied to the protocol of the efficacy study. On the basis of these results, deuterium is to be given to the mother 2 weeks before the 2-day dietary intake measurement, and the education intervention started immediately after this. Breast milk intake will be measured by deuterium oxide during this 2-week period. One week after the dietary intake study a second dose of deuterium oxide is given for measurement of breast milk intake during the period of the intervention when the mother is implementing the improved complementary feeding practices (hopefully!). The second dose of deuterium is given between 21 and 25 days after the initial dose, so, on the basis of the present results, there should be no problem with residual deuterium at that time.

There is a tendency for the measured breast milk intake volume by the test weighing method to be less than by deuterium oxide method, results similar to those of other studies [17]. Nevertheless in the efficacy study we have no option other than to use the test weighing because of the sample size required to measure differences in total energy and nutrient intakes with or without an education intervention. Obviously the results indicate that one method is not comparable with the other in the same study, i.e. cannot use 12-hour intake by test weighing and 24 hour intake by deuterium oxide, it needs to be the same method for each determination. However, the regression coefficient using formula 2 is interesting and we should like to discuss using this formula to adjust the breast milk intakes for all the children in the efficacy trial.

The results of the comparison of the measurement of deuterium oxide and volume of breast milk consumed using the mass spectrometry and infrared methods are interesting. We await further results and discussion to understand the significance of this. It will be important to see whether there are differences in the breast milk intakes using the results from the 2 methods.

The food intakes from foods other than breast milk in the children studied are low, in fact even lower than in other studies with larger sample sizes. This is the reason for conducting the efficacy trial, to try and improve complementary feeding practices,

which are currently deficient in many sectors of the population in Peru, and to explore the effect of this on breast milk intake.

4.1. Current situation

As explained above we are currently initiating the application of the technique to the community efficacy trial for measuring total dietary intake and the different dietary components as a result of an intensive education campaign. Sample collection will be completed in February and the results and final analyses are planned for March 2000.

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TABLE I

NUMBER AND AGES OF CHILDREN WHO PARTICIPATED IN BREASTMILK INTAKE MEASUREMENT BY DEUTERIUM OXIDE

AGE (months)	NUMBER CHILDREN
6	5
7	9
8	2
9	7
10	6
11	1
TOTAL	30

TABLE II

NUMBER OF CHILDREN WHO PARTICIPATED IN MEASUREMENT OF BREASTMILK INTAKE: DEUTERIUM OXIDE AND TEST WEIGHING

AGE (months)	NUMBER CHILDREN
6	4
7	3
8	1
9	4
10	2
TOTAL	14

TABLE III

SUBJETSNo. 2

DATOS DE LA MADRE

edad	31.00	anos
peso	54.00	kg

DATOS DEL BEBE

edad	7.10	meses
peso-inicial	7.23	kg
peso-final	7.27	kg

DOSIS

dosis 30.06 g

(1) Datos de saliva de la mama

tiempo	ppm	ppm	promedio	sigma	cv	ppm calc	chi squared
						1039.21	
1	887	887	887	0.00	0.00	894.38	54
2	774	776	775	1.28	0.16	766.06	87
6	427	397	412	20.85	5.06	412.52	0
14	119	119	119	0.00	0.00	121.46	5
						sum =	147

(2) Datos de saliva del bebe

tiempo	ppm	ppm	promedio	sigma	cv	Body Water	del calc	chi squared
						4.48	0.00	
1	137	137	137	0.43	0.31	4.48	148.74	141
2	245	245	245	0.00	0.00	4.48	251.56	45
5	373	365	369	5.53	1.50	4.48	363.66	30
6	377	369	373	5.96	1.60	4.48	362.96	102
13	215	200	207	10.64	5.13	4.50	217.13	97
14	205	188	196	11.49	5.85	4.50	196.90	0
							sum =	415

COMPOSICION DE LA MADRE

D space	28.89	kg
Lean Mass	38.06	kg
Body fat	15.94	kg
grasa	29.52	%
wat intake	4.43	kg.day-1

DATOS CINETICOS

Cm(0) =	1039.21	ppm
k(mm) =	0.15	day-1
k(bb) =	0.22	day-1
F(bm) =	0.79	kg.day-1
k(bm) =	0.03	day-1

LECHE	0.91	**kg.day-1**
F(bo) =	0.12	**kg.day-1**
Total error	**23.72**	

TABLE IV

TOTAL VOLUME (kg) OF BREASTMILK PER CHILD AS MEASURED BY DEUTERIUM OXIDE (formulas 1 and 2) AND TEST WEIGHING, AND THE DIFERENCES BETWEEN THE METHODS

CHILD	AGE	By Deuterium oxide		T.W.	Difference: D20 - T.W.			
		Formula 1	Formula 2	+ 3%	F1 - T.W.	F2 - T.W.	%F1 - T.W.	%F2 - T.W.
2	7.1	0.86	0.91	0.70	0.16	0.20	18.74	22.46
3	9.5	0.82	0.86	0.68	0.14	0.18	16.70	20.63
4	9.2	0.85	0.84	0.62	0.23	0.22	26.96	25.86
6	6.6	0.68	0.71	0.61	0.08	0.11	11.21	15.16
9	10.1	0.70	0.85	0.68	0.02	0.17	2.50	19.98
15	9.2	0.73	0.77	0.49	0.23	0.28	32.31	36.54
16	7.6	1.40	1.19	1.08	0.32	0.10	22.52	8.58
17	6.6	1.00	0.97	0.94	0.06	0.03	6.30	3.00
18	6.6	0.79	0.83	0.88	-0.09	-0.05	-11.52	-6.14
21	7.4	0.91	0.91	0.79	0.12	0.12	13.65	13.39
25	6.9	0.96	0.92	0.85	0.10	0.07	10.73	7.66
27	8.2	0.98	0.86	0.67	0.31	0.19	31.34	21.75
28	10.4	0.91	0.89	0.74	0.17	0.16	18.88	17.39
31	9.1	1.04	0.86	0.59	0.45	0.27	43.56	31.80
MEAN	**8.2**	**0.90**	**0.88**	**0.74**	**0.16**	**0.15**	**18.24**	**16.55**

TABLE V

ANALYSIS OF D_20 IN SALIVA AS MEASURED BY MASS SPECTROMETRY (CHILE) AND INFRARED SPECTROMETRY (PERU)

Child number	Mass spectrometry ppm D_20	Infrared ppm D_20	Difference	Percentage
AB19-01	96.157	107.163	-11.006	-11.45
AB19-05	240.615	164.638	75.977	31.58
AB19-13	156.419	180.123	-23.704	-15.15
AB20-01	162.519	139.650	22.869	14.07
AB20-06	356.798	224.912	131.886	36.96
AB20-13	209.035	206.136	2.899	1.39
AB21-01	131.623	130.933	0.690	0.52
AB21-06	377.086	386.582	-9.496	-2.52
AB21-13	259.582	272.350	-12.768	-4.92
AB22-01	129.19	140.096	-10.906	-8.44
AB22-06	341.669	360.926	-19.257	-5.64
AB22-13	195.26	196.907	-1.647	-0.84

TABLE VI

FOOD CONSUMED BY THE CHILDREN DURING THE 2 DAY TEST WEIGHING PERIOD (n=14)

FOOD CONSUMED	TOTAL NUMBER OF PREPARATION IN 2 DAYS
Bread, biscuits	21
Stews, purees, potato	17
Milk, milk and cereal	16
Soups, broths	10
Puddings, jelly	8
Tea, herbal infusions, fruit juices,etc	8
Banana	5
Egg	4
Oatmeal	2
Potato,	2
Tuna fish	1
Rice	1

FIGURE1

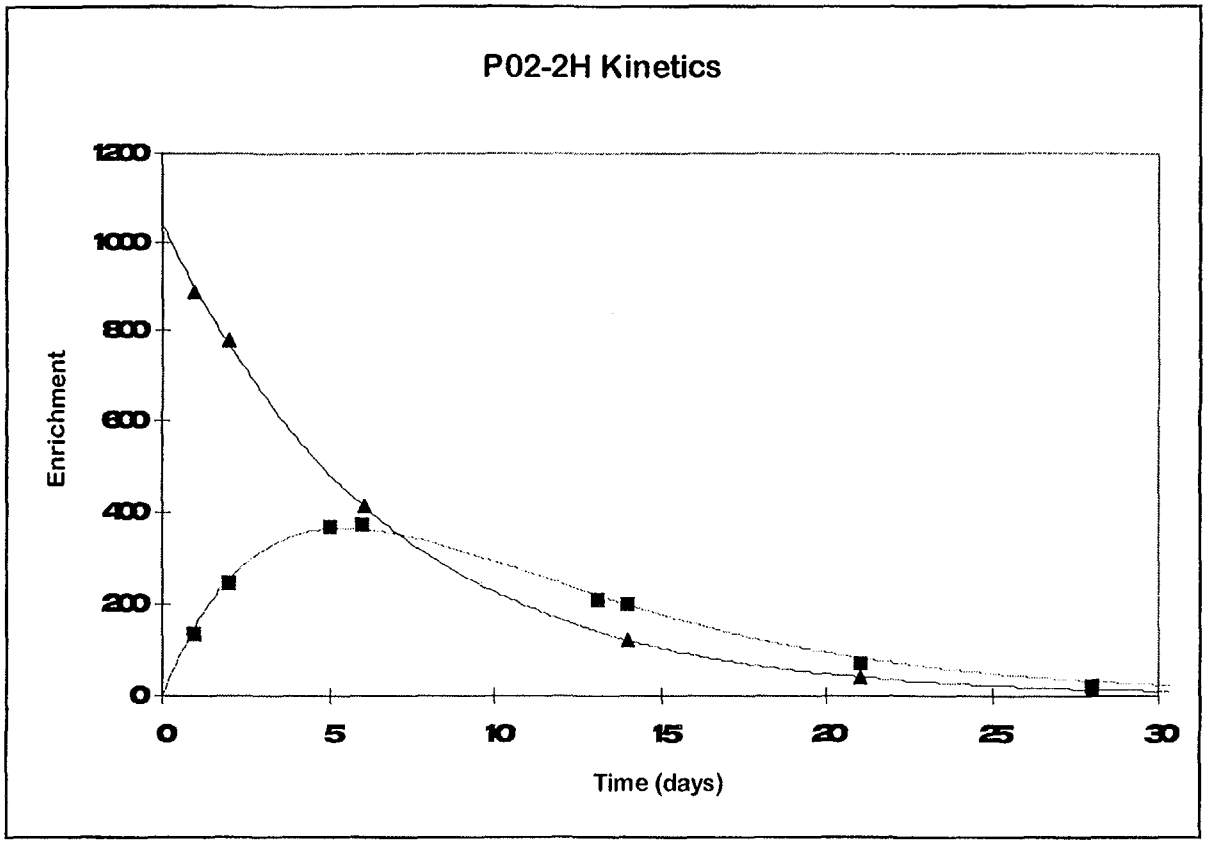

P02-2H Kinetics
Enrichment
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Time (days)

FIGURE 2
PERCENT DIFFERENCE IN AMOUNT OF BREASTMILK BETWEEN D_2O AND TEST WEIGHING METHODS BY CHILD

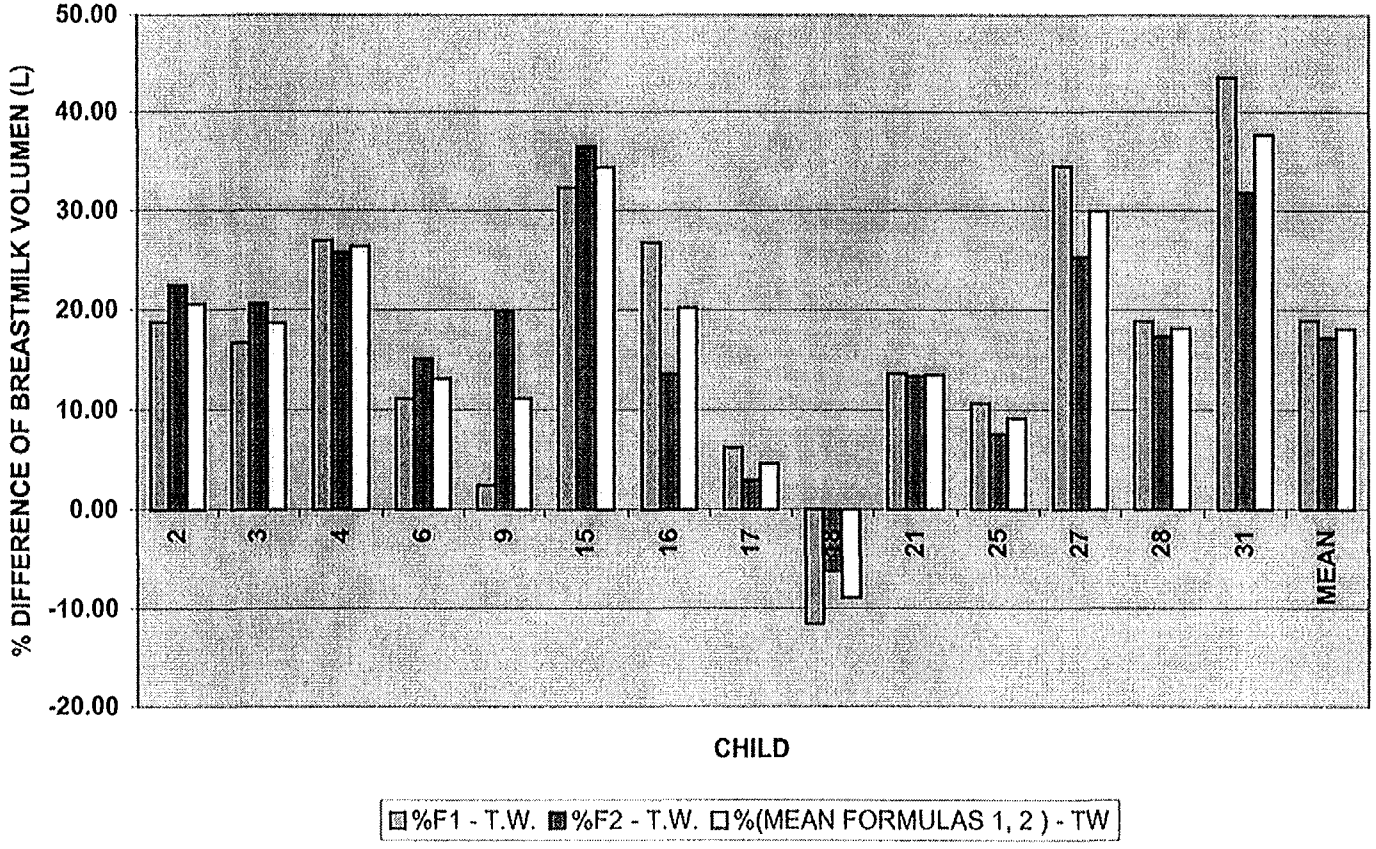


FIGURE 5
LINEAR REGRESSION OF COMPARISON OF
BREASTMILK INTAKE BETWEEN D2O
TEST WEIGHING METHODS
(Mean of Formula 1 and 2)

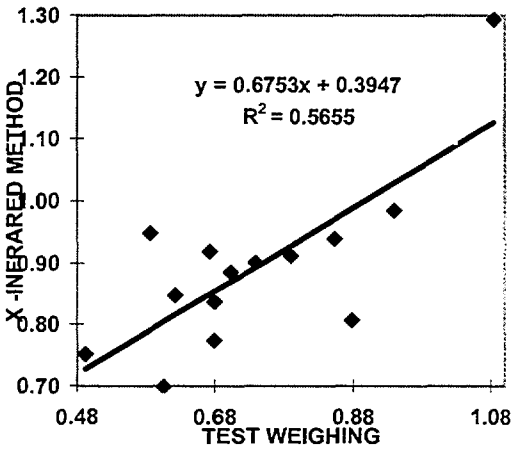


FIGURE 3
LINEAR REGRESSION OF COMPARISON OF
BREASTMILK INTAKE BETWEEN D2O
TEST WEIGHING METHODS
(Formula 1)

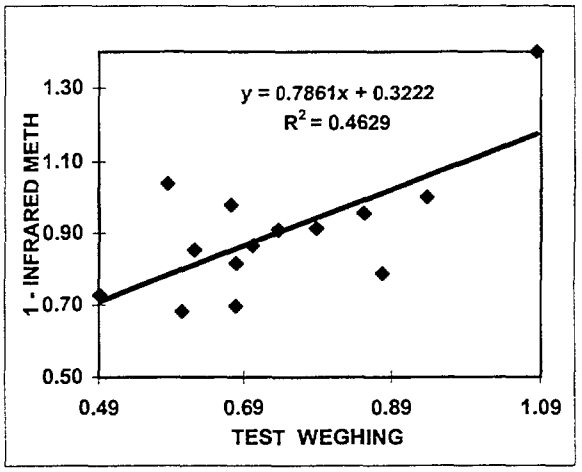
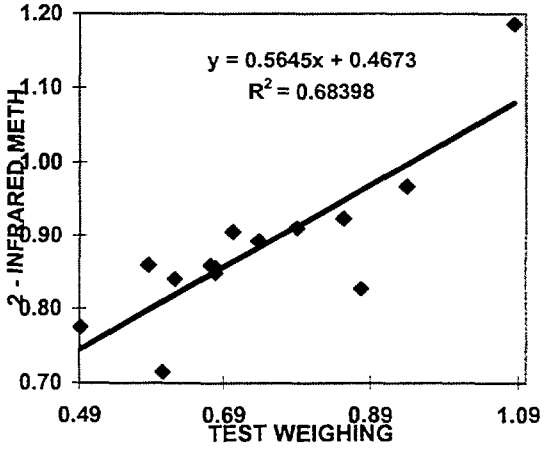


FIGURE 4
LINEAR REGRESSION OF COMPARISON OF
BREASTMILK INTAKE BETWEEN D2O
TEST WEIGHING METHODS
(Formula 2)



FIGURES 6 -9

RESULTS OF D2O MEASUREMENT IN SALIVA BY MAS SPECTROMETRY (CHILE) AND INFRA RED SPECTROMETER (PERU)