22. CAPACITY OF HABITUAL GUATEMALAN DIETS TO SATISFY PROTEIN REQUIREMENTS OF PRE-SCHOOL CHILDREN WITH ADEQUATE DIETARY ENERGY INTAKES

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Objective

To assess whether a diet considered customary among certain segments of the pre-school-age population of Guatemala supplied adequate amounts of protein, provided that:

1. Energy intakes corresponded to the estimated requirements.
2. There were no constraints in the amounts of staple foods available to the children.
3. The children were encouraged to eat as much food as they wanted to.
4. The children were in good health and nutritional status at the beginning of the study.

Experimental Details

1. Subjects

a. Eleven boys of mixed Maya and Caucasian descent (Ladino).

b. Chronological age: 37 ± 4 months (range: 29 to 46).
Height-age: 20 ± 4 months (range: 13 to 26).

c. All had been treated for moderate or severe protein-energy malnutrition. They had recovered fully at least two months before beginning the studies, based on clinical, anthropometric, and biochemical criteria (plasma proteins, non-essential/essential amino acid ratio, haematological indices).

d. Weight: 11.69 ± 0.82 kg (range: 10.47 to 13.00).
Height: 83.8 ± 4.3 cm (range: 76.1 to 89.4).
Weight-for-height, percentage of expected: 99 ± 5 per cent (range: 92 to 109).

e. The children were happy and active throughout the study, except for short disease episodes. Children (I.D. nos. 404, 405, 408, and 410) had febrile infections in weeks three, one, four, and six, respectively, that lasted from four to seven days. Only child 410 received antibiotics, to treat a periodontal infection. All others received symptomatic medication for upper respiratory infections. During week three, child 406 had an afebrile exanthema of unknown aetiology that disappeared spontaneously, and child 407 had an afebrile upper respiratory infection. Finally, all children except 405 had an afebrile upper respiratory infection characterized by catarrh and coughing during the last one or two weeks of the study.

A few children vomited occasionally and sometimes had slight increments in rectal temperature (<38.5° C) without other signs or symptoms of disease.

2. Study Environment
INCAP's Clinical Centre in Guatemala City; 1,500 metres above sea level. Temperature 18° to 24° C. Relative humidity 40 to 60 per cent. All children spent four to six hours each day outdoors on the grounds and playing area around the Clinical Centre, except on rainy days.

3. Physical Activity
The children were encouraged to be as physically active as healthy children who live in a good home environment. This was done through daily outdoor walks in the areas around the Clinial Centre and participation in games and other activities that required walking, running, jumping, or climbing. They were never forced to participate in those activities when they did not feel like doing so, nor were they ever pushed to exhaustion. Such activities alternated with periods of rest or sedentary play to avoid boredom or fatigue.

4. Duration of the Study
The children ate the experimental diets for at least 11 weeks, divided into the following periods:

a. Two weeks: *ad libitum* intake of indigenous diet to measure each child's individual food intake.
b. One week: *ad libitum* intake of indigenous diet with a higher density, whenever necessary, to ensure intakes of 87 to 97 kcal/kg/day.
c. Eight weeks: *experimental period.* Metabolic and other measurements while eating the diet with 87 to 97 kcal/kg/day in *ad libitum* amounts. The results were analysed as two separate four-week periods, as described below.

TABLE 1. Average Composition and Frequency of Consumption of Diets Customary for Many Children of Pre-school Age in Guatemala

Food	Intake per day (g)	Frequency of intake (days/week)	Weekly intake		
			Amount (g)	Protein (g)	Energy (kcal)
Corn tortilla flour	105	7	735	67.6	2,734
Black bean flour	18	7	126	27.8	423
Bread (sweet roll), fresh	37	7	259	19.7	1,000
Vegetables (chayote, squash, or potatoes), raw	44	7	308	5.8	163
Milk products (as fluid milk equivalents)	100	3	300	9.9	195
Fruit (orange, apple, banana), fresh	30	4	120	0.6	60
Egg, fresh	43	2	86	9.9	142
Meat, raw (as beef equivalent)	40	1	40	7.6	97
Sugar	42	7	294	–	1,176
Oil or lard	5	7	35	–	315
Total intake per week				148.9	6,305
Mean intake per day				21.3	901
Mean intake/kg/day (assuming weight of 12 kg)				1.78	75

Bean: corn ratio = 15:85 by weight and 29:71 by protein contents

Animal protein = 18% of total

Energy from fat (including natural fat content of all foods) = 17% of total

TABLE 2. Menus Offered to the Children[a]

Days	Breakfast (7 a.m.)	Lunch (11 a.m.)	Dinner (6.30 p.m.)
	corn and beans [b]	corn and beans sweet bread[c]	corn and beans sweet bread
	+	+	+
Mon.	egg (1 unit) [a]	apple	chayote [d]
Tue.	potatoes	apple	potatoes
Wed.	chayote	beef (40 g) [a]	apple
Thu.	squash	potatoes	potatoes
Fri.	egg (1 unit) [a]	squash	squash
Sat.	potatoes	apple	potatoes
Sun.	chayote	chayote	potatoes

Afternoon snack (3 p.m.):
sweet bread (13 g) [a]
+
Mon., Wed., Sat. : milk (100 ml) [a]
Tue., Thu., Sun. : lemonade (200 ml) [a]

Night drink (8 p.m.):
lemonade or water

[a] All foods offered *ad libitum*, except when noted otherwise.
[b] Corn-based beverage (*atole*), soft corn bread (*tamal*), and mashed black bean purée.
[c] Sweet bread dough prepared with sugar and lard.
[d] Chayote = *Sechium edule.*

5. Diet

a. The diet was based on the proportions of foods eaten by pre-school children from poor socio-economic rural families (see table 1). For practical reasons of food preparation in the metabolic kitchen, fluid milk was used as the only dairy product and the vegetables and fruits were limited to those shown in table 2.

b. The children ate three daily meals and a mid-afternoon snack (see table 2). All meals included the basic staple foods of the region (corn and black beans) plus one or two other foods that varied from meal to meal and from day to day. The snack consisted of a sweet roll and half a cup of milk or lemonade. The daily menus were repeated at seven-day intervals; that is, there were menus for Sundays, Mondays, Tuesdays, and so on. The children ate as much as they wanted and the nurses played the role of mother at meal times, encouraging but not forcing the children to eat all that was served. If a child asked for more of a food served in a meal, it was given to him except for the foods that are scarce and limited in the home environments represented by the study (meat: maximum of 40 g once weekly; egg: maximum of one twice weekly; milk: maximum of 100 ml three times weekly). No child was forced to eat if he refused to do so. Lemonade or water was offered to the child before retiring at night. By eating *ad libitum*, each child determined his own intake of proteins and other nutrients for the day. Energy intake, however, was adjusted each week, based on the preceding week's intakes, to approximately 92 ± 5 kcal/kg/day. The adjustments were made by adding more or less oil to the black beans and more or less sugar to the lemonade, or substituting water for the latter before retiring. Regardless of the preceding week's intakes, no more dietary adjustments were made in the last four weeks of the study.

c. Since the purpose of the study was to evaluate protein and energy nutriture, the diet was supplemented with vitamins and minerals, as shown in table 3.

6. Indicators and Measurements

a. Metabolic-balance studies: Most of the children were not toilet-trained. To avoid excessive limitations of physical activity, complete 24-hour urine and faecal collections were obtained at 4-day intervals so that every 28 days excreta corresponding to the seven different menus for each day of the week were collected. Urine collection began after the first morning micturition and ended after a micturition 22 to 26 hours later; volumes were adjusted to 24-hour periods. Faeces were collected between carmine red and charcoal faecal markers fed with breakfast on 2 consecutive days. When the two markers were excreted together or when there were other problems, such as losses of excreta, collections were repeated on the same day of the following week. Faeces were homogenized and dried. Their nitrogen and energy concentrations were measured in aliquots of powdered faeces by Kjeldahl analysis and bomb calorimetry, respectively. Urinary nitrogen was also determined by a Kjeldahl procedure. Each food was served in a separate dish or cup at every meal. The amounts eaten by a child were measured by weighing the corresponding containers before and after each meal, accounting for any additional servings or for losses by spillage. Aliquots of each food, as served to the children, were analysed at least four times during the study using the same methods as those for faeces. Tryptophan and benzoic acid were used as standards in each Kjeldahl and bomb calorimeter run, respectively. Total daily intakes of protein (nitrogen x 6.25) and gross energy (by bomb calorimetry) were calculated multiplying by the amounts of each food ingested.

Nitrogen balance ("apparent") was calculated by subtracting urinary and faecal nitrogen from intake. No corrections were made for integumental and other insensible nitrogen losses.

TABLE 3. Vitamin and Mineral Supplements Administered Daily

Vitamin A	2,500	I.U.
Vitamin B_1	1	mg
Vitamin B_2	0.5	mg
Niacinamide	5	mg
Vitamin B_6	0.5	mg
Pantothenic acid	5	mg
Folic acid	30	mcg
Vitamin B_{12}	2	mcg
Biotin	50	mcg
Vitamin C	25	mg
Vitamin D	500	I.U.
Vitamin E	1.5	mg
Iron (as ferrous sulphate)	60	mg
Iodine (as KI)	100	mcg
Manganese sulphate	0.9	mg
Zinc sulphate	1	mg

Net energy intake was calculated as the gross value intake minus faecal energy (by bomb calorimetry). This value was used to calculate the contribution of dietary protein to total energy intake (P%), assuming that each g of protein ingested corresponded to 4 kcal of metabolized energy. The dietary energy retained was calculated by subtracting urinary nitrogen energy (estimated as 5 kcal/g urinary nitrogen) from the net intake. Energy balance was calculated by subtracting the total energy expenditure, as described below, and sweat losses (estimated as 0.1 kcal/kg/day, based on 8 kcal/g sweat nitrogen) from the dietary energy retained.

The daily metabolic-balance data were combined in 28-day periods that included intakes and excreta corresponding to each of the 7 days of the week. These periods were termed I and II.

Apparent digestibility of nitrogen and apparent absorption of energy were calculated from the combined gross intakes and faecal excreta of a 28-day period.

It was assumed that collection days when a child ate little food or did not defecate much would be balanced by other collection days with higher intakes or greater faecal excretions. During collection days the children who were not toilet-trained remained in a metabolic bed during the hours in which it was expected that they would defecate and while they slept; at other times they moved and played around freely while wearing urine collection bags.

b. Basal oxygen consumption: This was measured with an oxygen diaferometer at 18-day intervals, each time on two separate occasions not more than 3 days apart; the lower of the two results was considered as basal. Basal conditions were defined as after a minimum of eight hours of sleep and ten hours of fasting. Measurements were done while the child was sleeping, sometimes after oral administration of chloral hydrate (4 mg/kg). Energy expenditure was calculated by indirect calorimetry, assuming a respiratory quotient of 0.82.

c. Total energy expenditure: Physical activity and energy expenditure were quantified by monitoring the children's heart rate (HR) throughout the day and calculating energy expenditure from individual determinations of heart rate and oxygen consumption (VO_2). The HR-VO_2 relationship was determined in each child at 14- to 21-day intervals. Heart rate was continuously monitored for at least 10 days within ± 7 days of determining the HR-VO_2 relationship. Total daily energy expenditure was calculated from each child's heart rate and his corresponding heart-rate–energy-expenditure relationship from 6 a.m. to 8 p.m. (14 hours), and from his basal energy expenditure from 8 p.m. to 6 a.m. of the following day (10 hours).

d. Anthropometry: The children were weighed naked before breakfast each morning. Body length ("height"), right arm circumference, and subcutaneous skin-fold thickness (tricipital, subscapular, and paraumbilical) were measured initially and at 14-day intervals.

e. Urinary creatinine excretion: This was measured in the 24-hour urine collections obtained for nitrogen balance. An alkaline picrate method (Jaffe) was used. The creatinine-height index (CHI) was computed, and running or weekly averages were calculated, including and excluding data from the days when meat was eaten.

f. Other biochemical and haematological determinations: Venous blood was drawn initially and at 18-day intervals. Packed cell volume (microcentrifuge) and the concentrations of blood haemoglobin (cyanomethaemoglobin), plasma proteins (refractometry), and serum albumin (bromcresol purple) were determined, as well as the ratio of serum non-essential/essential amino acids (Whitehead).

g. Statistical analysis: Changes in weight, anthropometry, and CHI were calculated by regression analysis. Data calculated at 7-, 14-, or 18-day intervals were also computed by analysis of variance. Differences between the 28-day periods were examined by the student's paired t test.

Unless otherwise noted, the data in the text and tables are expressed as the mean ± standard deviation, and in the figures as the mean ± standard error of the mean.

Summary of Main Results

1. Food Intake

Although there were differences among children, on a group basis food intake did not differ significantly from week to week. Febrile episodes were usually accompanied by anorexia, resulting in diminished food intakes. In most cases these episodes were followed by a transient increase above the food intake preceding the illness.

2. Growth

Table 4 and figure 1 show the anthropometric changes.
a. Weight: One child (402) did not gain weight and two (401, 410) gained at a rate slower than the 0.45 to 0.50 g/kg/day expected for healthy children of the same height-age. In contrast, two children (404, 405) gained weight at more than twice that rate.

b. Height: Five children grew at the expected rate of 0.30 to 0.34 mm/day. The other six children grew more (0.43 to 0.64 mm/day). This resulted in some catch-up growth, as shown in figure 2.

c. Weight-for-height: Maximum individual changes were ± 3 per cent. There were no changes on a group basis.

d. Other anthropometric measurements: A small decrease in tricipital skin-fold thickness resulted in a slight increment of lean arm diameter, since there were no changes in arm circumference.

3. Protein Intake, Digestibility, and Balance

Figure 3 and table 5 give the individual and group data. Protein intake accounted for 8.8 ± 1.1 per cent of the net dietary energy. Mean protein intakes were high (1.75 ± 0.22 g/kg/day), and apparent digestibilities were about 72 ± 5 per cent, greater in period I than II by 3 per cent. "True" digestibilities were about 7 per cent higher than apparent digestibilities. The average amount of protein "truly" absorbed was 1.46 ± 0.17 g/kg/day.

Apparent nitrogen balance was also high. All children retained at least twice the

TABLE 4. Average Values and Rates of Change in Anthropometric Measurements and CHI of 11 Children during Periods I and II

Average values	Period I	Period II	Mean of I and II	Paired t I vs. II[a]
Weight (kg)	11.98 ± 0.80*[b]	12.12 ± 0.89	12.05 ± 0.83	2.367
Height (cm)	84.5 ± 4.3*	85.7 ± 4.3	85.1 ± 4.2	6.120
Weight-for-height (%)[c]	99 ± 4	100 ± 5	99 ± 4	0.810
Arm circumference (cm)	16.1 ± 0.8	16.0 ± 0.9	16.0 ± 0.8	0.088
Lean arm diameter (cm)[d]	42.9 ± 1.5*	43.6 ± 1.6	43.2 ± 1.6	3.169
Subcutaneous skin-fold thicknesses[e]	17.6 ± 3.1*	16.5 ± 3.2	17.0 ± 3.1	2.300
CHI (units)[f]	80 ± 0.13	0.82 ± 0.12	0.82 ± 0.12	1.847
Rates of change				
Weight (g/day)	10.6 ± 8.7	3.7 ± 6.4	7.2 ± 8.2	1.987
Weight (g/kg/day)	0.87 ± 0.70	0.29 ± 0.54	0.58 ± 0.68	1.955
Height (mm/day)	0.41 ± 0.16	0.46 ± 0.23	0.43 ± 0.12	0.565
Lean arm diameter (mm/day)	0.02 ± 0.04	0.02 ± 0.04	0.02 ± 0.02	0.140
Subcutaneous skin-fold thicknesses (mm/day)	−0.01 ± 0.06	−0.04 ± 0.03	−0.03 ± 0.04	1.342
CHI (units/period)	0.035 ± 0.103	0.011 ± 0.106	0.023 ± 0.103	0.812

a For 10 degrees of freedom $p<0.05 = 2.228$ and $p<0.01 = 3.169$.
b Mean ± standard deviation.
c Weight expected for height: 100 per cent = 50th percentile of Boston standards.
d Corrected for subcutaneous skin-fold thickness.
e Sum of 3 sites: tricipital, subscapular, and paraumbilical.
f Creatinine-height index calculated from urine excreted on days without meat ingestion.
g Weight changes calculated by individual regression analyses over 28 days. All other changes by individual differences between days 0 and 28 (period I) and between days 28 and 56 (period II).
* Mean values of the two periods differ (see paired t value).

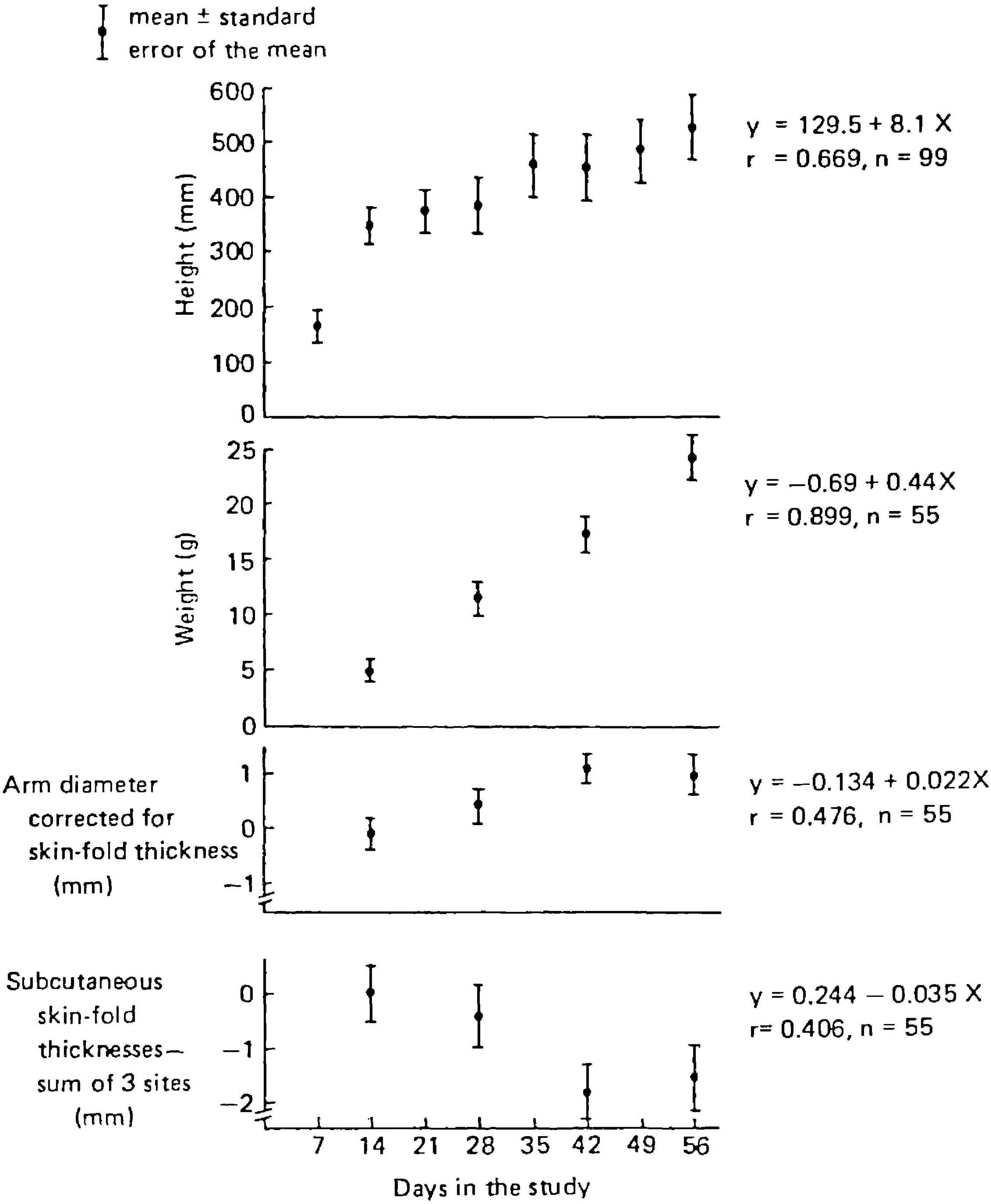


FIG. 1. Cumulative Anthropometric Changes — Periods I and II (11 children)

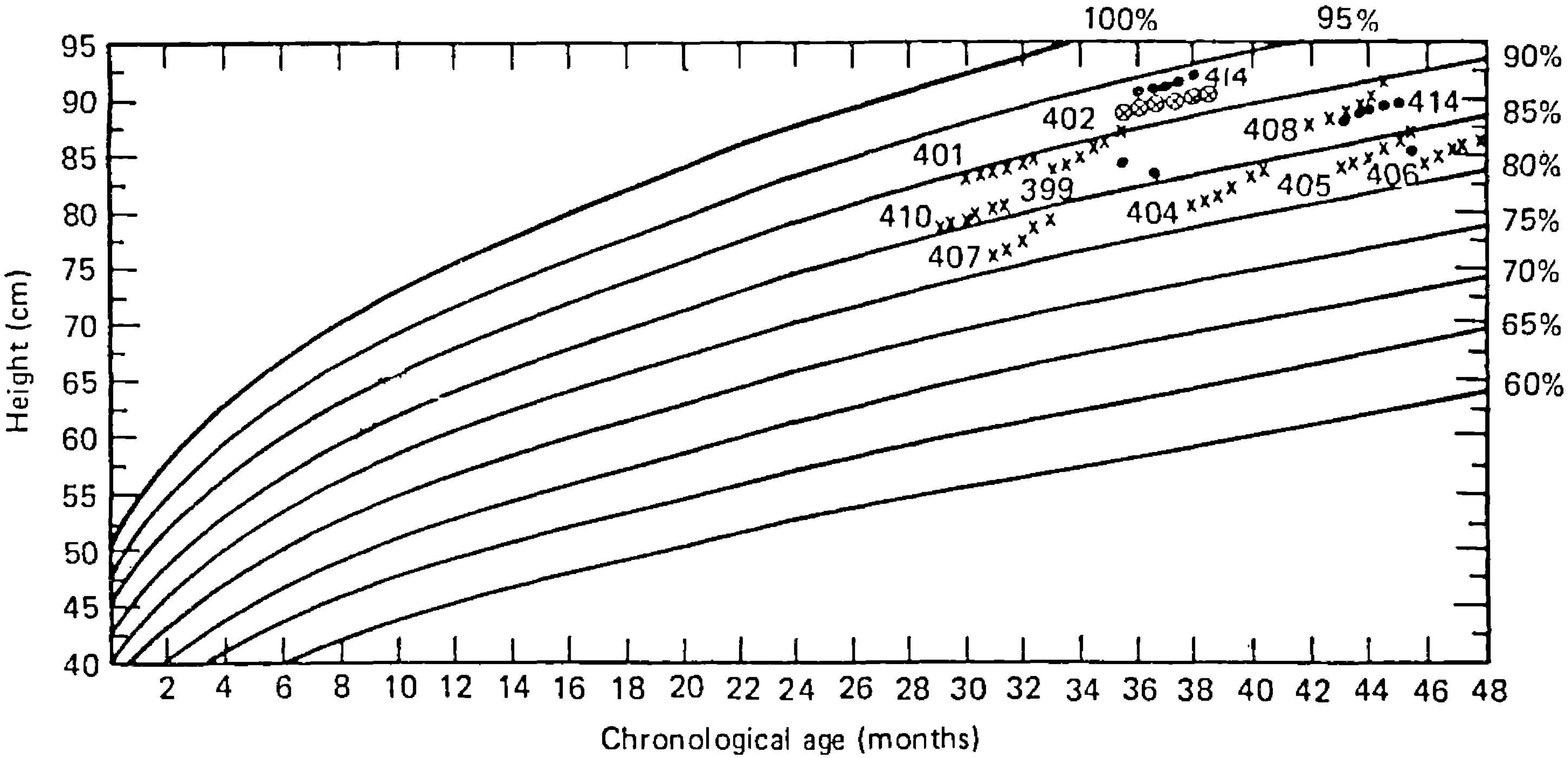
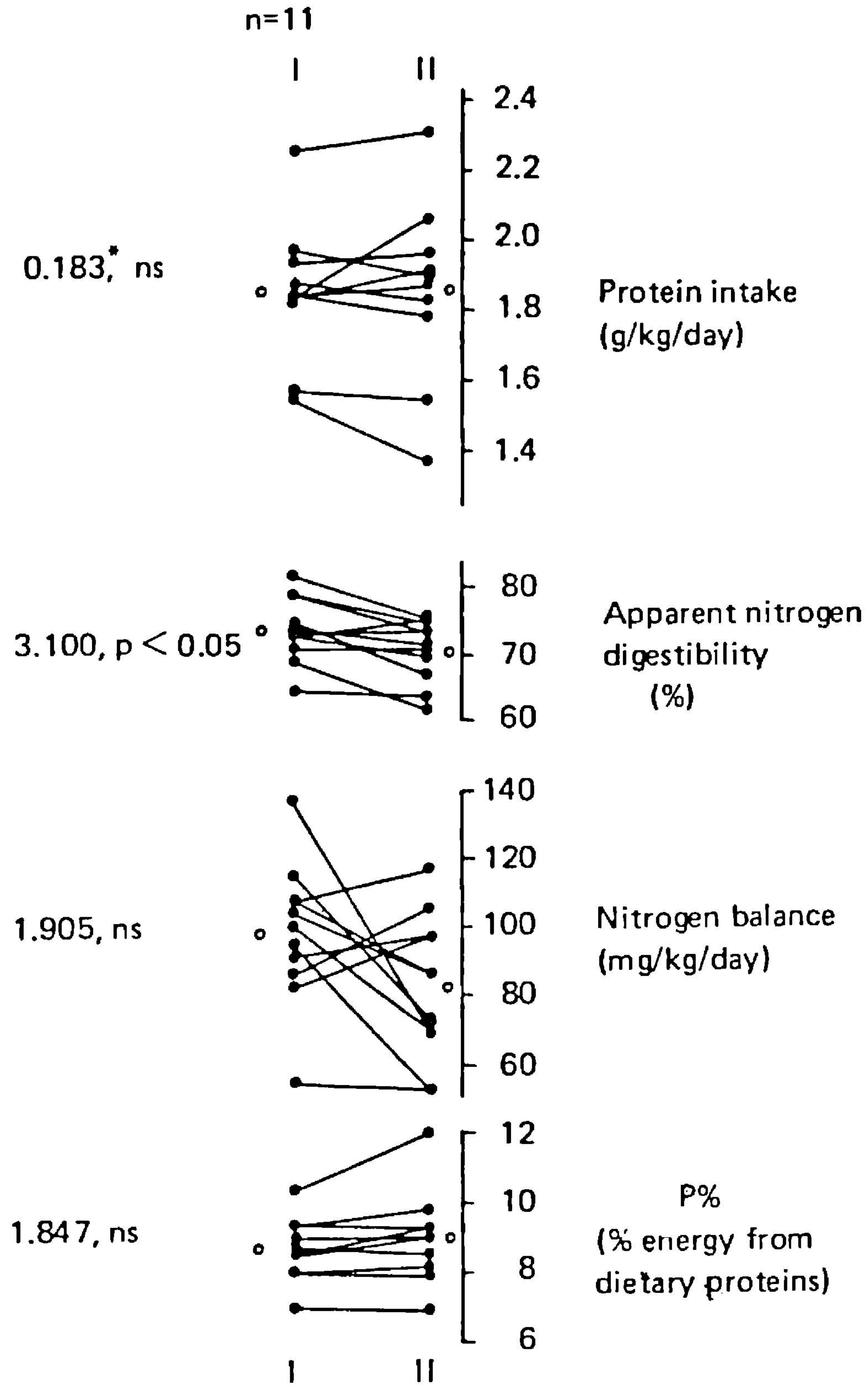


FIG. 2. Curves of Height Growth



* Student's paired "t."
• Mean value for the period.
I, II : Average data of consecutive 28-day periods.

FIG. 3. Nitrogen Balance Study

TABLE 5. Metabolic Balance Studies and Energy Expenditures in Periods I and II

(11 Children) [a]

	Period I	Period II	Mean of I and II[b]	Paired t I vs II[b]
Protein				
Protein intake[c] (g/kg/day)	1.85 ± 0.19[d]	1.85 ± 0.25	1.85 ± 0.22	0.183
Apparent digestibility. (%)	73.6 ± 4.6*	70.6 ± 4.7	72.1 ± 4.8	3.100
"True" digestibility[e] (%)	80.4 ± 4.6*	77.4 ± 4.7	78.9 ± 4.8	
Nitrogen balance[f] (mg/kg/day)	98.0 ± 20.6	82.3 ± 20.6	90.2 ± 2.16	1.905
p%[g] (% energy)	8.6 ± 0.9	8.9 ± 1.3	8.8 ± 1.1	1.847
Energy				
Gross intake[h] (kcal/kg/day)	93.6 ± 4.6	90.9 ± 4.8	92.3 ± 4.8	1.415
Apparent absorption, (%)	91.9 ± 1.6	91.2 ± 1.7	91.6 ± 1.6	1.326
Net intake[i] (kcal/kg/day)	85.9 ± 4.3	83.2 ± 4.8	84.6 ± 4.7	1.383
Total energy expenditure[j] (kcal/kg/day)	76.6 ± 8.6	73.0 ± 6.3	74.8 ± 7.6	2.188
Energy balance[k] (kcal/kg/day)	8.2 ± 10.1	9.7 ± 6.2	9.0 ± 8.2	0.589
Basal energy expenditure, (kcal/kg/hr)	–	–	1.33 ± 0.25	–
($kcal/m^2/hr$)	–	–	54.2 ± 5.4	–

a Balance data and digestibilities calculated from intakes and excreta collected seven times at 4-day intervals in each 28-day period.

b For 10 degrees of freedom $p.<0.05 = 2.228$ and $p<0.01 = 3.169$.

c Protein = nitrogen (Kjeldahl) x 6.25.

d Mean ± standard deviation.

e "True" digestibility calculated assuming obligatory faecal nitrogen loss of 20mg/kg/day.

f Nitrogen balance (apparent) = intake – urinary excretion – faecal excretion. No allowance made for sweat and other insensible losses.

g P% = proportion of dietary energy derived from proteins = (protein intake, g x 4) + net energy intake x 100.

h Gross energy intake determined by bomb calorimetry of the foods ingested.

i Net energy intake = gross intake – faecal energy (bomb calorimetry).

j Total energy expenditure calculated from heart rate and the corresponding heart rate – energy expenditure relationship during 14 hours of the day (6 a.m. to 8 p.m.) and from basal energy expenditure during 10 hours (8 p.m. to 6 a.m.).

k Energy balance = net intake – urinary losses (5 kcal/g urinary nitrogen) – sweat losses (8 kcal/g sweat nitrogen = approximately 0.1 kcal/kg/day) – total energy expenditure.

* Mean values of the two periods differ (see t value).

amount estimated for normal growth and to compensate for insensible losses (about 15 plus 9 mg N/kg/day, respectively).

Figure 3 indicates that only two children (405, 411) had protein intakes below the population estimates given in table 1. Their P%'s were also the lowest in the group, and their apparent digestibilities were near the group mean. Child 405 had the lowest nitrogen balance and child 411 retained 70.5 mg N/kg/day in period II when his intake was only 1.36 g protein/kg/day. Neither child had clinical signs of protein deficiency, both grew well, and their CHI, haemoglobin concentrations, and other biochemical meaurements did not differ from the group. Child 404 had the highest food and protein intakes and the highest P% of the group. He had a high faecal output and his apparent nitrogen digestibility was 64 per cent in both periods of the study. As a result of this, he absorbed 1.46 g protein/kg/day in the two periods and his nitrogen balance was only 53.1 mg N/kg/day in period II; in period I it was 92.3 mg N/kg/day.

4. Basal and Total Energy Expenditure

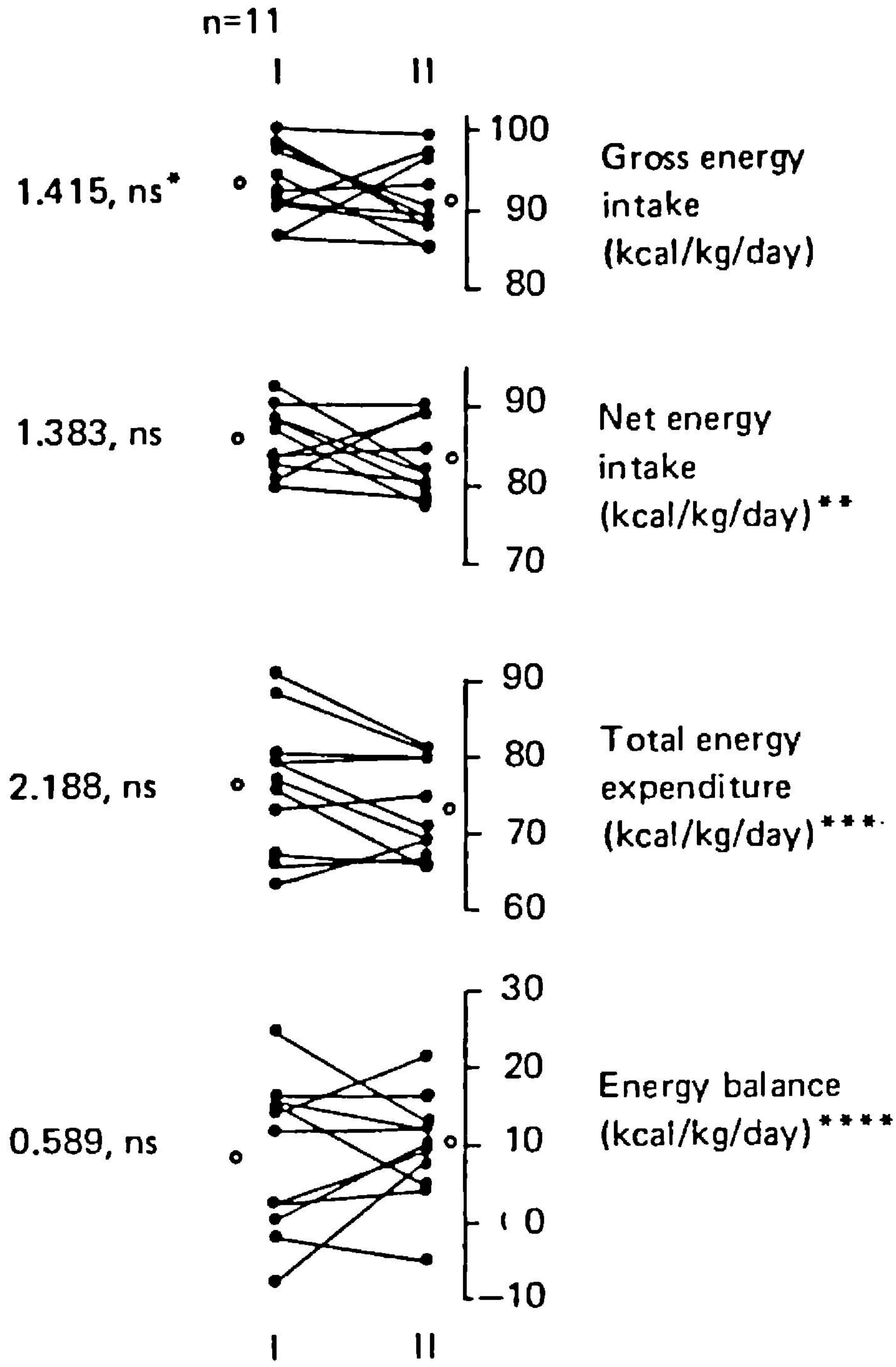
Each child's basal energy expenditure varied little throughout the study. Therefore, each child's mean value was used to compute his total energy expenditure. Table 5 shows the group's basal expenditure. Child 414 was higher than the rest, with 67.2 $kcal/m^2/hr$ (2.88 kcal/kg/hr). Basal expenditure varied among the other children from 47.7 to 58.4 $kcal/m^2/hr$ (2.11 to 2.65 kcal/kg/hr). These values agree with those of similar children measured by the same method.

Figure 4 shows the range of individual total daily energy expenditures. The average daily energy expenditure did not vary between periods and the medical and nursing staff did not notice changes in the pattern or duration of the children's physical activity, except when they were ill.

5. Energy Intake, Absorption, and Balance

Figure 4 and table 5 give the individual and group data. Gross intakes during the days of excreta collection ranged from 85 to 100 kcal/kg/day. These were to a certain extent independent of total food intake during period I, since the energy density of each child's diet was adjusted when the preceding week's intake was not between 87 and 97 kcal/kg/day. Apparent absorptions had a low coefficient of variability (1.7 per cent), and, except for child 414 who absorbed 88.6 per cent of the energy ingested, ranged from 91 to 94 per cent. Net energy intakes were, on the average, 8.4 per cent lower than gross intakes.

The estimates of urinary and sweat energy losses varied from 0.8 to 1.1 kcal/kg/day. Total daily energy expenditures and the energy balance results coincided with those from another study with similar children and equivalent dietary energy intakes. The energy balance ranged between −7.6 and 24.6 kcal/kg/day in period I, and between −4.8 and 21.2 kcal/kg/day in period II (see figure 4).



* Student's paired t.
** Net energy intake = gross intake (bomb calorimetry) – faecal energy (bomb calorimetry).
*** Calculated from heart rate-energy expenditure and basal metabolism.
**** Energy balance = net intake – urinary and sweat losses – total expenditure.
I, II Average values for two consecutive 28-day periods.
o Mean value for the period.

FIG. 4. Energy Expenditure and Balance Studies

TABLE 6. Blood Chemistry and Haematology during Periods I and II

		Days on the study			
		0	18	36	54
Packed red cell volume	%	36 ± 2*	35 ± 2**	37 ± 1	37 ± 2
Haemoglobin	g/dl	12.4 ± 0.8	12.2 ± 0.7	12.4 ±0.6	12.3 ± 0.6
Plasma proteins	g/dl	7.2 ± 0.4	7.0 ± 0.4	7.1 ± 0.3	6.8 ± 0.4[t]
Serum albumin	g/dl	3.8 ± 0.6	4.1 ± 0.6	3.8 ± 0.4	3.7 ± 0.7
Serum amino acid ratio non-essential/essential		1.68 ± 0.44	1.61 ± 0.40	1.38 ± 0.31	1.42 ± 0.35

* Mean ± standard deviation, n = 11.
** Lower than on days 36 and 54. $F_{(3,40)} = 3.072, p < 0.05$, L.S.D. = 2.
t Lower than initial (day 0) values, student's paired $t = 3.253$, $p < 0.01$.

6. Haematological and Biochemical Analyses

Table 6 gives the results of the analyses performed on blood, serum, and plasma. Analyses of variance indicated a small, transient decrease in packed red blood cell volume on day 18 ($p < 0.05$). The analysis of variance did not show differences among the other haematological and biochemical determinations. However, the paired comparison of initial and final values indicated decrease in total plasma protein concentration ($p < 0.01$) not accompanied by a decrease in albumin concentration.

Comments

1. The results indicate that the diet used in this study satisfied the protein needs of healthy, well-nourished, pre-school children. In contrast with the most common dietary practices among people of low socio-economic conditions in developing countries, the preparation and administration of the foods differed in three important ways: (a) the increment in energy density achieved by the addition of oil and sugar to some foods; (b) the availability of staple foods in sufficient amounts to satisfy the children's appetites; and (c) the preparation and administration of the foods in the same way as for adults (e.g., feeding whole beans to small children instead of only a watery bean soup). These three conditions can be met by an increment in local food resources, including fats, combined with an educational programme for the use of such resources.

2. It is not necessary to use large amounts of proteins from animal sources or of commercially processed foods, as these tend to be more expensive and less regularly available and may imply greater changes in food preparation and eating habits than the mere addition of fats and carbohydrates to staples. The diet used in this investigation included small amounts of animal proteins on some days. It remains to be shown if these small additions of high-quality proteins are essential to other diets of exclusively vegetable origin. Our previous investigations with diets based on corn and black beans indicated that such additions or other protein fortification measures are not necessary, provided that the vegetable foods are eaten in *such quantities and proportions that a good complementary vegetable protein mixture is absorbed* by the children. In any event, the present study demonstrated that it is not necessary for such high-quality protein "complements" to be ingested with every meal or every day.

3. The use of mixed diets with varied menus and free choice of intake departed from the traditional patterns of metabolic studies. However, it resembled everyday life since children do not eat the same foods or the same amounts day after day, and all the nutrients in their diet are not always digested and absorbed simultaneously or in similar time sequences. This experimental design also accounted for some of the possible interactions of various nutrients and other food components, such as the effects that the presence of different amounts and types of dietary fibres may have on nitrogen and fat absorption on different days.

4. Complete collections of daily excreta might yield more accurate metabolic-balance results, but this is feasible only with older children and adults. The 4-day interval surrogate used in this study was adequate, as shown by the replication of the 28-day periods. The results did not vary unless the children became sick for several days with fever and marked anorexia; illness and mild symptoms did not produce any changes in metabolic balances.

5. Growth rates were also similar in the two consecutive periods. Therefore, a 28-day period might be adequate to assess the effects on body weight and metabolic balance of local diets eaten in the usual patterns *if the children do not become ill.* Otherwise, 28-day replications are necessary. The evaluation of other anthropometric changes, such as length, lean arm diameter, and subcutaneous skin-fold thickness, requires a longer period (42 to 56 days).

6. There is no satisfactory explanation for the apparent high nitrogen retentions. This is known to occur in children and adults with protein intakes well beyond their requirements. The important observation derived from this study was that nitrogen balance remained positive and sufficed to cover the estimated needs for growth and insensible nitrogen losses. This is supported by the stability of the CHI and the small, but consistent, increment in lean arm diameters.

7. The energy expenditure and balance data confirm our previous observations that mean net energy intakes (i.e., dietary intakes measured by bomb calorimetry and

corrected for faecal losses) of 82 to 85 kcal/kg/day are adequate for active, well-nourished children two to four years old. The energy not available from the protein absorbed, calculated from urinary nitrogen excretion, was about 1 kcal/kg/day. Consequently, the net energy intake is similar to that calculated from food composition and the Atwater energy factors for proteins, fats, and carbohydrates. Since the coefficient of variability of the mean net intake was 5 to 6 per cent, it seems safe to recommend energy intakes of 92 to 95 kcal/kg/day (mean ± 12 per cent) for well-nourished children of this age group.

8. The question remains open as to whether diets of the types used in this investigation will allow children with mild-to-moderate nutritional deficiencies to catch up. This is highly relevant, since such children make up the majority of the pre-school-age population of low socio-economic groups in the developing world. The catch-up in length and high rates of weight gain observed in this investigation suggest that this might be possible. The answer can be explored through a similar study on apparently healthy children with small weight deficits (5 to 15 per cent below that expected for their heights) who do not have clinical signs of malnutrition except for their leanness. A longer period of time (e.g., 84 instead of 56 days) may be necessary to assess the rates of catch-up growth and to account for the impact of disease that might occur.

Conclusions

1. With the diet used in this study, protein intakes were adequate when energy intakes corresponded to the estimates of energy requirements.

2. The protein and energy needs of well-nourished children were satisfied by the diets used in this study if: (a) dietary energy density was increased, and (b) sufficient amounts of the staple foods were available.

3. If the diet is supplemented with foods of animal origin or other high-quality proteins, there is no need to provide them every day of the week. Some local vegetable-based diets may not require such supplements.

4. It is not necessary to change the composition of the diet during short, acute episodes of disease or during convalescence from them if the foods are offered to the child in sufficient amounts to satisfy his appetite.

5. The energy needs of most well-nourished children two to four years old can be satisfied with dietary intakes of 92 to 95 kcal/kg/day.

6. Measurements made at 4-day intervals over 28 days yield good metabolic-balance data. A 28-day period of observation is adequate to assess the dietary effects on body

weight and metabolic balance if the child does not become ill. In that event, other 28-day replications are necessary. The evaluation of other anthropometric changes requires a longer time.

7. It is still necessary to show whether or not the preceding conclusions are correct for pre-schoolers with mild-to-moderate protein-energy deficiencies.

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