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Determination of the Absorption of Zn by Preterm Infants, Using ^{70}Zn Enriched Tracer

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Abstract : Absorption of zinc by preterm infants from a synthetic milk labelled extrinsically with ^{70}Zn tracer was determined by faecal monitoring. Six male preterm babies with a mean gestational age of 31±2.9 weeks and a mean birth weight of 1.23±0.24 kg were studied for three balance periods of 72 h with intervals ranging from 6 to 30 days. The babies were fed with the milk containing zinc with added ^{70}Zn tracer. The net (NA) and true (TA) absorption, and endogenous loss (EL) of zinc were determined by the classical (chemical) balance and isotopic approaches. The mean TA, NA and EL were 615±79, -341±665 and 956±647 μg kg^{-1} d^{-1} respectively from a mean Zn input of 839±93 μg kg^{-1} d^{-1}. The significant correlation between absorption of zinc and weight gain of the babies during the study period implied that the minimum absorption of Zn to keep weight at birth should be 500 μg kg^{-1} d^{-1}.

The observed differences between the NA, TA and EL obtained by the two approaches demonstrate that net intestinal loss or secretion of Zn can occur simultaneously with an efficient uptake of the element from the luminal pool, which can only be determined by the use of isotopic tracers.

Key Words: Stable Isotope, Zinc Absorption, Premature Infants

Introduction

Chemical (classical) balance is a method for the determination of mineral absorption by faecal monitoring (1). The difference between the element content of intake and faecal output (which are determined by, for example, atomic spectrophotometer) is considered to be the net amount absorbed. This approach does not take account of the contribution of endogenously excreted elements to faecal losses, which are particularly significant for elements such as Zn and Cu, for which homeostasis is maintained by the intestine (2,3). A measure of true absorption and the endogenous faecal loss can be obtained by using radioactive or stable isotopic tracers. The use of radioactive tracers on children and pregnant women is not appropriate due to risks associated with radiation exposure. The use of enriched stable isotopic tracers with determination by neutron activation analysis (NAA) or mass spectromety (MS) enables the determination of true absorption without any radiological risk.

In the stable isotope approach, subjects are fed a diet which is usually extrinsically labelled with a suitable stable isotope, and the absorption is derived from the degree of enrichment in faeces or blood (4). Tracer concentrations in body fluids and urine are much lower than in faeces, and repeated sampling of body fluids may be limited by ethical considerations. Measurement of absorption by faecal monitoring is the most frequently applied approach in view of the requirement of reduced inputs of isotopic tracers, and has been applied to elements such as zinc and copper. Absorption results obtained by faecal monitoring can be compared with those obtained in balance studies in which total element input and output are determined by atomic absorption spectrophotometer (AAS).

The nutritional needs of growing low birth weight infants have been based on estimates of the daily intrauterine increment of the body content of each nutrient and on estimates of gastrointestinal nutrient absorption and maintenance nutrient requirements (5). Measurement of gastrointestinal absorption by chemical balance often produces variable results due to non-random collection errors and irregularities in faecal excretion, causing overestimates of both absorption and retention, and its inability to measure endogenous faecal nutrient loss causes underestimates of absorption.

This study is a part of an investigation of the absorption of trace elements from synthetic infant formula milk, to correlate uptake with metabolic and nutritional state, protein source, extent of supplementation, and inter-element effects. Part of the investigation, including Fe and Cu absorption, and protein turnover by the enriched stable isotopic approach have been reported elsewhere (6,7,8). The principle concern of this paper is the determination of absorption of zinc from the infant formula milk in preterm infants by the use of ^{70}Zn enriched tracer. The absorption pattern of zinc in the infants is also considered.

Materials and Methods

Subjects and ethical approval

Six male preterm babies (gestational ages 27-35 weeks), whose physical characteristics are given in Table 1, were investigated. Ethical approval was provided by the Joint Ethical and Medical Committee of Grampian Health Board and the University of Aberdeen (Scotland, UK).

Supplementation of diet and sample collection

To avoid production variations, a single batch of diet containing no added minerals was allocated to the investigations. ^{70}Zn enriched stable isotope was added as part of the customary fortification of the 100 mL ready to feed infant diet. For ^{70}Zn with a natural abundance of 0.6%, an enrichment factor of about 80 was used. 400 μg of ^{70}Zn was added together with the required amount of Zn in natural abundance so that the final Zn content was 840 μg $(100\ mL)^{-1}$. Formulations were confirmed by analysis of diet. The diet was the sole source of the mineral input during the period of investigation.

The investigation was carried out for three balance periods with intervals ranging from 6 to 30 days. Four of the infants were studied for two and one for three balance periods. Each balance was performed for 72 hours, the first and final feeds were labelled with 50 mg of carmine red as a non-absorbable marker. Isotopically enriched diet was provided in the first 12 hours and the feeding was continued with unlabelled diet thereafter. The consumption was calculated by weighing the bottles before and after feeding.

Table 1. Physical characteristics of six preterm babies at the birth and the balances studied.

Subject No	1	2	3	4	5	6	Mean±SDM
Gestation, wk	29	27	35	33	30	31	31±2.9
Birth weight, kg	0.82	1.08	1.36	1.46	1.35	1.33	1.23±0.24
Balance I							
Age at study, d	19	31	17	18	29	13	21±7
Post-conceptional age, wk	31.7	31.4	37.4	35.6	34.1	32.9	33.8±2.3
Body weight, kg	0.85	1.34	1.46	1.54	1.78	1.41	1.40±0.31
Weight gained from birth, kg	0.03	0.26	0.10	0.08	0.43	0.08	0.16±0.15
Balance II							
Age at study, d	35	39	31	24	-	-	32±6
Post-conceptional age, wk	34	32.6	39.4	37.7	-	-	35.9±3.2
Body weght, kg	1.22	1.51	1.86	1.74	-	-	1.58±0.28
Weight gained from birth, kg	0.40	0.43	0.30	0.28	-	-	0.35±0.07
Weight gained from bal. I, kg	0.37	0.17	0.40	0.20	-	-	0.29±0.12
Balance III							
Age at study, d	54						
Post-conceptional age, wk	36.7						
Body weight, kg	1.62						
Weight gained from birth, kg	0.80						
Weight gained from bal. II, kg	0.40						

Stools were collected in de-mineralised disposable nappies and removed immediately from soiled nappies. Samples were collected for about 72 hours starting with the samples in which the first marker appeared and continued until the appearance of the second. Individual stool samples were analysed to confirm total output of enriched isotopes that appeared between the markers. Collections of stools for each balance were pooled and homogenised in an homogeniser with titanium-coated blades. The samples were then freeze-dried and ashed at 400ºC in silica (6).

Analysis of samples

The ^{70}Zn content of ashed faecal samples was determined by NAA with pre- and post-irradiation separation procedures. FAAS was used for the determination of elemental Zn contents of the samples to account for the isotopic contribution of Zn of the natural composition to enrichment in faecal pools. FAAS was also employed for the determination of Zn contents of the diet. All chemicals used in sample preparation and analysis were of high purity and suitable for trace metal analysis.

Duplicate fractions of ashed samples were subjected to a pre-irradiation chemical separation of the major elements by APDC (ammonium pyrolidine dithiocarbamate) precipitation to reduce exposure to unnecessary radiation during post-irradiation manipulations (9). For determination of the procedure yield, Zn-65 was used as a radioactive tracer. Precipitates were separated with centrifugation and dissolved in conc. HNO_3 and transferred to polypropylene irradiation vials. Zn standard solutions labelled with ^{70}Zn enriched tracer were also prepared in irradiation vials. The contents of the vials were evaporated and the vials were sealed.

The samples and standards were irradiated in the pneumatic transfer system facility of the research reactor of the SURRC (Scottish Universities Research and Reactor Centre, UK) in a flux of $2.4x10^{12}$ n cm^{-2} s^{-1} for 1 hour. Further purification was achieved to remove Mn by post-irradiation separation involving twice the precipitation of MnO_2, as described by Ting et al. (9) and Gökmen et al. (10). The ^{70}Zn content of the samples was determined by the activity of ^{71m}Zn, that is the activation product of ^{70}Zn, which emits a gamma ray of 386 keV with a half life 3.9 h. A fraction of the standard was also run throughout the procedures to yield the post-irradiation separation. Samples and standards were then counted on a 130 cc HPGe detector. The counting results were processed by data acquisition and an analysis system associated with a microprocessor. The ^{70}Zn contents of the samples were determined with reference to those of the standards by using an NAA program, taking account of the chemical yield.

Fractions of ashed samples were dissolved and diluted to appropriate volume, and their elemental Zn contents were determined by FAAS. The accuracy of the results was confirmed by analysis of NBS Bovine Liver reference standard material together with the samples. The same analytical procedure was applied for analysis of the diet administered when it was analysed by NAA or FAAS.

Fractional net absorption of zinc (FNA) was calculated from the ratio of total intake (E_d) minus faecal excretion (E_f) to E_d for each balance. The term "net" as used here is synonymous with "apparent" (11,12).

$$FNA=(E_d- E_f)/E_d$$

Fractional true absorption of zinc (FTA), defined as absorption of the ^{70}Zn label, was calculated from

$$FTA=1-(I_f / I_d)$$

If,d is the amounts of ^{70}Zn enriched in faeces and diet, given by

$$I_{f,d}= W_{f,d}C_{f,d} -E_{f,d}R_e$$

where $W_{f,d}$ is weights of 3 days faecal pool and the diet consumed labelled with ^{70}Zn; $C_{f,d}$ and $E_{f,d}$ are ^{70}Zn (determined by NAA) concentration and total elemental Zn (determined by AAS) content of the pool and the diet; and Re is the natural abundance of ^{70}Zn (0.006).

The net (NA) and true absorption (TA), were calculated by multiplication of FNA and FTA with the total elemental Zn intake (E_d) for each balance period. FNA and FTA were multiplied by 100 to obtain percentage values of the NA and TA.

Endogenous faecal loss of Zn (EL) was calculated from

$$EL=E_f - (1-TA)E_d$$

The ratio of TA/EL was also calculated to obtain which fraction of endogenous loss was met by the true absorption.

A two-tailed t-test and linear regression analysis were applied for statistical evaluation of the results.

Results

Elemental and isotopic contents of input and output, the NA and TA, EL of zinc (as the specific amounts, i.e.

micrograms per kg of body weight of the babies per day, μg kg^{-1} d^{-1}), and TA/EL for each balance period are given in Table 2 and Table 3. A summary of the data obtained from overall studies for a total of 11 balance studies performed on the 6 babies is given in Table 4.

When the values of TA of zinc were compared with weights gained from birth (as the specific weights gained i.e. gram per kg of body weights of the babies at birth per day, g kg^{-1} d^{-1}), a significant linear relation was found, $p<0.01$ (Figure).

Table 2. Elemental and isotopic contents of input and output for each baby at the balance studied (μg kg^{-1} d^{-1}).

Subject No	1	2	3	4	5	6	Mean±SDM
Balance I							
Zn consumed (E_d)	797	755	880	789	695	794	785±60
Faecal Zn lost (E_f)	2674	677	1217	1019	947	929	1244±677
^{70}Zn consumed (I_d)	112.2	98.3	114.0	98.7	93.2	106.6	103.8±8.4
Faecal recovery of ^{70}Zn (I_f)	35.0	23.7	42.4	35.2	6.5	31.7	29.1±12.6
Balance II							
Zn consumed (E_d)	883	824	968	830	-	-	876±67
Faecal Zn lost (E_f)	2155	784	797	1176	-	-	1228±644
^{70}Zn consumed (I_d)	108.5	109.0	105.7	105.7	-	-	107.2±1.8
Faecal recovery of ^{70}Zn (I_f)	25.3	29.7	22.0	27.1	-	-	26.0±3.2
Balance III							
Zn consumed (E_d)	1015						
Faecal Zn lost (E_f)	611						
^{70}Zn consumed (I_d)	113.1						
Faecal recovery of ^{70}Zn (I_f)	33.6						

Table 3. True (TA), net (NA) absorption and endogenous lost of Zn (EL) as specific amounts and percentages of input of zinc, and the ratio of TA/EL for each baby for each balance studied.

Subject No	1	2	3	4	5	6	Mean	SDM
Balance I								
NA, μg kg^{-1} d^{-1}	-1877	77	-337	-230	-252	-134	-459	709
TA, μg kg^{-1} d^{-1}	548	572	552	507	647	559	564	46
EL, μg kg^{-1} d^{-1}	2425	494	890	737	693	898	1023	732
NA, %	-235.5	10.2	-38.3	-29.2	-36.2	-16.9	-57.6	88.9
TA, %	68.8	75.8	62.8	64.3	93.0	70.3	72.5	11.0
TA/EL	0.23	1.16	0.62	0.69	0.93	0.62	0.71	0.31
Balance II								
NA, μg kg^{-1} d^{-1}	-1272	40	171	-347	-	-	-352	652
TA, μg kg^{-1} d^{-1}	677	600	767	617	-	-	665	75
EL, μg kg^{-1} d^{-1}	1949	559	596	963	-	-	1017	648
NA, %	-144.1	4.9	17.7	-41.8	-	-	-40.8	73.4
TA, %	76.7	72.7	79.2	74.3	-	-	75.7	2.8
TA/EL	0.35	1.07	1.29	0.64	-	-	0.84	0.42
Balance III								
NA, μg kg^{-1} d^{-1}	404							
TA, μg kg^{-1} d^{-1}	714							
EL, μg kg^{-1} d^{-1}	310							
NA, %	39.8							
TA, %	70.3							
TA/EL	2.30							

Table 4. Mean of overall results obtained from 6 babies studied for the 11 balances (see Table 2 and Table 3 for details).

Mean of overall results (n=11)	Mean	SDM	Range
Zn consumed (E_d), μg kg^{-1} d^{-1}	839	92	(695)-(1015)
Faecal Zn lost (E_f), μg kg^{-1} d^{-1}	1180	649	(611)-(2674)
NA, μg kg^{-1} d^{-1}	-374	633	(-1877)-(404)
TA, μg kg^{-1} d^{-1}	615	79	(615)-(767)
EL, μg kg^{-1} d^{-1}	956	647	(310)-(2425)
NA, %	-42.7	79.9	(-235.5)-(39.8)
TA, %	73.4	8.2	(62.8)-(93.0)
TA/EL	0.90	0.57	(0.23)-(2.30)

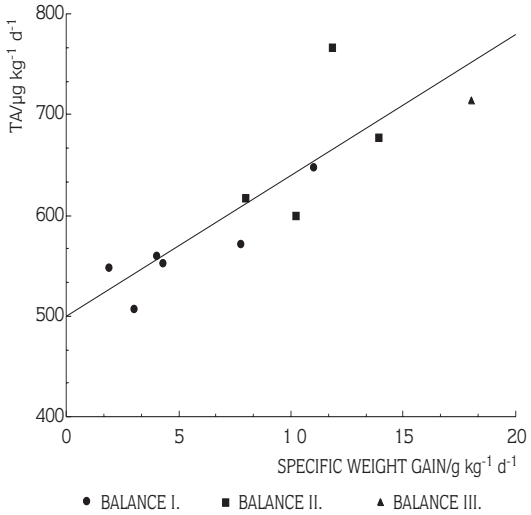

Figure Relations between specific absorption of zinc (TA) and specific weights gain from brith (WG) [TA=14WG+499, r=0.857, p<0.01].

Discussion

Overall mean input and output of Zn were 839±92 μg kg^{-1} d^{-1} and 1180±649 μg kg^{-1} d^{-1} respectively. From these, an overall mean of NA was found to be -341±665 μg $kg^{-1}d^{-1}$ (-42.7±79.9% of the input) by the chemical balance approach, whilst the mean of true absorption was 615±79 μg kg^{-1} d^{-1} (75.4±8.2% of the input of ^{70}Zn tracer) by the stable isotopic tracer approach (Table 4). The mean TA/EL, which refers to the fraction of zinc lost by endogenous secretions (956±647 μg kg^{-1} d^{-1}) met by the TA, was found to be 0.90±0.57. The observed differences between the absorption results obtained by the two approaches (net and true absorption) demonstrate that net intestinal loss or secretion of Zn can occur simultaneously with an efficient uptake of the element from the luminal pool. The negative balance that occurred could largely be explained by the immaturity of the gastrointestinal tract of the premature infants which fails to reabsorb endogenous zinc that is present in pancreatic and intestinal secretions, and in discarded epithelial cells, as suggested by Ehrenkranz et al. (12).

The positive slope found for the correlation between TA and weight gain (Figure) indicates that the absorption of zinc increased accordingly with the increasing weight gained. The intercept obtained from the linear relation implies that for the babies involved in this investigation, the minimum amount of Zn required at birth is 499 μg kg^{-1} d^{-1}. However, specific absorption increased with increasing weights, from 564±46 μg kg^{-1} d^{-1} for 1.40±0.31 kg mean weights at the 1st balance to 665±75 μg kg^{-1} d^{-1} for 1.58±0.28 kg mean weights at the 2nd balance. Although no such comparison was possible for the 3rd balance, in view of only one result being available, the increasing specific absorption with increasing body weight for the subject studied for three balance periods was also worthy of note. For this infant, TA/EL values were 0.23, 0.35 and 2.30 for the consecutive balance periods, which increased with the weights gain from birth (0.03, 0.40 and 0.80 kg for the corresponding balance periods). A similar trend was also observed for the other infants studied twice (Table 3).

A significant relation between net and true percentage absorption of zinc from various preterm formulas in preterm babies was reported (5), where the number of cases studied was 50 for 41 subjects. However, no such relation was found in this investigation. No significant relation was found between the absorption and any other physical characteristics of the babies that changed during the investigation ($p>0.05$). This is attributable to the fact that the study was performed within a relatively short time period, as reported by Ehrenkranz et al. (5).

In conclusion, this investigation has demonstrated that the extrinsic stable isotopic tag ^{70}Zn can be used to study absorption of dietary zinc in premature infants and indicated the clinical significance of the true determination of Zn absorption. We used NAA for the determination of ^{70}Zn enriched tracer. For such investigations, mass spectrometry (ICP-MS in particular), which is commonly used for analysis of isotopic tracers (13), should be considered. In investigations on the relevance of absorption of essential minerals with regard to health and diseases, stable isotopic tracers provide the most reliable results, although the costs are considerable.

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