PROTEIN REQUIREMENTS OF PRESCHOOL CHILDREN: OBLIGATORY NITROGEN LOSSES AND NITROGEN BALANCE MEASUREMENTS USING COW'S MILK

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SUMMARY

Obligatory N losses through feces (FN) and urine (UN) were measured in five children and N balance was measured in them and in five others (23 ± 4 months old) using four levels of cow's milk intake. FN, UN and FN + UN were 19.5 ± 6.9, 34.0 ± 5.3, and 53.7 ± 8.1 mg N/kg/day, respectively. The ratios of FN, UN and total obligatory losses (FN + UN + sweat and integumental N) to basal energy expenditure were 0.38, 0.64 and 1.11 mg N/basal kcal, respectively. The two latter values are 32 and 45% lower than the 1971 FAO/WHO estimates.

Mean N requirement determined by factorial calculations using a correction factor of 1.3 and by N balance techniques was 98 mg N or 0.61 g milk

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protein/kg/day, which is 33o/o lower than the FAO/WHO estimates. Depending on the allowances made for inter-individual variability, safe levels of protein intake ranged from 0.79 to 0.94 g/kg/day, 33 to 21o/o lower than FAO/WHO recommendations. Apparent and "true" milk protein digestibilities were 80 ± 4o/o and 94 ± 4o/o, respectively.

INTRODUCTION

The estimates of protein requirements most widely used for preschool aged children are those recommended by an Expert Committee convened in 1971 by the United Nations' Food and Agriculture Organization (FAO) and World Health Organization (WHO) (1). The lack of adequate experimental data forced the Committee members to adopt as average requirements those obtained from factorial calculations corrected by a factor of 1.3, "which agreed with the few directly determined values for milk and egg proteins" (1). The factorial calculations, however, were based on the assumption that total obligatory nitrogen losses maintained the same relationship with basal energy expenditure as that observed in adults (i.e., 2 mg N/basal kcal). This would correspond to 100 mg N/kg/day for a 2-year-old child. Those figures are still used although there is now evidence that the total obligatory N losses are significantly lower in young children (2, 3).

The FAO/WHO Committee also concluded that the requirements for maintenance (obligatory losses) and growth, which are the basis of the factorial calculations, have a coefficient of variation of approximately 15o/o. Therefore, in addition to the correction factor of 1.3 to calculate the average requirements, an additional 30o/o would cover the needs of the great majority of individuals. These "safe levels of protein intake" corresponded to 1.19 g milk or egg protein/kg/day for a 2-year-old child.

The present studies, based both on factorial calculations and on nitrogen balance techniques, suggest that the average protein requirements and safe level of intake for a 2-year-old child may be 33o/o lower than those suggested by the 1971 Joint FAO/WHO Expert Committee.

MATERIALS AND METHODS

The investigations were carried out as two separate studies.

In one of them, the obligatory N losses through urine (UN) and feces (FN) were determined on five children. In the other study, the average N requirement was determined by N balance techniques on the same five children and on five additional ones.

Children

Table 1 shows their characteristics at the beginning of the first study in which they participated. All had been admitted to INCAP's Clinical Center with edematous protein-energy malnutrition and achieved complete nutritional recovery 1-3 months prior to initiating the study, based on rate of growth and on clinical, anthropometric and biochemical characteristics. One child (MV) had relatively low weight-for-height and creatinine-height index (CHI) but he was healthy, active, and growing at a steady rate. The absence of further catch-up in weight-for-height and CHI suggested that he was in the lower end of the normal distributions of weight and urinary creatinine excretion.

The children remained in the Clinical Center for an extended period of time at their parents' request. It was made clear to them that participation in these studies was *not* a requirement to remain in the Clinical Center. The parents agreed to their children's participation in the study after its objectives and experimental details were clearly explained. Any child who might show signs of nutritional derangement or of an infection would have been returned immediately to a normal, highly nutritive diet. A program of games which involved running, walking and climbing stairs allowed the children to maintain an active physical life at the Clinical Center.

Obligatory Losses Protocol

Four children were studied for nine days. A fifth child was studied only seven days when initial results indicated this was sufficient for the purpose pursued. The children drank a milk formula which provided 2 g protein and 100 kcal/kg/day for at least eight days before being placed on the nitrogen-free diet shown in Table 2. They were returned to a relatively high protein diet, fed *ad libitum*, immediately afterwards. The diets were supplemented with vitamins, minerals and electrolytes; water was also offered *ad libitum*. Complete urine collections were obtained at 24-hour intervals. Fecal collections were begun on day 2 as 48-hour pools,

TABLE 1

CHARACTERISTICS OF CHILDREN WHO PARTICIPATED IN THE STUDIES

Child	Study[1]	Age months	Height-age, months	Weight, kg	Height, cm	Weight-for-height, o/o of expected[2]	CHI[3]
CR	O, BD	25	16	10.86	80.0	98	0.86
WM	O, BA	17	10	8.82	72.9	96	1.02
IG	O, BD	24	17	10.98	81.0	98	1.04
HA	O, BD	25	15	10.66	78.5	100	0.89
AA	O, BD	31	23	11.96	86.4	97	0.92
WG	BD	21	11	10.29	74.0	104	1.08
DV	BD	24	13	9.45	76.7	93	0.94
AZ	BA	19	14	9.98	77.9	95	0.96
IT	BA	21	9	9.03	71.3	100	0.96
MV	BA	25	14	9.28	77.6	88	0.84
Mean		*23*	*15*	*10.13*	*77.6*	*97*	*0.95*
SD		*4*	*3*	*1.00*	*4.4*	*4*	*0.08*

1 BA = Nitrogen balance, ascending design. BD = Nitrogen balance, descending design. O= Obligatory N losses.

2 Based on 50th percentile of Boston Standards (4).

3 Creatinine-height index (5).

separated by alternating carmine red and brilliant blue as fecal markers given with breakfast on days 2, 4, 6, 8 and 10.

Nitrogen Balance Protocol

The study lasted 36 days. The children drank milk formulas which provided 0.5, 0.75, 1.0 and 1.25 g protein and 100 kcal/kg/day; 30o/o of the energy was derived from cottonseed oil. The composition of the diet providing 1.25 g protein/kg/day is detailed in Table 2. A single batch of spray dried skim cow's milk was used. Its amino acid composition, based on 24 and 88-hour hydrolysis, is shown in Table 3. The protein content of the diet was increased (ascending design, A) or decreased (descending design, D) by 0.25

TABLE 2

COMPOSITION OF DIETS

Ingredients	Milk formula providing 1.25 g protein/kg/day			Protein-free formula		
	g/kg	g prot/kg	kcal/kg	g/kg	g prot/kg	kcal/g
Dried skim milk	3.55	1.25	12.2	–		
Cornstarch	1.50		6.0	2.5		10.0
Sucrose	13.25		53.0	15.15		60.6
Cottonseed oil	3.26		28.8	3.33		29.4
Mineral mixture*	0.61			0.61		
Water	77.83			78.41		
Total	100.0	1.25	100.0	100.0	–	100.0

* Provides (in mEq): K^{+} 6; Na^{+} 1; Ca^{++} 1; Mg^{++} 0.4; Cl^{-} 6; PO^{-3} 1; CO_3^{-2} 1; SO_4^{-2} 0.4.

g/kg/day at 9-day intervals. The changes were isoenergetic with carbohydrate replacement of proteins and viceversa. Six children began with 1.25 g protein/kg/day (D design) and four with 0.5 g/kg/day (A design); diets were supplemented with vitamins, minerals, electrolytes and water. Urine and feces were collected during the last four of each 9-day period, using brilliant blue or carmine red as fecal markers. Apparent N balance was calculated as intake minus urinary and fecal excretions. Allowances for growth (16 mg N/kg/day) (1) and miscellaneous insensible losses (8 mg/kg/day) (2, 3) were made to calculate mean requirements and safe levels of intake. Prior to the study, the children ate for several days a diet which provided 2-3 g protein and 100 kcal/kg/day and during the four days preceding the first experimental period they ate a diet with either 2 (D design) or 1.5 (A design) g milk protein and 100 kcal/kg/day.

Nitrogen Analyses

Feces were dried in an oven until they achieved constant weight; the dried powdered feces were homogenized and aliquots

TABLE 3

AMINO ACID COMPOSITION OF SKIM MILK PROTEIN BASED ON 88-HOUR HYDROLYSIS FOR ISOLEUCINE AND VALINE AND ON 24-HOUR HYDROLYSIS FOR ALL OTHERS (mg/g protein)*

Essential			Non essential	
Histidine		34.5	Alanine	34.0
Isoleucine		56.4	Arginine	32.8
Leucine		98.8	Aspartic acid	78.3
Lysine		85.8	Glutamic acid	235.0
Total sulfur a.a.		38.0	Glycine	19.9
Methionine	27.6		Proline	104.5
Cystine	10.4		Serine	55.0
Total aromatic a.a.		93.0		
Phenylalanine	52.7			
Tyrosine	40.3			
Threonine		44.1		
Tryptophan		19.8		
Valine		64.2		

* Analyzed by column chromatography. Courtesy of Drs. F. Steinke and D. Hopkins, Ralston-Purina, Co., St. Louis, Mo.

were then analyzed in duplicate by a micro-Kjeldahl technique. Aliquots of diets and urines were analyzed in the same manner. If duplicates did not coincide within 10%, the analysis was repeated. Dietary and fecal results were multiplied by 1.05 based on 95% recovery of tryptophan standards. No corrections for recovery were made in urinary nitrogen.

RESULTS

Obligatory Nitrogen Losses

Figure 1 demonstrates that UN reached a plateau after four days on the N-free diet, whereas mean FN was stable from days 2 to 9. These children ate low residue diets for several days before

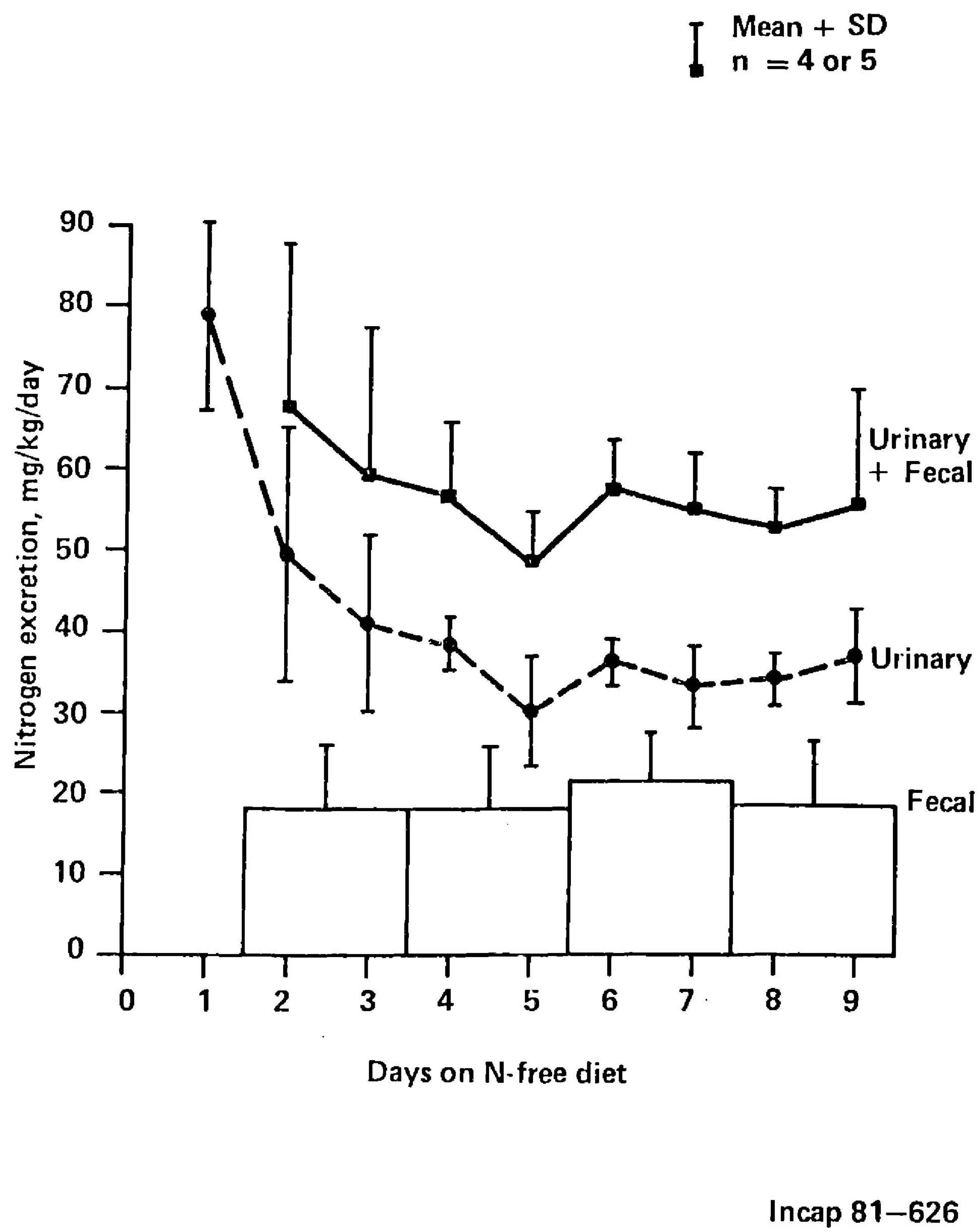

FIGURE 1

Urinary and fecal nitrogen excretion on a nitrogen-free diet and during the study; this may have induced the early FN plateau.

The mean and standard deviations for the combined data of days 5 to 9 were 34.0 + 5.3 mg N/kg/day for UN, 19.5 ± 6.9 for FN, and 53.7 ± 8.1 for the sum of both. Viteri and Martínez (2) reported 36 ± 7 for UN and 19 ± 2 for FN in 8 children 14-24 months old between days 7 and 10 on a nitrogen-free diet. If the present study had been carried out only for six days and the means of days 5 and 6 would have been used, the corresponding values would have been 33.2 ± 5.9 for UN, 19.9 ± 6.8 for FN and 53.0 ± 7.7 for the sum.

Nitrogen Balance Study

The regression equations of apparent balance (intake-fecal and urinary excretion) on intake for each child and for the pooled data are detailed in Table 4. The mean regression coefficients (*b*) of the individual equations agreed with those of the pooled regressions within 6% and *b* was 5% greater with the descending than with the ascending design. The mean N requirement to retain 24 mg N/kg/day for growth and to compensate for miscellaneous insensible losses was quite consistent whether based on the mean individual or pooled regression equations and on the A, D or both designs. It ranged from 96 to 100 with and overall average of 98 mg N/kg/day, which corresponds to 0.61 g protein/kg/day.

"True" protein digestibilities calculated with 20 mg/kg as the daily FN were similar at all levels of intake in both designs. The average "true" digestibility was 94% of intake (Table 4).

DISCUSSION

Factorial Calculations

Assuming that miscellaneous insensible N losses were of the order of 5 mg N/kg/day while eating a N-free diet (2), total obligatory N losses were 59 mg N/kg/day, which are 41% less than the current FAO/WHO estimates (1). After adding 16 mg N/kg/day to allow for growth of children of the same height-age and multiplying by the 1.3 correction factor, the mean N requirement for these children would be 97.5 mg/kg/day, equivalent to 0.61 g milk protein/kg/day. The coincidence with the results obtained in the nitrogen balance study suggests that the empirical factor of 1.3

TABLE 4

DATA DERIVED FROM NITROGEN BALANCE STUDY

Child	Digestibility %o of intake app.	"true"[1]	Regression of apparent balance (y) on intake (x), mg N/kg/day[2] y = a + bx	r	N intake required to retain 24 mg N/kg/day[3]
Descending design					
AA	81	96	−68 + 0.690x	.991	133
CR	76	91	−74 + 0.922x	.970	106
IG	88	104	−16 + 0.652x	.994	61
HA	78	92	−24 + 0.546x	.972	88
WG	76	91	−49 + 0.765x	.988	95
DB	78	92	−42 + 0.718x	.992	92
Mean	*80*	*94*	*−46 + 0.716x*		*96*
SD	*5*	*5*	*23 .125*		*24*
Pooled data (n= 22)[4]			*−51 + 0.766x*	*.914*	*98*
Ascending design					
WM	83	97	−34 + 0.725x	.995	80
MV	75	91	−72 + 0.642x	.997	150
AZ	82	96	−51 + 0.816x	.999	92
IT	78	93	−16 + 0.552x	.958	72
Mean	*80*	*94*	*−43 + 0.684x*		*99*
SD	*4*	*3*	*24 .113*		*35*
Pooled data (n= 16)			*−48 + 0.718x*	*.836*	*100*
Both designs					
Mean	*80*	*94*	*−45 + 0.703x*		*97*
SD	*4*	*4*	*22 .115*		*27*
Pooled data (n= 38)			*−49 + 0.740x*	*.877*	*98*

1 "True" digestibility assumes obligatory fecal N loss of 20 mg/kg/day.
2 Apparent balance = Intake − urinary N − fecal N.
3 Allowing 16 mg N/kg/day for growth and 8 mg N/kg/day for insensible losses.
4 Using all data for the corresponding design; n = number of determinations.

applied by the FAO/WHO Expert Committee (1) to estimate mean N requirements from the factorial method is a good approximation.

Mean Protein Requirements

Two different experimental approaches were followed: a) factorial calculations based on actual measurements of obligatory N losses, and b) multi-level N balance measurements with ascending and descending designs. Both gave a result of 0.61 g milk protein/kg/day, which suggests that the FAO/WHO Committee overestimated the protein requirements for 2-year-old children by about 33%.

Safe Levels of Protein Intake

The addition of 30% to the mean requirements to account for inter-individual variability, as proposed in 1971 and reiterated in a more recent FAO/WHO Memorandum (6), indicates that 0.79 g/kg/day is the safe level of milk protein intake. Table 5 shows that 0.79 g protein/kg/day from the milk used in this study provides the recommended intakes of essential amino acids established at INCAP for similar children (7, 8). This lends further support to our conclusion of an overestimation in the 1973 FAO/WHO report.

The coefficients of variation observed in our measurements of UN + FN (8.1/53.7 = 15%) and in the mean regression coefficients of N balance on intake (0.115/0.703 = 16%) agree with the FAO/WHO estimates of variation. However, the coefficient of variation of the mean N intakes necessary to retain 24 mg N/kg/day, therefore allowing for growth needs and insensible losses, was larger (27/97 = 28%). If an allowance of 56% (i.e., two times that coefficient of variation) were made for interindividual variability, the safe level of intake would become 151 mg N or 0.94 g protein/kg/day. A similar figure is obtained from the 95% confidence bands of the pooled regression analysis of N retention on intake (Figure 2) as suggested by MIT investigators (9, 10), considering a retention of 24 mg N/kg/day as the adequate N "balance" for children of this age group. The intake of 150 mg N/kg/day would satisfy the child with the highest needs who participated in the present nitrogen balance study (child MV, Table 4). If that child were excluded from the analysis based on his low

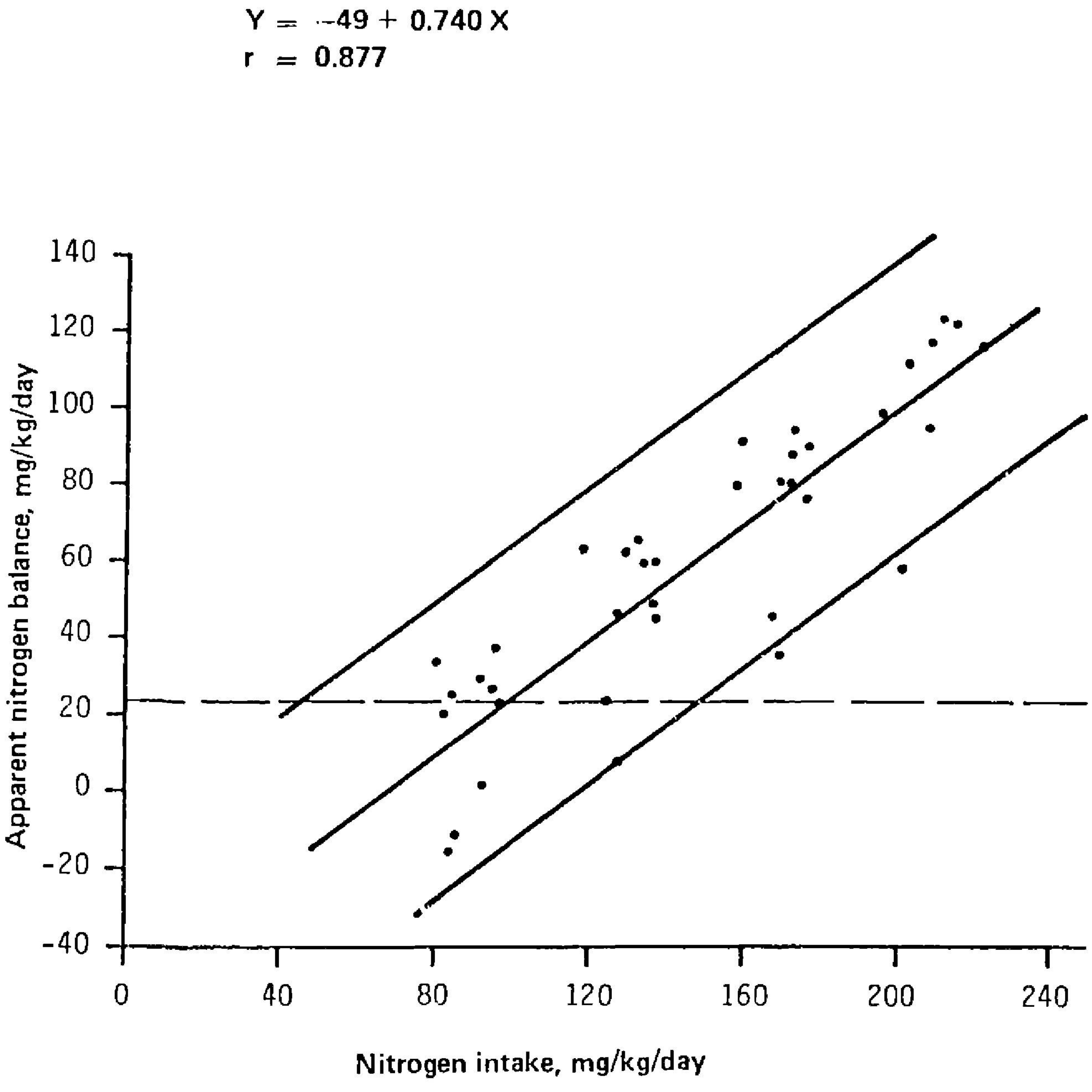

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FIGURE 2

Apparent nitrogen balance [Intake — (Urinary N + Fecal N)] as function of intake. Pooled data of 38 measurements on 10 children with 95% confidence bands

TABLE 5

ESSENTIAL AMINO ACID CONTENT, mg, OF THE SAFE LEVEL OF MILK PROTEIN INTAKE SUGGESTED BY THIS STUDY

Essential amino acid	Content in 0.79 g milk protein	Amino acid recommendations established at INCAP[1]
Histidine	27	n. i.[2]
Isoleucine	45	3[illegible]
Leucine	78	n. i.
Lysine	68	6[illegible]
Methionine + cystine	30	2[illegible]
Phenylalanine + tyrosine	73	n. i.
Threonine	35	> 37, < 53
Tryptophan	16	> 12, < 17
Valine	51	3[illegible]

1 From Pineda *et al.* (7), and Torún *et al.* (8).
2 Not investigated.

weight-for-height and CHI (Table 1), the mean N requirement for the remaining nine children would be 91 ± 21 mg/kg/day (coefficient of variation = 23%). The safe level of intake would then be estimated as 133 mg N or 0.83 g protein/kg/day. The safe level based on the 95% confidence band of the pooled regression analysis would be 130 mg N/kg/day after excluding MV's data.

The issue of inter-individual variation is yet to be resolved in order to recommend safe levels of intake from mean requirement measurements. But even if the largest allowance mentioned above (56%) was made, current FAO/WHO recommendations would still be too high by 21%.

Obligatory N Losses and Basal Energy Expenditure

The relationship between total obligatory N losses and basal energy expenditure (BEE) in preschool children is lower than the 2 mg N/basal kcal reported for adults (1). The average BEE measured by two of the authors (Viteri and Torún, unpublished data) in many children similar to those in the present study was 53 kcal/kg/day, which agrees with the values notified by others for children of the same weights (11). Therefore, the ratio of FN to BEE was 0.38 mg of N per basal kcal, which coincides with observations made by Huang, Lin and Hsu (3) and with the value accepted by the FAO/WHO Committee for all ages after infancy. The ratio of UN to BEE, however, was 0.64 mg N/basal kcal, lower than the values of 1.4 and 0.95 mg N/basal kcal accepted by the Committee and by Huang, Lin and Hsu, respectively. The seemingly low urinary N excretions in the present study are not artifacts; their validity was supported by the measurements done in 44 trials carried out to develop a short-term nitrogen balance assay for protein quality (Torún, unpublished observations). Children similar to those who participated in this study ate a nitrogen-free diet for two days. On the second day their urinary N had fallen to 42.9 ± 11.4 mg/kg/day which is similar to the values shown in Figure 1. As a consequence of the low UN/BEE, the total obligatory N losses of 59 mg/kg/day correspond to 1.11 mg of N per basal kcal, which is around 45% less than the estimates of 2 mg N/basal kcal derived from adult data.

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RESUMEN

REQUERIMIENTOS PROTEINICOS DE NIÑOS PREESCOLARES: PERDIDAS OBLIGATORIAS DE NITROGENO Y DETERMINACIONES DE BALANCE NITROGENADO USANDO LECHE DE VACA

Se midieron las pérdidas obligatorias de N en heces (NF) y orina (NU) de cinco niños y su balance de N, así como el de otros cinco niños más (edad: 23 ± 4 meses) usando 4 niveles de ingestión de leche de vaca. Los valores de NF, NU y NF + NU fueron de 19.5 ± 6.9, 34.0 ± 5.3 y 53.7 ± 8.1 mg N/kg/día, respectivamente. Las proporciones de NF, NU y pérdidas obligatorias totales (NF + NU + N en sudor e integumentario) en relación a gasto energético basal fueron 0.38, 0.64 y 1.11 mg N/Kcal basal, respectivamente. Los dos últimos valores son 32% y 45% más bajos que los estimados por FAO/OMS en 1971. El requerimiento promedio de N determinado por cálculos factoriales usando un factor de corrección de 1.3 y por medio de los estudios de balance de N, fue 98 mg N ó 0.61 g proteína de leche/kg/día, que es 33% menos que el estimado por FAO/OMS. Así, dependiendo de las estimaciones que se hagan por variabilidad inter-individual, el nivel seguro de ingesta proteínica oscilaría entre 0.79 y 0.94 g/kg/día, o sea entre 33 y 21% menos que las recomendaciones de FAO/OMS. Las digestibilidades aparente y "verdadera" de la proteína de leche fueron de 80 ± 4% y de 94 ± 4%, respectivamente.

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