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ESTABLISHMENT AND APPLICATION OF DEUTERIUM DILUTION METHOD FOR MEASURING BREASTMILK INTAKE OF PAKISTANI INFANTS

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Abstract

We established the deuterium dilution method in our laboratory and have applied it in the field for quantification of milk intake of babies. A comparison of the test weighing (TW) data with the D_2O data is also being made. The growth of children fed exclusively on breast milk is also being monitored. The method once established will be made available for various projects where correct estimation of milk intake/out put is desired. First year of the project was devoted to standardization of the D/H preparation from biological fluids using Zn shot method. During the report period, the D_2O dilution methodology for measuring the breast milk intake of infants was streamlined. 21 infant-mother pairs have been recruited and data including deuterium dilution assay was collected at 4 weeks of age. Growth of the infants is being followed up-till six month. Analysis of 21 infant mothers data is presented. Samples of six infant mother pairs collected at 13^{th} week of age are waiting to be analyzed.

The mean breast milk intake of infants measured by deuterium dilution method is 801± 262 gm at first sampling at 31.15 ± 7.7 days of age (n=18). The milk intake determined by test weighing was significantly higher than estimated by D_2O dilution. The values were 1169 ± 384 per day as compared to 705 ± 129 per day for TW and D_2O method respectively (n=13). The growth data collected over six month period for 21 infants showed that they fall within 5 percentile of NCHS for weight. The height of boys is in 50 percentile whereas for girls it at 5 percentile. The data collected so far suggest that although majority of infants are exclusively breast fed and the intake values are normal yet their growth is far from optimum. This emphasizes the need to take up the studies involving quality of milk in relation to the nutritional status of the mothers.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

There is an increasing awareness of positive effects of breast feeding (BF) on health of infants worldwide. Breast milk (BM) provides all macro and micro nutrients in most palatable form and in appropriate quantity and quality. Most mothers are aware of this fact and wish to breast feed their babies. However, their breast feeding behavior may not be optimum.

Breast feeding provides a multitude of advantages for infants including protection from infections. The maximum beneficial effects of BF however can only be obtained with exclusive breast feeding, which should be practiced at least for the first 4-6 months of life. Thereafter, BF should be supplemented with other foods [1,2]. With formula milk usually given in bottles, the baby not only is deprived of the many advantages of BF but is also exposed, especially in developing countries to a very high risk of acquiring intestinal, respiratory and other infections resulting in disease and death [3].

Breast feeding adequacy is determined by studying the infant growth. The growth standards currently being used are based on formula fed infants. Hence there is a requirement to reassess the growth standards and this envisages the need for determining the amount of BM intake and weight gain by infants. Moreover, there is a common belief that the nutritional status of the mother affects the BM output. Although some studies carried out in UK [4] found no relationship between body mass index and volume of milk produced. The authors concluded that "lactation performance is extremely robust".

Pakistan is a country of large population of about 140 million, of which nearly 45% are children under 15 years who suffer from severe nutritional problems. The worst affected are very young ones. Lack of appropriate breast feeding is a major factor in wide spread prevalence of malnutrition and infections. According to UNICEF [5] 38% of children below five years of age are under weight, while 13% are severely so in Pakistan. Breast feeding status in Pakistan has been very well documented through various studies [6]. They show an increased prevalence of bottle feeding, especially in urban areas. Though most mothers still breast feed, this is not optimum. It is rarely exclusive even in first weeks of life [7]. Supplementary water, other fluids, milk and foods are commonly introduced after the first or second months of life. These foods are likely to be contaminated. Initiation of breast feeding is delayed for 2-3 days and prelacteal feeds are commonly used [6].

Owing to the multitude of advantages associated with breast feeding there is a profound need to promote BF in its optimum form, so that full benefits can be obtained. A lactation management clinic was set up at the Children's hospital, Islamabad. Its main objectives were to help mothers to maintain BF as long as possible by counseling according to WHO guidelines. The conceptual model for the lactation management clinic is to identify underlying problems when lactation process is not successful and when mothers find it difficult to breast feed. The common complaint of mothers attending the lactation management clinic is insufficient milk which according to a study was perceived in 50% of the cases [8].

There are a number of instances where it is important to accurately determine the breast milk intake and output. The conventional methods generally used for measurement of breast milk output is by test weighing, flow meter method and more recently is tracer technique based on measurement of water turnover rate [9]. Conventional methods are most prevalent, since facilities for performing the sophisticated isotopic methods are not available in many countries. However, the stable isotopic methods though not very simple and straight forward are gaining popularity due to a multitude of advantages over traditional methods.

The isotopic method can be with tritium or deuterium labeled water. It is ethically acceptable and safe to use stable rather than radioactive isotopes where infants and women of child bearing age are involved. The dose of deuterium can be given to the baby [10] or to the mother [11] The method has been validated [12,13] and is increasingly been used the world over.

The deuterium dilution, especially dose to the mother method provides not only an accurate estimate of total body water, which can in turn be used to calculate milk intake/ output but also the maternal body composition [14,15], and estimates of non milk water intake. With an additional dose of ^{18}O, it is possible to calculate energy expenditure and body composition of the infant also.

Radiation and Isotope Applications Division (RIAD) of PINSTECH has all the facilities for doing the preparation and analysis of all major isotopes of interest. The mass spectrometry group has streamlined expertise for accurate and precise analysis of ^{2}D, ^{13}C, ^{15}N, ^{18}O from biogenic and abiogenic samples. The Life Science Group of RIAD is the pioneer in the country for application of stable isotopes in nutrition and health related research.

The current project was initiated in collaboration with the Department of Pediatrics, The Children's Hospital Islamabad and is continuing with the involvement of Islamic International Medical College. The project has following objectives:

- Establishment of D_2O dilution method for measuring the BM output.
- Apply the established methodology for determining the BM intake of Pakistani Infants.
- Monitor the growth of infants fed exclusively on BM up to 4-6 months of age.

It is envisaged that method once established will prove to be a very useful tool in addressing a number of questions e.g., nutritional requirements and lactation performance and other factors affecting breast milk production [16,17], energy requirements of the mother for optimal output in developing countries (recommendations come from west might not apply directly to the developing country situation); total weight gain in the mother and distribution in various compartments during pregnancy and the utilization of fats during lactation; water turnover and its relationship to the milk output [18]. All these issues can be addressed using an appropriate study design, whereby milk output and body composition of the mother is determined simultaneously.

Most studies done so far have relied on test weighing for milk intake and anthropometric methods for body composition.

The execution of this IAEA project has brought prospects of utilizing the stable isotopic method not only for milk intake but simultaneous determination of body composition. Once refined the more sensitive and sophisticated methodology can assist in generating the data on a number of problems regarding maternal and child health nutrition.

2. METHODS

2.1. Subjects

The mothers residing in the Barakahu area, who were breast feeding their infants were interviewed and recruited post-natally. Twenty one infant mothers have been recruited and assayed.

2.2. Inclusion criteria

The volunteer mothers were required to meet the following selection criteria (Parity 1 or 2) Gravid 2+, no abnormality at Labor, birth weight >2.5 Kg, no prenatal problems in the baby or mother, willing to breast feed, willing to be visited.

2.3. Study design

The mothers meeting the selection criteria were pre-empted by the hired lady health worker, who was in charge of that area. When the infants were 25-30 day of age the research team visited them at their homes to explain the study objectives in detail and collect the data. Anthropometric measurements were obtained on the mother and infant at first visit. Pertinent information regarding civic facilities, socioeconomic status, nutritional habits of mother were also recorded at this stage.

The base line samples of saliva/breast-milk and urine were collected and dose of deuterium dilution given to the mothers. Explicit instructions were given to the LHW and the mother regarding collection and timing of post dose saliva and urine samples and their storage. The LHW was asked to keep a record of the time of collection of samples and to record any deviation from the stipulated time of sample collection.

Although study protocol specified exclusive breast feeding, the non compliance to study protocol along with reason was recorded. The study procedure were postponed if either mother or infant were ill or unavailable at the expected time. The determination of milk intake was done at 25-30 days (4^{th} week) and then again at 13^{th} week. Test weighing was usually conducted at 6 or 7^{th} day of D_2O sampling.

The monitoring for infant growth were performed at monthly interval. The actual ages of infant at the times of D_2O were 4.5±1.2weeks (Table II) and 31.15±7.7 days (Table III). All study procedures were conducted at homes under the supervision of LHW.

2.4. Test weighing

The amount of milk ingested over a 24 h period was determined by weighing the infant before and after each feeding. The LHW was instructed to use an automatic, electronic balance (Tanita, Japan), which integrates repetitive weighing and displays figures when constant. The precision stated by the manufacturer is ±0.05 gms. A diaper was placed on infant before and during feeding, to keep a record of loss of urine during feeding. Any losses of milk during feeding were not recorded.

The test weighing procedure was conducted at 6^{th} or 7^{th} day of D_2O assay. Only day time readings were recorded. It was not possible to find a subject where night time feeding could be weighed. The number of night time feedings were based on mothers recall. The mean intake per feed during day was averaged and multiplied by the number of feeds in 24 hrs to calculate milk intake per day.

2.5. Anthropometry

Infant weight was recorded using Tanita electronic balance and infant length was measured on an infant board. Head circumference was measured by a soft tape. Most anthropometry readings were collected from LHW record, however readings at alternate months were taken by the research team.

2.6. Deuterium dilution method for measuring breast milk intake

The dose to the mother method of Coward *et.al.*, [19] was adopted with some modifications.

2.7. Preparation and administration of deuterium oxide

The 99.9% D_2O (CIL Andover, MA 01810 USA) was prepared as follows for oral administration to the mothers. Seven gram of 99.9% D_2O was made up to 70ml with distilled water and given to subject 1 and two. Later, bulk dose was prepared for entire study and stored as diluted dose. At each field trip 70 grams of dose aliquots were weighed and carried in the field pack.

After taking the pre dose saliva or milk sample from the mother, she was given the dose aliquot. The bottle was rinsed with water and mother was asked to drink the washings too. The time of dose administration was noted.

2.8. Sample collection and storage

The saliva samples were collected either through cotton wool placed in the mouth of the mothers or by direct collection by the subject into collection vial. The urine samples from the baby were collected, either through cotton wool placed in diaper or by direct collection into the bottles. The samples from the mother and baby were collected on day 0, 1, 2, 3, 4, 5, 13 and 14. The collected samples were stored in refrigerator, while in the field and later, in the lab at −15 °C in a deep freezer.

2.9. Sample preparation

The samples were thawed at room temperature and then centrifuged at 15,000 rpm for 15 min. The supernatant was separated and stored in Eppendorf tube. For D/H analysis, 8μl of samples was reduced to H_2 gas using Zinc shot method [20]. The details of procedure adopted and modifications were reported earlier [21].

2.10. Accuracy and precision of standards

Before the initiation of D/H analysis in our laboratory, the enriched and natural standard samples from IAEA were analyzed. The IAEA standards used were IAEA-302A, GISP, I-S-8A and I-S-9 along with internal laboratory standards. The results obtained are presented in Table I.

TABLE I. PRECISION OF IAEA-302A, GISP, I-S-8A AND I-S-9 OF IAEA AND INTERNAL LABORATORY STANDARDS

	δD vs. V. SMOW (‰)			
	IAEA-302A	**GISP**	**I-S-8A**	**I-S-9**
	511.04 505.71 507.31	-189.72 -190.62 -189.34	1.10 0.83 1.03	-140.58 -140.29 -139.95
Average	**508.02**	**-189.89**	**0.99**	**-140.27**
SD	2.23	0.66	0.14	0.32

1. Average IAEA-302A value determined by the IAEA: **508.4** and range **505.5-511.3.**
2. Average GISP value determined by the IAEA Laboratories: **-189.50 ± 1.0**

2.11. Sample analysis (D/H determination)

The D/H was determined on at least five samples from the mother and seven samples from the baby (baseline and day 1,2, 13 and 14 from the mother and baseline and day 1,2,3,4 ,13 and 14 from the baby) and were analyzed using isotope ratio mass spectrometry (Modified Finnigan Mat GD-150). Preparation and analysis of sample were performed in duplicate for D/H abundances.

3. RESULTS AND DISCUSSION

3.1. Subjects

Twenty one women were enrolled. The data for 1st sampling is available for 21 mothers. Of these 21 mothers, two subjects discontinued and left during first sampling (M-3 and M-17). Two could not comply to BF protocol (M-4 and M-20).

M-4 and M-5 data is excluded due to inappropriate sample collection.

The women were in low socioeconomic status. Mean maternal age was 23.85± 4.48 year and mean weight was 54.35±8.75 kg at the beginning of study (one month post natal) and a BMI of 22.66±3.62, mean level of education was less than 5 year in school. Maternal characteristics are presented in Table II .The mean birth weight of 17 infant was 3.02±0.37 kg (range 2.2 to 3.5 kg). The mean gestational age could not be ascertained. There were 16 boys and 5 girls. Table III shows the characteristics of the babies included in the study.

3.2. Determination of milk intake

The milk intake of the infants calculated from water intake using deuterium dilution method was 801±262 ml/day (n=18) (Table IV). Although most mothers promised to comply to exclusive breast feeding protocol, yet the non milk intake of infants as determined by deuterium dilution method was up to 120 ml per day. In some cases negative values of Fbo were obtained. No explanation for that could be found. The matter warrants to be discussed with the technical officer of the project.

3.3. Comparison of D_2O vs test weighing

The milk intake was determined at 4.5±1.2 week by D_2O dilution method as well as by test weighing method. The average milk intake determined by D_2O method was 705±129 ml (n=13) whereas by test weighing it was 1169±384 ml (n=13) and is presented in Table V and Fig 1.

Possible reason for high values in test weighing could be that only day time readings were recorded. Mothers recall was relied upon for the total number of feeds during night time, which might be a source of error. Moreover, since the number of feeds per 24 hours were up to 20, consequently the amount milk per feed was smaller. One of the inherent problems of test weighing method in developing countries, where feeds are more frequent and, consequently small, induce error in weighing.

Deuterium dilution method values for BM intake are also on higher than what one would normally expect at four weeks of age of infants. This envisages the need to look into the accuracy and precision of analysis as well as more careful sampling schedule (How much is that likely to effect the results. To be discussed with technical officer or experts in the field). The results of standards analyzed in our laboratory show high accuracy and precision (Table I). However, handling and preparation of biological samples in our laboratory needs to match the accuracy and precision that is normally attainable for all other hydrological samples [Ref]. It is expected that with a little more expertise and experience we will be able to resolve the problem of accuracy in analysis of biological samples also.

The data on water intake of mother is also erroneously high in cases where BM intake values are significantly higher than normal. The probable reasons for that need to be discussed with the CRP members and experts.

3.4. Growth

The mean birth weight of all infants was 3.02±0.37 kg (Table III) and at first sampling at 4.5±1.2 week it was 3.92±0.57 kg (Table IV) . The weight for age and length for age data is given in Fig. 2 and Fig. 3. The growth data collected over six month period for 21 infants showed that they fall within 5 percentile of NCHS for weight. The height of boys is in 50 percentile whereas for girls it is much below 5 percentile.

3.5. Intake/growth

The normal daily intake of water and calorie requirement of infants at four weeks of age is up to 100 to 120 ml/kg/day. [22]. The estimated milk intake of infants in the study 202±62 (Table III) should provide needed energy for optimum growth. However, the data obtained is contrary to what is expected. More data is therefore needed to verify the findings. Moreover, the amount of milk intake determined by either methodology need to be reviewed. The growth standards used as reference are of NCHS. The WHO growth charts should be used as reference.

4. PROBLEMS ENCOUNTERED

4.1. Samples

Initially plan was to collect saliva from the baby and breast-milk from the mother for D/H determination. However, at first sampling i.e., when infants were 25 days old it was not possible to collect any saliva. Baby's mouth was quite dry. Therefore it was decided to collect urine samples from the baby instead.

The breast milk sample collection posed no problem. However, the results of D/H determination from BM samples was highly variable. Since the bench top microfuge was not available, to spin down the probable particles, we therefore, changed from BM to saliva for the mothers.

4.2. Sample collection

The collection of samples over 15 day sampling for D_2O presented certain problems. The families sometimes moved to some other town without informing. This created difficulty in keeping to the strict time schedule for collection of samples. Although effort was made, by explaining to mother, the importance of keeping the time schedule. The test weighing were usually scheduled for the 5^{th} & 6^{th} day of deuterium study, so that data from all subjects could be standardized.

4.3. Sampling

The D_2O sampling was initiated a bit late because, the tracer (D_2O) ordered through IAEA procurement section could not be delivered as initially planned . However, Dr W.A. Coward was kind enough to supply some, through his own resources and later IAEA procurement rescheduled the order.

The Kontess ampoules, which are considered better than O-ring type J-young type ampoules, could not be procured. The glassware required for sample preparation arrived quite late (because of re-ordering after May 29th).

The sampling for D_2O dilution involved a number of precautions, which became more clear, once the analysis of samples were made.

The LHW normally available, are not quite trained in the type of sampling that was involved in the method. The variation in the D_2O and test weighing could be due to the timings of sample collection and the test weighing only during day time.

A few assays conducted in strict supervision of the research team gave comparable estimates e.g., (B-10 and B-11) of Kg/day by D_2O method and by TW (Table V).

Since the TW gives immediate estimates whereas, the D_2O dilution estimates come when the samples are analyzed in the laboratory and are plotted through Microsoft Excel. It was therefore not possible to keep strict scrutiny over the field workers who were mainly responsible for collecting the samples routinely. The data of D_2O was the last one to compile. Therefore no further precautions was possible on already collected samples.

5. PLANS FOR 2000

- Follow-up the remaining recruited infants at 4 month (13 week) of age for milk intake by D_2O and test weighing methods.
- Follow-up the growth of infants using anthropometry up to 1 year of age.
- Work on accuracy and precision of analysis of D/H samples.
- Work on dose administration, sampling accuracy and test weighing procedure.
- Analyze the samples already collected, to compute the milk intake.
- Interpret and analyze when all the data is available, for the following: a) correlation of milk output with the body composition of the mother, b) milk intake and growth of the baby at 1 and 4 months of age, c) correlation of milk output and nutritional status of the mother, d) correlate the illness record during first six months of age as possible explanation of sub optimum growth.
- Perform ^{13}C urea breath test on malnourished children at six months of age, to account for *Helicobacter pylori* colonization, which might provide some clue for growth failure.

5.1. Conclusions so far

The results obtained so far suggest that the D_2O method once streamlined is a useful and accurate method to provide the quantitative data on milk intake of infants. Besides milk intake data, additional useful information on maternal body composition is also obtained if dose to the mother method is used.

The volume of intake that baby ingested was adequate, however, the observed growth pattern of the baby suggest that the nutritional requirements were not met. This envisages the need to look at the maternal nutritional status and also the milk composition besides other environmental factors like infections which might explain to the sub optimal growth of the infants.

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TABLE II: CHARACTERISTICS OF MOTHERS ENROLLED IN THE STUDY

Sr.No	Code No	Age (yrs)	Height	Wt (kg)	Wt (Kg)	Difference	BMI	Education
			Meter	4 wk PN	12 wk PN			
1	M-1	26	1.55	50	45	-5	22.22	UM
2	M-2	22	1.61	63.5	61.6	-1.9	24.5	UM
3	M-3				Volunteer discontinued			UM
4	M-4	28	1.47	67.8	71.5	3.7	31.38	UM
5	M-5	23	1.55	65.9	64	-1.9	27.42	UM
6	M-6	25	1.61	na	60	na	23.14	UM
7	M-7	19	1.674	59	64	5	22.5	UM
8	M-8	22	1.45	56	51.5	-4.5	26.7	UM
9	M-9	19	1.55	30.6	33	2.4	13.18	UM
10	M-10	35	1.5	54.6	57	2.4	24.26	UM
11	M-11	21	1.55	45	48	3	18.73	UM
12	M-12	27	1.643	51	51	nil	19.92	UM
13	M-13	28	1.473	49	nd	_	22.6	UM
14	M-14	21	1.575	46..5	44.5	-2	20.5	UM
15	M-15	23	1.65	58	58	0	22.13	UM
16	M-16	26	1.575	48	50	2	19.5	UM
17	M-17	15	1.524	49	Volunteer discontinued		21.2	UM
18	M-18	25	1.524	51	57		22.07	matric
19	M-19	30	1.6	57	56	-1	22.2	UM
20	M-20	24	1.55	63	58.8	-4.2	26	UM
21	M-21	18	1.473	50	47.5	-2.5	23.1	UM
	Mean	**23.85**	**1.55**	**53.24**	**54.35**		**22.66**	
	SD	**4.48**	**0.0618**	**8.31**	**8.75**		**3.62**	
	Range	**15-35 yr**		**30-67**	**33-64**		**13.18-31.38**	

Body mass index was calculated according to the following formula:

BMI = Wt / (length)2

Ref: VINCENT HEGARTY(1988), Decissions in Nutrition, Pb.Times mirror/ Mosby College

Body Mass Index: Reference values from encylopedia Brittanica

20-24 Desir 25-29.9 Over weight 30 or above = Obese

TABLE III: CHARACTERISTICS OF INFANTS INCLUDED IN THE STUDY

Sr.No	Code	Birth wt (Kg)	delivery	infant wt/age perentile(birth)	age (week) 1st sampling	Sex	Remarks
1	B-1	3	normal	25	4	male	
2	B-2	2.5	normal	5	4	male	
3	B-3	NA	normal	x	3	female	left the study
4	B-4	2.5	c-section	5	4	male	Excluded
5	B-5	3	normal	25	7	female	Excluded
6	B-6	NA	normal	x	7	male	
7	B-7	3.5	normal	75	7	male	
8	B-8	3.5	normal	75	7	male	
9	B-9	3.5	normal	75	4	male	
10	B-10	2.8	normal	10	3	male	
11	B-11	NA	normal	x	4	male	
12	B-12	3	normal	25	5	female	
13	B-13	2.2	normal	<5	5	female	
14	B-14	3	normal	25	4	male	
15	B-15	3	normal	25	4	male	
16	B-16	3	normal	25	4	female	
17	B-17	3	normal	25	4	male	left the study
18	B-18	NA	normal	x	4	male	
19	B-19	3	normal	25	4	male	
20	B-20	3.5	normal	75	4	male	Non compliance
21	B-21	3.4	normal	50	4	male	
	Average	**3.02**			**4.5**	**Male =16**	
	SD	**0.37**			**1.2**	**Female=5**	

Table 4: Breast milk and non breast milk intake of infants at 4 week of age Determined by deuterium dilution method

Sr.No	Infant Code	Wt. (kg)	Age(d)	HC (cm)	Height (1st sampling)	F(mo)	M* (ml)	F(bm) (ml)	gm/kg	Fbo(gm)
1	B-1	3.4	30	nd	nd	3.72	690	600	202.9	60
2	B-2	4.04	23	36	nd	4.83	1660	1450	410.89	90
3	B-3					excluded from the study				
4	B-4	2.66	23	34.5	50	excluded from the study				
5	B-5	4.22	51	36	56	excluded from the study				
6	B-6	5.54	50	40	nd	3.09	900	790	162.45	70
7	B-7	4.64	37	38	57	10.34	1040	910	224.13	-470
8	B-8	4.28	35	38	57	6.29	540	470	126.16	-10
9	B-9	4	24	37	55	3.07	870	760	217.5	30
10	B-10	3.4	19	34	51	2.82	520	460	152.9	10
11	B-11	3.75	28	37	55	3.29	790	690	210.6	-50
12	B-12	3.75	34	37	53	2.93	720	630	192	-40
13	B-13	3.32	33	35	51	2.91	610	530	183.73	40
14	B-14	4.04	30	35	53	4.65	680	590	168.3	90
15	B-15	4.08	28	35	51.2	6.2	890	780	218.13	-290
16	B-16	4.02	30	36	52	6.04	730	640	181.5	50
17	B-17	3.84	31	37	53	8.63	1040	900	270.8	-320
18	B-18	3.14	30	36	54	3.5	670	590	213.3	-60
19	B-19	4.04	30	37.5	57	12.3	880	760	217.8	-50
20	B-20	3.96	30	37	54	14.32	720	630	181.81	120
21	B-21	4.22	27	36	58	4.72	470	410	111.3	46
Mean	**(n=18)**	**3.92**	**31.15**				**801**		**202**	
SD		**0.57**	**7.7**				**262**		**62.26**	

TABLE V: COMPARISON OF TEST WEIGHING AND D20 DILUTION METHOD

Sr.No	code No	TW-1	D2O-1
1	B-1	1.58	0.69
2	B-8	1	0.54
3	B-9	0.8	0.87
4	B-10	0.52	0.52
5	B-11	0.86	0.79
6	B-12	1.032	0.72
7	B-14	1.5	0.68
8	B-15	1.1	0.89
9	B-16	1.55	0.73
10	B-18	1.6	0.67
11	B-19	1.77	0.88
12	B-20	0.672	0.72
13	B-21	1.22	0.47
	Mean	1169	705
	SD	384	129

Data for 13 subjects was available for comparison

Fig 1: Comparison of test weighing and D2O dilution method

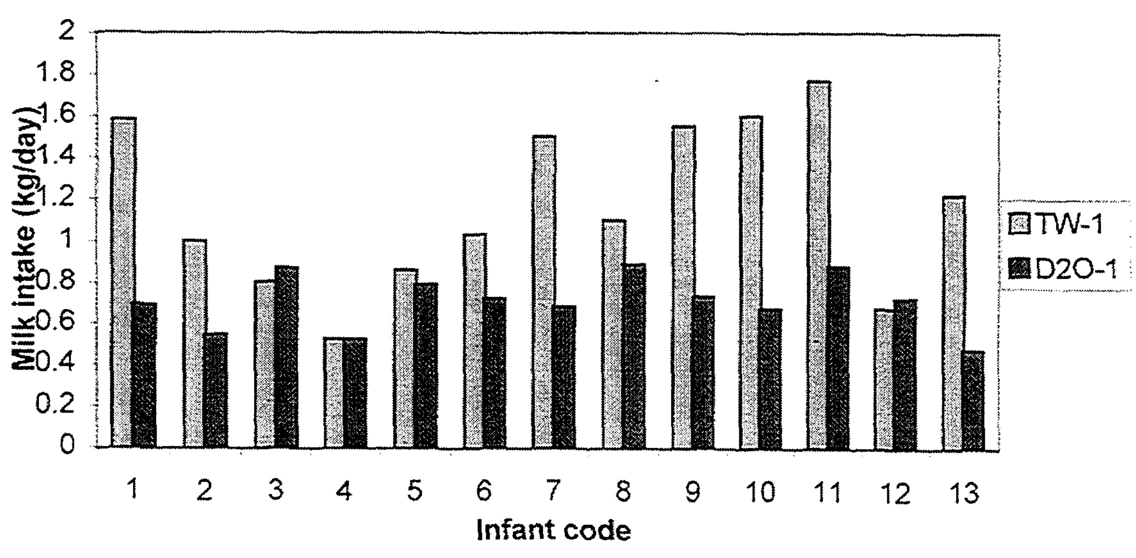
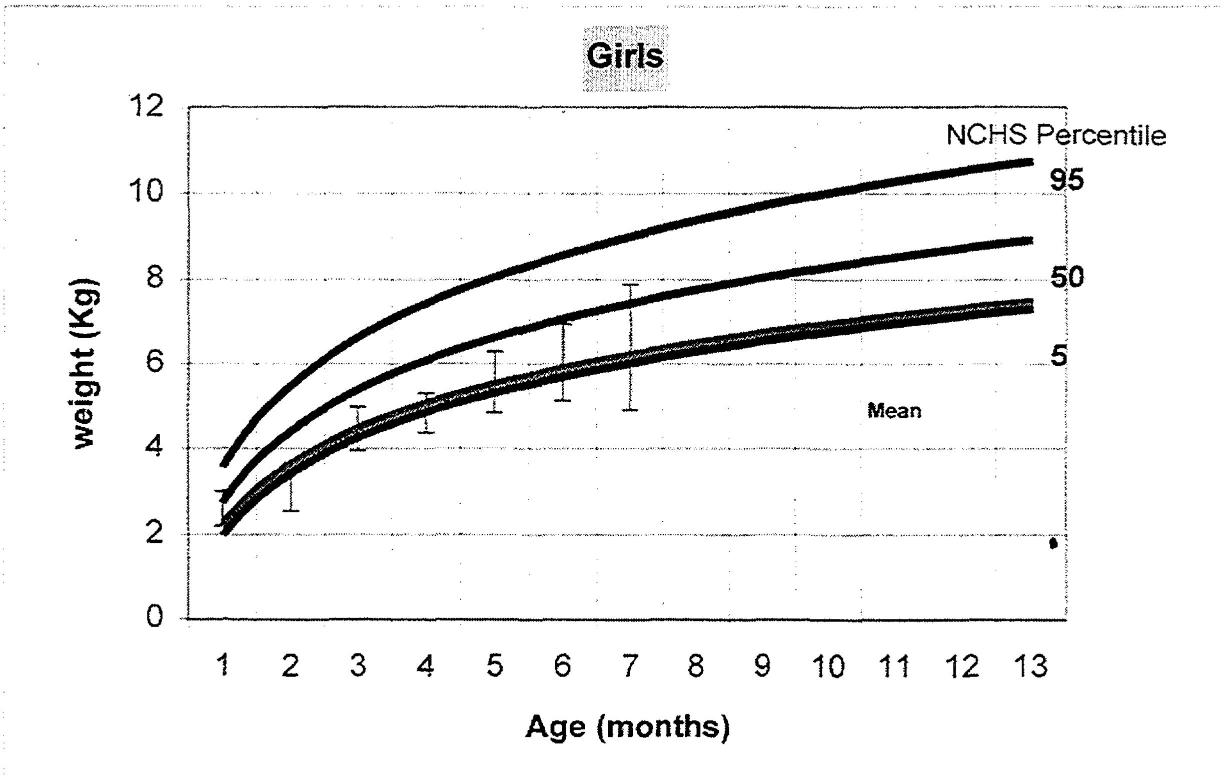
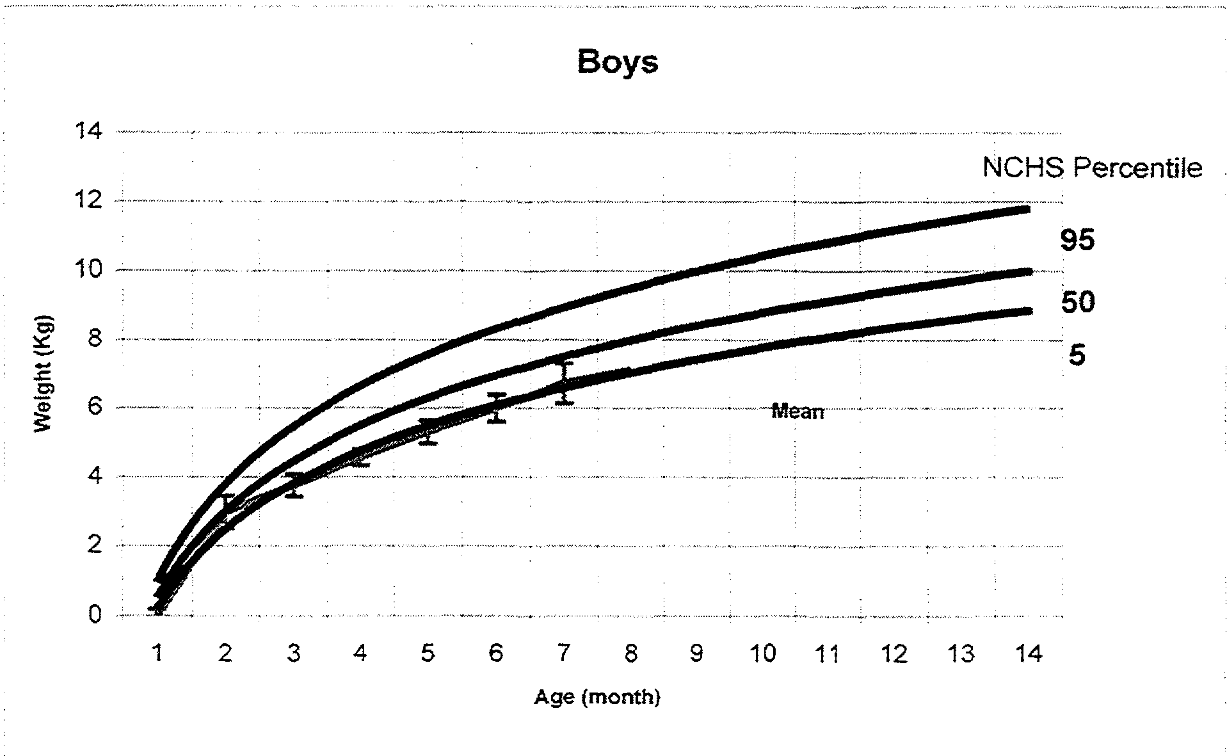


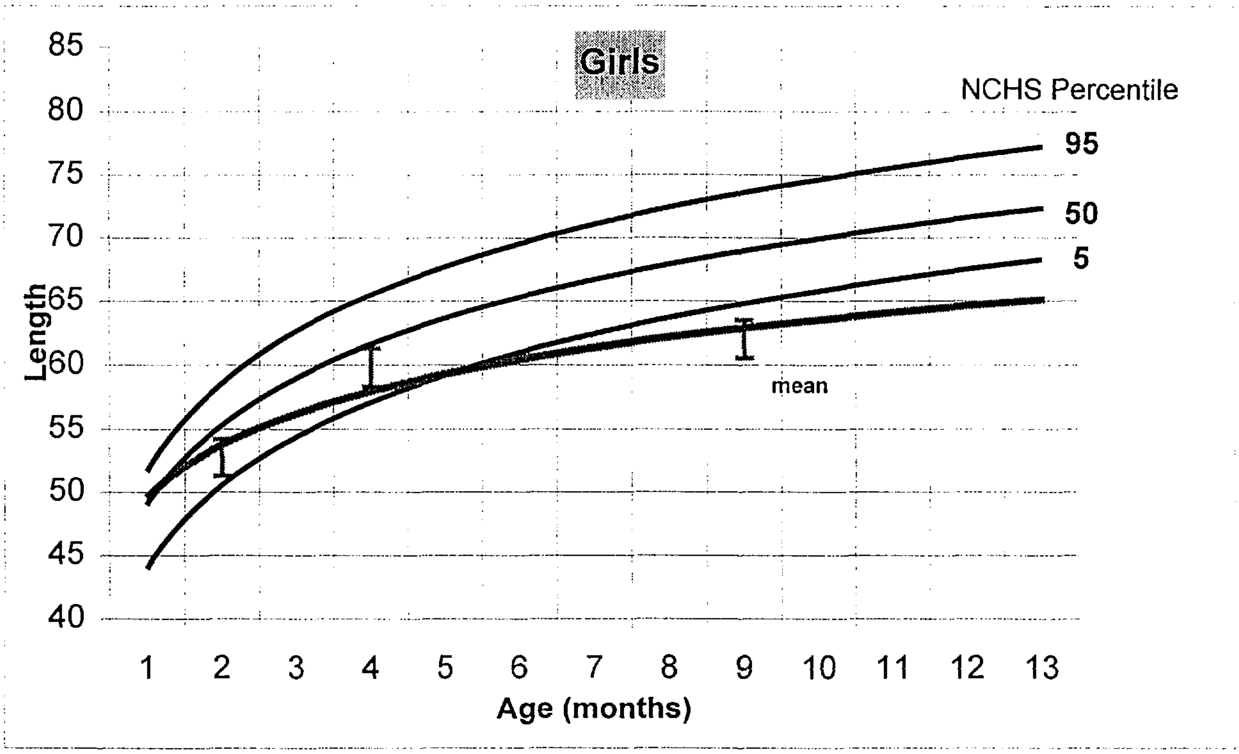
Fig 2: Weight for age of infants (mean +/- SD compared to NCHS

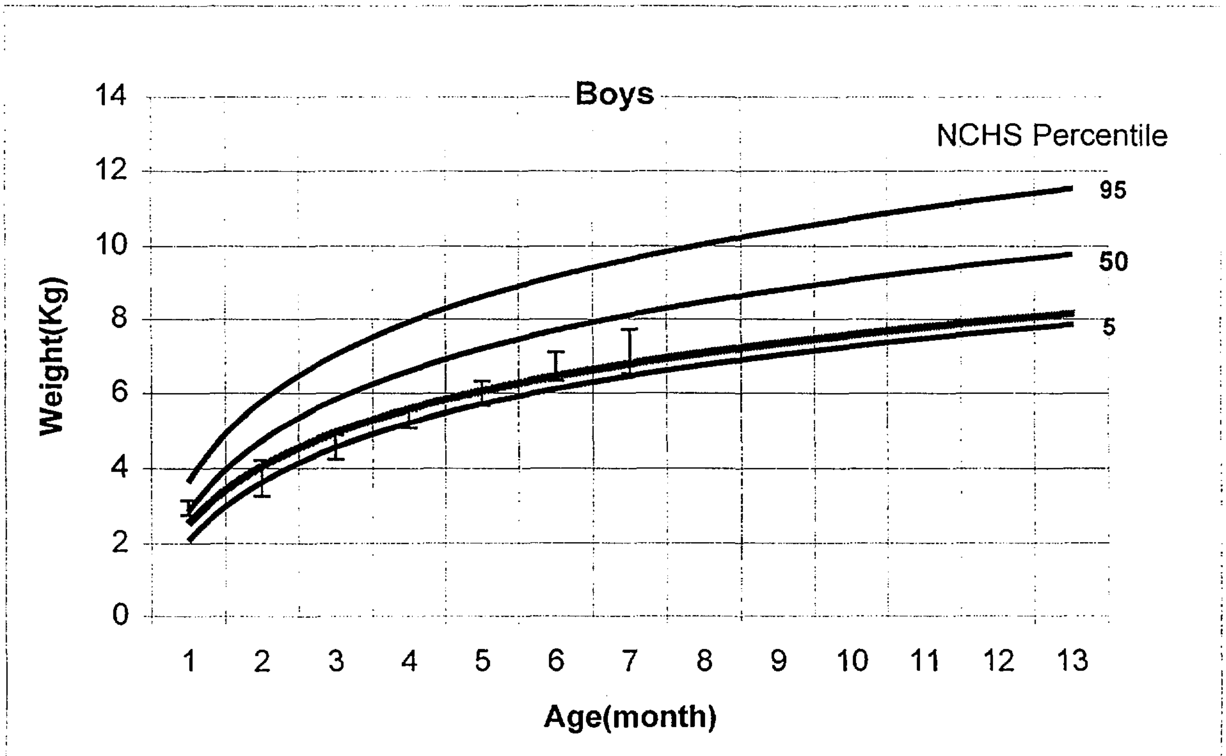




Ref: Hamill et.al., (1979) Physical Growth: National Centre for Health Statistics Percentiles.
Am. J. Clin. Nutr. 32:607-629

Fig 3: Length for age of infants (mean +/- SD compared to NCHS





Ref: Hamill et.al., (1979) Physical Growth: National Centre for Health Statistics Percentiles. Am. J. Clin. Nutr. 32:607-629