Protein-Energy-Requirement Studies in Developing Countries: Results of International Research

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THE UNITED NATIONS UNIVERSITY

WHTR-8/UNUP-481

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Report of a workshop of the International Union of Nutritional Sciences held in Berkeley, California, USA, 10–14 August 1981, to consider research organized by the United Nations University, the Food and Agriculture Organization, and the World Health Organization

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5. ADDITIONAL STUDIES ON VERY SHORT-TERM PROCEDURES TO EVALUATE PROTEIN QUALITY IN ADULT HUMAN SUBJECTS

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It is a common practice to evaluate the protein quality of diets, ingredients, protein supplements, and new protein sources by the use of animal assay. A test with human subjects is of interest to reinforce animal data and to demonstrate their applicability to protein quality for human consumption. Protein quality assays in humans are, however, costly and of relatively long duration. Therefore, it would be desirable to have an assay of shorter duration, practical and valid, which would be capable of giving results comparable to those of other assays. This report presents additional information on a short-term assay for protein quality that may be useful for routine use.

Objectives

1. To compare the relationship of nitrogen intake to nitrogen retention using ascending and descending sequences of protein feeding.

2. To study the effect of variations in the descending sequence on the relationship between NI and NB.

Experimental Details

Subjects

Study 1: Standard Short-Term Ascending-Descending Assay

Ten young men from 19 to 30 years of age, in good physical health with an average weight of 55 kg, were the subjects of study 1. The physical characteristics of these subjects are described in table 1.

Study 2: Modified Descending Assay

Thirteen healthy young men from 23 to 30 years old, with an average weight of 55.1 kg, were the subjects of study 2, whose physical characteristics are described in

TABLE 1. Physical Characteristics of Experimental Subjects: Standard Short-Term Ascending-Descending Assay

Subject	Age (years)	Weight (kg)	
		Initial	Final
F.M.	24	60.4	58.2
O.B.	26	59.1	58.3
R.A.	28	58.2	56.9
J.P.	25	58.2	57.4
R.S.	23	56.6	55.9
O.Bo.	24	58.2	57.7
H.R.	25	49.5	49.1
M.R.	30	62.7	60.5
O.H.	24	46.5	44.2
N.R.	19	46.6	44.5
$\bar{x} \pm$ S. error	24.8 ± 1.0	55.6 ± 1.9	54.3 ± 2.0
Standard dev.	2.9	5.8	6.0

TABLE 2. Physical Characteristics of Experimental Subjects: Modified Short-Term Descending Assay

Subject	Age (years)	Weight (kg)	
		Initial	Final
R.A.	28	58.2	57.8
R.S.	23	57.8	59.1
O.Bo.	24	56.7	58.7
H.R.	25	59.5	59.6
J.P.	25	59.5	59.6
D.V.	30	49.3	50.0
G.C.	23	58.2	57.9
A.G.	28	58.0	57.9
A.O.	23	50.9	51.4
R.C.	28	50.0	50.9
O.B.	24	55.9	53.6
A.A.	26	60.0	59.5
C.P.	23	50.9	50.9
$\bar{x} \pm$ S. error	25.4 ± 0.6	55.1 ± 1.1	55.4 ± 1.0
Standard dev.	2.4	4.0	3.8

TABLE 3. Composition of the Protein-Free Basal Diet: First Assay

Ingredients	Grams
Instant coffee	3
Apple marmalade	20
Sugar	25
Water-melon	200
Artificial drink fruit flavour (glasses)	2
Mineral and vitamin supplement (tablets/day)[a]	1
Calorie Sources to Meet Requirements	**Units**
Carbonated drink (Pepsi-Cola)	1
Refreshments	Variable
Candies	Variable

a. UNICAP-T

table 2. In both studies the subjects were selected on the basis of their previous protein and energy intakes, as well as for their physical and general health conditions.

Study Environment: The subjects lived in Guatemala City and worked at INCAP. All of their meals were eaten in the Metabolic Unit of the Division of Food and Agricultural Chemistry. The daily ambient temperature ranged from 21 to 25°C, and relative humidity ranged from 72 to 85 per cent. Guatemala City is 1,510 m above sea-level.

Physical Activity: All men performed their usual chores at INCAP.

Experimental Diets

Basal diet

The diet described in table 3. Plantain flour made from half-ripe, drum-dried plantains was used to provide 25 per cent of the total energy intake equivalent to 45 kcal/kg/day. The protein source in all studies was provided by a pre-cooked bean flour containing 22.8 per cent protein. The protein intake per day was provided in three equal amounts at 7.30 a.m., 12 noon, and 5.30 p.m.

Experimental Design

Standard Ascending Assay

This is described in table 4. It includes an adjustment period with animal protein included in the basal diet for six days, with faecal and urine collections for the last

TABLE 4. Experimental Design of the Ascending-Descending Protocols

Protein Level (mg/kg/day)	Days		Marker
0.6	3	Standardization[a]	
0.0	3		Carmin
0.2	2		Carbon black
0.4	2		Carmin
0.6	2		Carbon black
0.6	3	Rest[a]	Carmin
0.6	2		Carbon black
0.4	2		Carmin
0.2	2		Carbon black
0.0	2		Carmin

a. Animal protein-based diet.

TABLE 5. Experimental Design for the Modifications on the Descending Protocol

Protein Level (mg/kg/day)	Days		Marker
Modification 1			
0.6	4	Standardization[a]	
0.6	2		Carmin
0.4	4		Carbon black
0.2	4		Carmin
0.0	2		Carbon black
Modification 2			
0.6	4	Standardization[a]	
0.6	2		Carmin
0.5	2		Carbon black
0.4	2		Carmin
0.3	2		Carbon black
0.2	2		Carmin
0.1	2		Carbon black

a. Animal protein-based diet.

TABLE 6. Nitrogen Retention at Different Levels of Protein Intake

Design	Subjects	Average Intake (mg/kg/day)	N Retention
Ascending standard	10	24.7	− 66.3 ± 14.1
		52.8	− 43.6 ± 8.4
		85.9	− 9.5 ± 6.9
		117.4	− 2.2 ± 7.0
Descending standard	10	119.5	− 11.4 ± 4.7
		85.9	− 43.9 ± 5.4
		60.4	− 42.4 ± 4.0
		25.4	− 54.7 ± 3.7
Descending modification 1	7	119.7	− 25.3 ± 7.8
		86.2	− 43.1 ± 5.4
		56.4	− 33.1 ± 2.7
		26.2	− 62.4 ± 4.6
Descending modification 2	6	120.5	− 29.8 ± 8.1
		103.2	− 33.0 ± 8.9
		86.8	− 33.5 ± 6.2
		73.7	− 36.1 ± 12.7
		51.9	− 51.9 ± 9.9
		43.5	− 45.3 ± 9.5

TABLE 7. Regression Equations between Nitrogen Intake and Nitrogen Retention

Assay	NI = a + b (NR)	r ($P < 0.01$)	NI for NR = 0 (mg/kg/day)
Ascending standard	− 81.68 + 0.73	0.77	112
Descending standard	− 68.98 + 0.42	0.71	162
Descending mod. 1	− 72.46 + 0.49	0.78	147
Descending mod. 2	− 66.62 + 0.39	0.44	171

three days for nitrogen balance calculation. The low N basal diet follows for three days with faecal and urine collections during the last two days. This is followed by three levels of protein feeding of 0.2, 0.4, 0.6 kg/day each for two days with no adaptation in between. Faeces and urine are collected daily and pooled for two-day nitrogen balance calculation.

TABLE 8. Comparison of Nitrogen Intake Values for Nitrogen Equilibrium of Various Proteins Using a Very Short and Short-Term Assays

Proteins	Author	Short	Very Short[a]
Animal			
Egg	Young et al.	88	—
	Puig	92 (42)	—
	Inove et al.		
	INCAP[a]	83 (16)	70 (4)
Milk	Wayler	93 (33)	—
	Wayler	133 (49)	—
	INCAP	88 (7)	74 (4)
Beef	Wayler	108 (20)	—
	Fajardo	83 (11)	—
	Puig	106 (19)	—
	INCAP	—	85 (9)
Vegetable:			
Soy isolates			
Supro 620	Inoue	130 (14)	—
	Puig	111 (15)	—
	Wayler	135 (28)	—
	INCAP	—	88 (13)
Supro 710	Wayler	114 (36)	—
	INCAP	—	82 (12)
Supro 220	INCAP	—	90 (20)
Cottonseed isolate			
Glandless	Thomas et al.	106	—
Glanded	Alford et al.	106	—
Glanded	INCAP	—	98 (18)

a. INCAP: Very short-term assays. Bressani and Navarrete.

Standard Descending Assay

This is described in table 5. It follows the ascending assay with a three-day rest period in between in which the basal diet with animal protein if fed at 0.6 g P/kg/day. Protein intake is then decreased from at 0.6 g/kg/day, to 0.4, 0.2, and the low N-basal diet with the test protein for two days each.

Modified Descending Assay

Two modifications were introduced into the standard descending design. One consisted of extending to four days the protein levels of 0.4 and 0.2. The second modification consisted of keeping the number of days constant, at two days per protein level, with protein levels decreasing from 0.6 to 0.5, 0.4, 0.3, 0.2, and 0.1 g/kg/day. These are shown in table 5.

Results

Table 6 summarizes the average nitrogen balance at the various levels of protein intake in the four designs studied, and table 7 summarizes the regressions of nitrogen intake to nitrogen retention. The greatest linearity, with the highest coefficient of regression, was observed with the ascending design. Among descending designs, which had similar coefficients, the standard and modification 1 showed the best linearity. Modification 2 of the descending designs had the lowest coefficient of regression and the lowest correlation. The table also shows the nitrogen intake needed for nitrogen equilibrium. These were 112, 162, 147, and 171 mg N/kg/day for the ascending and descending standard assays and for the descending modification 1 and 2, respectively. The average value of the three designs showing the greatest linearity between intake and retention was 140.

Conclusions and Comments

The ascending short assay has a tendency to overestimate the quality of the protein as well as the amount of nitrogen needed for nitrogen equilibrium. On the other hand, the descending design tends to underestimate the same values in its present sequence. Therefore, it seems justified to assay proteins by both protocols and calculate average values. It is of interest to point out that the ascending standard ranks proteins for their quality rather well.

This is shown in the values of table 8, which compares the nitrogen intake for nitrogen equilibrium of animal and vegetable proteins assayed by the very-short-term method and by other longer-term assays. Two conditions should be tried for the descending protocol; one is to increase the adjustment period and the other to extend the length of intermediate protein feeding periods, as in modification one of this report.