23. ENERGY REQUIREMENTS OF PRE-SCHOOL CHILDREN AND EFFECTS OF VARYING ENERGY INTAKES ON PROTEIN METABOLISM

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Objectives

1. Evaluation of the adequacy of a diet based on corn and beans, fed at recommended levels of protein intake with different levels of energy intake.

2. Measurement of total energy expenditure, energy balance, and energy requirements.

Experimental Details

1. Subjects

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

b. Chronological age: 30 ± 8 months (range: 22 to 40).
Height-age: 17 ± 5 months (range: 15 to 25).

c. All had been treated for severe, oedematous protein-energy malnutrition (kwashiorkor and marasmic kwashiorkor). They had recovered fully at least one month before beginning the studies, based on clinical, anthropometric, and biochemical criteria (plasma proteins, non-essential/essential amino acid ratio, haematological indices, urinary creatinine excretion, and creatinine-height index [CHI]).

d. Weight: 11.93 ± 0.95 kg (range: 10.85 to 13.25).
Height: 84.4 ± 3.5 cm (range: 78.2 to 88.6).

Weight-for height, percentage of expected: 104 ± 4% (range: 89 to 109).

e. All children were healthy throughout the study, except for occasional minor illnesses, such as upper respiratory infections of viral aetiology, that were treated symptomatically. In a few instances a child had fever for one to three days. If that happened immediately before or at the time scheduled for nitrogen-balance studies, the balance was postponed until seven days after the fever had subsided. Energy expenditure was not measured during days when a child had fever.

f. Intestinal parasites: Two children (nos. 387 and 390) had mild infestation with *Trichuris trichiura.* Two others (388, 394) had *Giardia lamblia,* and one of them (394) also had mild ascariasis. All were asymptomatic and none was treated before or during the study.

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, except during rainy days. All children spent two to four hours each day in outdoor playing facilities, except on rainy days.

3. Physical Activity

The children were encouraged to be about as active as those living in a free environment, rather than leading the sedentary life usually observed in children who live in an institution. This was accomplished by encouraging them to participate throughout the day in various games that required walking, running, and climbing ramps and stairs. They were never forced to participate, and the games never exhausted them.

4. Duration of the Study

Five children were studied for 120 days, 40 days with each of three levels of dietary energy. The sixth child was studied for 160 days with four levels of dietary energy, as described below.

5. Diet

a. The diets were based exclusively on vegetables, with 95 per cent more of the protein derived from black beans (*Phaseolus vulgaris*) and corn. The diets were designed to provide 1.75 g protein/kg/day. The proportion of black beans to corn protein was 42:58. Three different levels of energy intake were used: initially about 100 kcal/kg/day and after 40-day intervals about 92 and 83 kcal/kg/day. The 100 kcal level was that used in previous experiments when the requirement for corn-and-bean protein was established. This energy intake was excessive for several children who became obese during those experiments. Only one child (EG) ate a higher energy-dense diet after he failed to gain the expected weight with the initial intake of 100 kcal/kg/day and lost weight with 92 kcal/kg/day. His energy intake was then raised to 120 kcal/kg/day, and at 40-day intervals it was lowered to 110 and 100 kcal/kg/day. It should be pointed out that while this child was recovering from protein-energy malnutrition before participating in the present study he required a diet with 150 kcal/kg/day for a longer period than was needed by the other children for recuperation. The changes in dietary energy were made by adding more or less vegetable oil to the black bean preparations, as shown in table 1.

b. As the components of the diet were those used locally in Central American rural and low-income urban homes, no multivitamins and mineral supplements were added, except for supplementary iron and vitamin A provided with sugar fortified with NaFeEDTA (13 mg of iron per 100 g of sugar), and retinol palmitate (15 micrograms of vitamin A in 1 g of sugar).

c. Table 2 shows the proportions of energy provided by fat, protein, and carbohydrate. The diet provided 0.54 g crude fibre/kg body weight/day.

d. The foods were prepared and cooked in ways similar to those followed in Guatemalan homes, except that with the higher levels of energy intakes more oil was used than is customary in low-income homes. Table 3 shows the foods served in every meal. The same menus and amounts of food were served each day throughout the study, adjusted for each child's average weekly body weight.

6. Indicators and Measurements

a. *Nitrogen and energy balance:* Every 20 days, urine and faeces were collected for 96 hours and analysed to determine their nitrogen and energy contents. The same was done with an aliquot of the diets. Nitrogen was determined by an automated method using alkaline phenol-hypochlorite-nitroprussiate (Berthellot reaction) after a Kjeldahl digestion; food and faecal aliquots were homogenized and solubilized with H_2O_2-H_2SO_4 before digestion. "True" nitrogen balance was calculated by subtracting urinary, faecal, and insensible losses from the amount of nitrogen ingested. It was assumed that the insensible losses were 5 mg/kg/day.

Energy in diets and faeces was measured by bomb calorimetry using benzoic acid standards. Energy balance was calculated by subtracting from the dietary energy intake (measured by bomb calorimetry): the faecal energy (measured by bomb calorimetry); urinary energy losses (estimated as 5 kcal/g urinary nitrogen); sweat (estimated as 0.1 kcal/kg/day, based on 8 kcal/g sweat nitrogen; and the total energy expenditure, as described below.

b. *Absorption:* Twenty mg N/kg/day were used as the obligatory faecal losses to calculate "true" nitrogen digestibility.

c. *Total energy expenditure:* Physical activity and energy expenditure were quantified by monitoring the children's heart rate throughout the day and calculating energy

TABLE 1. Ingredients and Amounts of Foods Eaten

	Amount (g)	Protein (g)	Fat (g)	Energy (kcal)	Amount eaten per kg/day
Black bean paste (purée)					16.6 g/kg
Black bean flour	18.1	4.2	0.4	61	
Salt	0.7	–	–	–	
Vegetable oil	11.8	–	11.8	106	
	(6.3)*		(6.3)	(57)	
	(0.0)		(–)		
Water enough to reach	100.0				
Total	100.0	4.2	12.2	167	
			(6.7)	(118)	
			(0.4)	(64)	
Corn tamale					15.1 g/kg
Lime-treated corn flour	35.4	3.1	1.5	131	
Salt	1.7	–	–	–	
Water	62.9				
Total	100.0	3.1	1.5	131	
Corn gruel (*atole*)					50 g/kg
Lime-treated corn flour	11.4	1.0	0.5	42	
Sugar	6.5	–	–	26	
Water	82.1	–	–	–	
Total	100.0	1.0	0.5	68	
Lemonade	– (10 g sugar/100 g ≠ 40 kcal/100 g)				25 g/kg
Banana	– (1 g protein and 106 kcal/100 g)				5 g/kg
Cooked carrots	– (0.2 g protein and 31 kcal/100 g)**			approx.	5 g/kg
Cooked squash	– (0.5 g protein and 20 kcal/100 g)**			approx.	2 g/kg
Boiled potatoes	– (1.1 g protein and 42 kcal/100 g)**			approx.	1.3 kg/kg
Cooked spinach	– (2 g protein and 17 kcal/100 g)**			approx.	1.7 g/kg

* 11.8 per cent oil added to provide a total of about 100 kcal/kg/day. Figures in parentheses correspond to diets that provided about 92 or 83 kcal/kg/day.

** Only two of these vegetables were served each day.

TABLE 2. Nutrient Contents of the Diet as Energy Sources*

% Energy from:	Theoretical dietary energy levels (kcal/kg/day)				
	120**	110**	100	92	83
Total fats	27.0 (23)+	24.8 (21)	22.5 (18)	15.5 (10)	5.9 (0)
Proteins	5.8	6.3	7.0	7.6	8.4
Carbohydrates	67.2	68.8	70.5	76.9	85.7

* Based on Atwater factors and proximal analysis of beans, corn, tamale, corn gruel, and lemonade, and on food composition tables for banana and other vegetables.

** Diets with 120 and 100 kcal/kg/day were consumed only by child E.G. (no. 387).

\+ In parentheses: percentage of energy derived from vegetable oil added to black beans.

expenditute from individual determinations of heart rate and oxygen consumption. The heart-rate-oxygen-consumption relationship was determined in each child at 20-day intervals. Total daily energy expenditure was calculated from each child's heart rate and his corresponding heart-rate–energy-expenditure relationship from 5 a.m. to 8 p.m. (15 hours), and from his basal energy expenditure from 8 p.m. to 5 a.m. of the following day (9 hours).

Basal energy expenditure was measured by indirect calorimetry with an oxygen diaferometer at two-day intervals, each time on two separate occasions not more than three 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 fast. 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.

d. *Growth and body composition:* The children were weighed naked before breakfast every morning. Anthropometric measurements were taken at 14-day intervals. Basal oxygen consumption was measured on 2 consecutive days at approximately 20-day intervals. Urinary creatinine excretion was determined at 20-day intervals when the nitrogen-balance determinations were carried out.

e. *Other determinations:* Initially and at two-day intervals, packed blood cell volume and total plasma proteins were determined.

f. *The experimental design* is summarized in table 4.

TABLE 3. Menu Used in the Study

Breakfast:	corn gruel (corn flour + sugar + cinnamon + calcium)*
	tamale (corn flour + salt + calcium)
	beans (black bean flour + salt + vegetable oil)
Mid-morning snack:	banana
	beans
	tamale
	lemonade (lemon juice & sugar)
Lunch:	corn gruel
	tamale
	beans
	cooked carrots or spinach
Mid-afternoon snack:	banana
	beans
	tamale
	lemonade
Dinner:	corn gruel
	tamale
	beans
	mashed potatoes or squash

* Ingredients are shown in parentheses.

TABLE 4. Summary of Experimental Design and Schedule

Experimental days	0	4	↑	8	12	↑	16	20	24		28		32	36	40
	↑							↑		↑		↑			↑
	↑	44	↑	48	52	↑	56	60	64		68		72	76	80
								↑		↑		↑			↑
	↑	84	↑	88	92	↑	96	100	104		108		112	116	120
								↑		↑		↑			↑
Procedures	A, B		BEE, HR			A		NE, B		A		BEE, HR			A,B,NE

Diet: Protein: 1.75 g/kg/day throughout the study. Energy: initially 100/kcal/kg/day and decreased to 92 and 84 kcal/kg/day on days 41 and 81, respectively (only exception: child E.G., see text).

A: Anthropometric measurements: height; perimeters of arm and leg; tricipital, subscapular, and abdominal subcutaneous skin-fold thicknesses. Weight was measured every day.

B: Packed red blood cell volume, plasma proteins, and urinary creatinine.

NE: Nitrogen and energy balance.

HR: Heart-rate-energy-expenditure relationship. Heart rate continuously monitored 5 days before and 5 days afterwards.

BEE: Basal energy expenditure.

TABLE 5. Mean Daily Intakes of Dietary Protein and Energy During the 4-Day Balance Periods

Patient	Protein (g/kg)	Energy (kcal/kg)*				
		120**	110	100	92	83
379	1.73 ± 0.03 (6) ***			99 (2)	92 (2)	83 (2)
387	1.75 ± 0.06 (8)	118 (2)	106 (2)	100 (3)	97 (1)	–
388	1.69 ± 0.04 (6)			99 (2)	89 (2)	78 (2)
390	1.75 ± 0.01 (6)			95 (2)	84 (2)	76 (2)
394	1.79 ± 0.01 (6)			102 (2)	94 (2)	86 (2)
395	1.63 ± 0.04 (6)			100 (2)	92 (2)	84 (2)
Average	1.73 ± 0.06 (38)	118 (2)	106 (2)	99 ± 3 (13)	91 ± 4 (11)	81 ± 4 (10)
Mean net energy intakes ****		106	96	90	82	71

* Gross intakes, i.e., bomb calorimetry values not corrected for faecal energy excretion.
** Theoretical levels of energy intakes.
*** Mean ± standard deviation (number of balance period).
**** Net intakes = gross intakes — faecal losses (bomb calorimetry).

Summary of Main Results

1. Dietary Intakes

Table 5 gives the average intakes of dietary energy and protein for each child and for the group.

2. Energy and Protein Absorption

Table 6 gives the apparent energy absorption of each child for all levels of energy intake. The overall average absorption was 88 ± 6 per cent. With gross intakes of 100, 92, and 83 kcal/kg, apparent absorptions were, respectively, 91 ± 6 per cent, 80 ± 7 per cent, and 85 ± 4 per cent. Although the mean values decreased with decreasing levels of intake, they were not different from each other.

Table 6 also gives the "true" and apparent digestibilities of nitrogen (i.e., with or without correction for endogenous faecal nitrogen). The average apparent digestibility was 59 ± 6 per cent for all levels of energy intake. With net energy intakes of 90, 82, and 71 kcal/kg/day, apparent nitrogen digestibility was 56 ± 7 per cent, 59 ± 6 per

TABLE 6. Absorption of Energy and of Nitrogen as a Percentage of the Amounts Ingested (Mean of all Levels of Dietary Energy)

Patient	Energy absorption, apparent	Nitrogen digestibility	
		apparent	"true"**
379	94 ± 3*	64 ± 4	71 ± 4
387	88 ± 4	59 ± 11	66 ± 11
388	84 ± 9	60 ± 3	67 ± 3
390	85 ± 4	61 ± 9	68 ± 9
394	88 ± 7	47 ± 4	54 ± 4
395	87 ± 5	65 ± 7	73 ± 7
Average	88 ± 6	59 ± 9	66 ± 9

* Mean ± standard deviation.
** Assuming obligatory faecal losses of 20 mg N/kg/day.

cent, and 62 ± 9 per cent, respectively. These values were not different from each other. "True" digestibilities were higher by about 7 per cent of nitrogen intake. One child (394) consistently had low nitrogen digestibilities. If his results were not included in the analysis, the average apparent digestibility for the other five children at all levels of energy intake would be 62 ± 4 per cent, and with the three levels of net dietary energy they would be 58 ± 6, 61 ± 3, and 66 ± 8 per cent. These values do not differ from each other.

There was no association between the apparent absorptions of energy and protein (r = 0.173).

3. Nitrogen Balance

The values of the two four-day balance periods at each dietary energy level were averaged for each child. Table 7 gives the nitrogen-balance data of five children at each level of energy intake. There were no differences among the three dietary energy intakes. Child 387 retained 54.1, 95.7, and 43.6 mg N/kg/day when he ingested 106, 96, and 90 kcal/kg/day, respectively. The mean and standard deviations of the seven balances performed on him were 61.5 ± 31.8 mg N/kg/day.

TABLE 7. Nitrogen Balance (Nitrogen Intake – Urinary Nitrogen – Faecal Nitrogen – 5 mg/kg/day) Expressed as mg N/kg/day

Child	Net dietary energy (kcal/kg/day)			Mean and S.D. of 6 balances*
	90	82	71	
379	76.6	51.4	59.9	62.6 ± 18.7
388	59.9	88.3	60.8	69.6 ± 20.6
390	51.6	53.1	86.7	63.8 ± 18.1
394	52.5	50.9	49.9	51.1 ± 14.6
395	78.1	69.4	66.0	72.2 ± 9.2
Average (n = 10)*	63.7 ± 11.5	58.0 ± 6.8	64.9 ± 15.2	

* Two balance periods with each level of energy intake.

4. Energy Expenditure

Table 8 gives the energy expended by five children at each of the three levels of dietary energy. The children spent more energy in physical activity with the highest level of intake ($p < 0.05$); there was no difference between the two lower levels of intake. The nurses and the investigators, however, did not notice any changes in the children's behaviour or in the pattern and intensity of their activities throughout the study. The sixth child (387) also expended more energy when he had the highest intake. His daily expenditure with net dietary intakes of 90, 106, 96, and 90 kcal/kg were 80.4, 90.4, 73.4, and 78.9 kcal/kg, respectively.

5. Energy Balance

Table 8 also gives the energy balance of the children. There were no differences in energy balance with the different levels of dietary energy intake. The corresponding figures for child 387 were +9, +14, +19, and +5 kcal/kg/day with net dietary energy intakes of 90, 106, 96, and 90 kcal/kg/day, respectively.

6. Anthropometry and Growth

The only consistent anthropometric change was a decrease in body-weight gain with the net dietary energy intake of 71 kcal/kg/day. Weight gains calculated in five children by regression analysis during the 40 days at each level of energy intake were (mean ± S.D.) 10.7 ± 4.8, 11.4 ± 3.4, and 0.6 ± 4.4 g/day for 90, 82, and 71 kcal/kg/

TABLE 8. Total Daily Energy Expenditure and Energy Balance (kcal/kg/day)*

Patient	Net dietary energy (kcal/kg/day)					
	90**		82		71	
	exp.	bal.	exp.	bal.	exp.	bal.
379	104.0	−11	89.8	−3	81.6	−7
388	84.8	7	76.0	4	63.3	5
390	77.8	0	77.8	−4	72.0	−10
394	93.5	−6	65.6	22	65.6	3
395	85.2	−3	69.7	13	74.9	−3
mean	89.1***	−3	75.8	6	71.5	−2
±						
S.D.	9.0	6	8.3	10	6.5	6

* Energy expenditure was calculated from heart rate and the corresponding heart-range-energy-expenditure relationship between 5 a.m. and 8 p.m., and from basal energy expenditure during the remaining 9 hours. Energy balance was calculated from energy intake (bomb calorimetry) − faecal energy (bomb calorimetry) − urine losses (5 kcal/g urinary nitrogen) − sweat losses (8 kcal/g sweat nitrogen = approx. 0.1 kcal/kg/day) − energy expenditure (as described above).

** Dietary-faecal energy, measured by bomb calorimetry.

*** Differs from the two other levels of intake, $p < 0.025$.

day, respectively. Paired comparisons confirmed that the last value differed from the preceding two ($p < 0.05$ and <0.01), respectively.

The sixth child (E.G., no. 387) lost 2 to 3 g/day with intakes of 90 and 82 kcal/kg/day. With 106 and 96 kcal/kg/day he gained 25 and 13 g/day, respectively, and only 3 g/day when he again received 90 kcal/kg/day.

The level of dietary energy did not affect growth in terms of height. Three children showed a continuous tendency to catch up, regardless of the amounts of energy intake.

7. Other Biochemical Measurements

Fluctuations within normal ranges were observed, without relation to the amounts of dietary energy intake.

Conclusions and Comments

1. An all-vegetable diet can fulfil the protein requirements of pre-school children when black beans and corn provide about 0.7 and 0.95 g protein/kg/day, respectively. This is true when the children are physically active and net dietary energy intakes range between 71 and 90 kcal/kg/day.

2. Within those ranges the level of energy intake did not affect nitrogen balance. The amounts of nitrogen retained by the children were greater than those that have been estimated to allow adequate growth, suggesting that the diets used in this investigation provided more protein than required. Therefore, it may be that the amounts of protein provided by the diet surpassed requirements to a point where the protein-sparing effect of energy was obscured, and it is conceivable that the amounts of dietary energy used might have influenced nitrogen balance if the protein intake had been closer to the requirement level.

3. Nitrogen retentions were high even though the "true" protein digestibilities were low, corresponding to an average of 1.14 g protein absorbed/kg/day (1.73 g/kg with a digestibility of 66 per cent). Nitrogen digestibility was lower than we have observed in other studies using similar diets (67 to 83 per cent), and it seemed to be related to large faecal volumes that averaged 197 g/day in this study. The apparent absorption of energy (88 per cent) differed less from our other investigations (89 to 92 per cent).

4. Diets of the type used in this study failed to fulfil pre-school children's energy requirements unless their energy density was increased. At least 10 per cent additional energy had to be added to the diets as vegetable oil to ensure adequate weight gains. This provided an average of 82 net kcal/kg/day.

5. There were no changes in weight gain when the net dietary energy decreased from 90 to 82 kcal/kg/day. However, this change was accompanied by a decrease in total energy expenditure, although no changes in physical activity patterns were noticed, and energy "balance" (defined as intake minus faecal, urinary, and sweat losses, and minus total energy expenditure) remained constant. Basal energy expenditure and the energy lost through excreta also remained constant. The children seemed to adjust to the initial decrease in intake by a decrease in physical activity or by a greater efficiency in performance (i.e., spending less energy to do the same things) and continued to gain weight at an adequate rate. These findings could also indicate that, within certain limits, the children avoid becoming obese by increasing their energy expenditure when there is an excessive dietary energy intake. When the net dietary intake was further decreased to 71 kcal/kg/day, energy expenditure did not diminish any more and the children lost or reduced their weight gains. Therefore, we conclude that a net dietary intake of 71 kcal/kg/day was insufficient and the children could not compensate for this low intake by a decrease in physical activity under the experimental conditions that prevailed.

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