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A CRITICAL SUMMARY OF A SHORT-TERM NITROGEN BALANCE INDEX TO MEASURE PROTEIN QUALITY IN ADULT HUMAN SUBJECTS

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The evaluation of protein quality in human subjects is a difficult problem mainly due to:
a) Continuous changes in the metabolic state taking place while attempting to measure the response of body protein metabolism to variable dietary intakes;
b) The determination of energy requirements;
c) The variability in the efficiency of protein utilization by individuals in different physiological states, and;
d) Interpretation of results in relation to the meaning of theoretical models and evaluation for practical purposes.

Because of these and other possible problems the cost of such assays is quite high, representing a significant constraint to the establishment of standard experimental conditions to obtain reproducible and constant responses to specific protein sources.

Presently, various approaches are utilized to measure protein quality. However, one which overcomes most of the difficulties is a multiple point assay originally known as the Nitrogen Balance Index (Allison, 1945; Bressani and Viteri, 1971; Bressani et al, 1973). This assay relates through a regression equation, the relationship between nitrogen intake or nitrogen absorbed to nitrogen retained, which according to theory should be linear in the region below and slightly above nitrogen equilibrium. The theoretical line is shown in Figure 1, which also shows the regression equation NB + K(NA) - Neo, where NB signifies nitrogen balance and NA nitrogen absorbed. The value K is equivalent to the efficiency of utilization of the protein under study and is the slope of the line; and Neo represents the total excretion of nitrogen of metabolic and endogenous origin from feces and urine.

We believe that in theory, the point enclosed by circle 1, in Figure 1, represents the sum of metabolic fecal and of

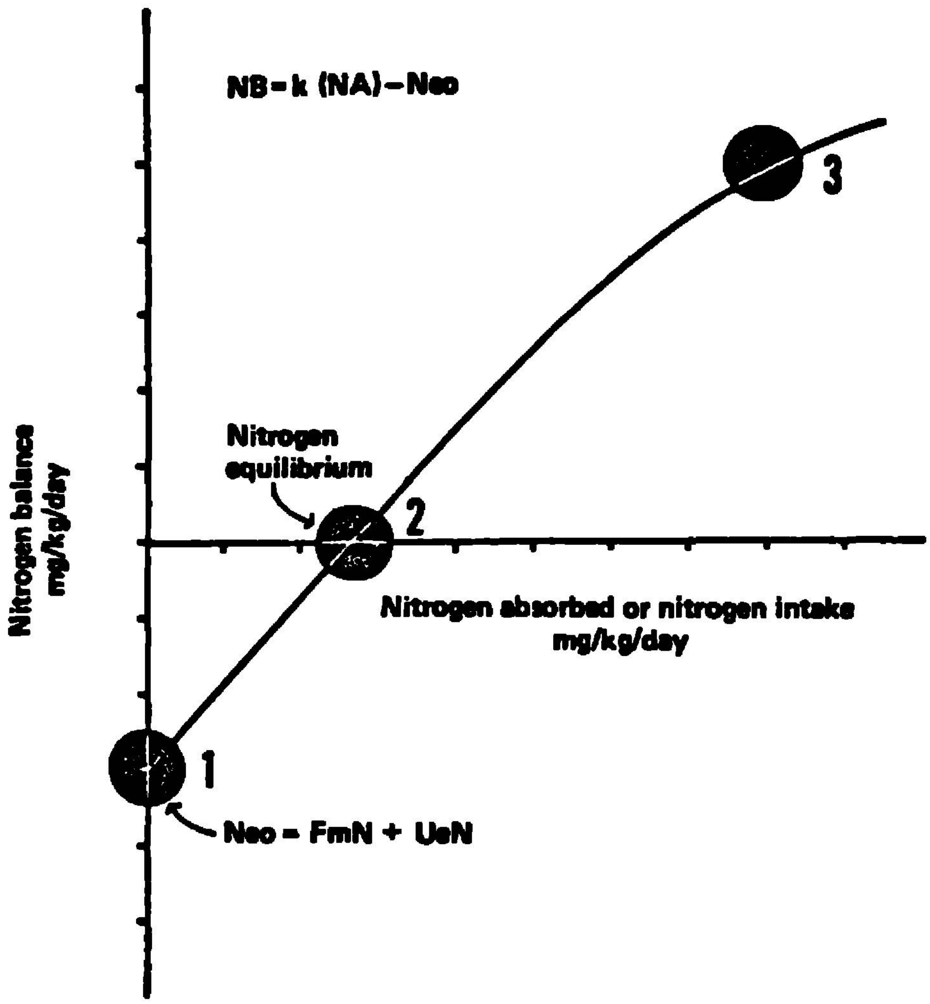

FIGURE 1. The basic principle of the nitrogen balance index.

endogenous urinary nitrogen, obtained by feeding a nitrogen-free diet. Only rarely does the extrapolated value (i.e., the intercept) from the regression equation coincide with that derived from the actual feeding of a nitrogen-free diet. Typical of this are results reported for egg and other proteins in recent publications (Young et al, 1977; Bressani, 1977). The reasons for such discrepancies are not known, but could be due to metabolic causes, to methodological technique, or both. In any case, it is an area which must be investigated. In Figure 1, circle 2 is the level of nitrogen absorbed to meet maintenance requirements. This level is determined mainly by the amino acid limiting the efficiency of utilization of the protein or, in other words, the most limiting amino acids needed to meet the maintenance requirement. These amino acids may be different in adults from those in young growing subjects. In theory, this point must be different in terms of nitrogen, for proteins of different quality.

If food proteins are limiting in different essential amino acids, but proportionally to the same extent, the amount of nitrogen retained at a given level of nitrogen intake should be the same for all of them. Furthermore, in theory, the minimum amount of nitrogen retained under normal and acceptable experimental conditions for a high quality protein, would take place when the N balance line crosses the N absorbed line at a 45^{o} angle. For high quality proteins, that is, for those with a high efficiency of utilization, the amount of nitrogen required for nitrogen balance should be numerically only slightly higher than the value for total metabolic, fecal and urinary endogenous nitrogen excretion. If the above is true, this point is quite meaningful, in terms of expressing protein quality, and can be used as a reference point to calculate relative protein quality values.

Circle 3 represents excess nitrogen intake, which is wasted, or utilized with a low efficiency of nitrogen utilization. This point is believed to be determined by overall absolute amino acid level in which the limiting amino acid of the protein no longer plays an important role in establishing true biological value.

The nitrogen balance values from area 3 are not sensitive to protein quality and in the past have led to erroneous conclusions with respect to the significance of biological value of proteins in human adults. The biological effects, if any, from such N retentions from poor quality proteins are not known.

In our opinion, the meaning of these points and the correlation between theoretical and experimental results should be assessed; a limitation, however, is the time required to run a test. Customarily, in nitrogen balance studies with humans, the length of each feeding period is of 10 to 14 days duration. The number of individuals needed for each test is a complicating factor. To be able to solve some of these problems, as well as to be able to develop a protein assay technique, we studied the possibility of reducing the experimental period, after obtaining promising results with adult dogs, as experimental animals (Bressani et al., 1978; Navarrete et al, 1977; Bressani et al, (in press); Navarrete et al, (manuscript in preparation). The experimental conditions established for adult human subjects are shown in Table 1.

Subjects between 20 and 35 years of age are chosen on the basis of good general health as judged by medical and biomedical parameters, ongoing dietary intake and physiological functions. In various experiments between 5 and 12 subjects were fed each diet protein. Once in the experimental run they are fed from 40 to 45 kcal/kg body weight/day, of which 30% of the calories are derived from fat. A constant and adequate water

Table 1. Basic Procedure Followed For Short Time Nitrogen Balance Index

Subject age:	20 - 35 years old
Calories/day:	40 - 45 kcal/kg
Water intake:	Adequate and Constant
Low nitrogen diet:	10 - 15 mg/kg/day
Protein intake g/kg/day	Days on balance
0.0	3
0.2	2
0.4	2
0.6	2

intake is provided. The order of levels of protein feeding are shown in the lower part of the Table. A multivitamin capsule is given daily to all subjects. With the aid of markers, feces are quantitatively collected, as well as urine, for a total period of two days.

Results using this modified technique as well as the conventional method are summarized in Table 2. The relationship between nitrogen intake (NI) to nitrogen balance (NB) is expressed as the regression coefficient, b. Although the number of conventional runs is small in comparison with the short-term approach, agreement between the two is quite good. Furthermore, the values found by the short-term assay agree with those reported by other workers (Bressani, 1977; Navarrete et al, 1977; Bressani et al, (in press); Navarrete et al, (manuscript in preparation). Of the group, beans show the poorest quality, needing around 116 mg N/kg/day to attain equilibrium. Without considering egg, the best quality protein is milk, given an intake of around 80 mg/kg/day for nitrogen equilibrium. Table 3 summarizes the same information; however, the relationship here is between NA and NR.

Based on the coefficient of regression which varies from 0.70 for casein to 1.06 for milk, the ten proteins so far tested are of high quality. Egg protein has been very erratic for reasons not yet known; however, the values found, except for the 1.02, fall within the range reported by other workers. The high values are probably due to prolonged time on the nitrogen-free diet. It is our intention to test intermediate and low quality proteins once the experimental conditions are well established. The values for the short assay are similar to those obtained from the conventional method in our laboratory.

Table 2. Relationship Between Nitrogen Intake (NI) and Nitrogen Balance (NB) as Obtained From a Short Term Nitrogen Balance Index In Adult Humans

Protein Source	Short Assay NR = a + b (NI)		Conventional Assay NB = a + b (NI)	
	a b	r	a b	r
Egg	-55.21 + 0.86	0.88	-57.58 + 0.70	0.89
	-63.64 + 0.57	0.78		
Milk	-78.80 + 0.91	0.84	-72.34 + 0.82	0.92
	-73.61 + 0.98	0.92	-70.38 + 0.77	0.91
	-81.30 + 1.03	0.84		
	-70.60 + 1.00	0.82		
Casein	-60.28 + 0.64	0.90		
Soybean-TVP	-65.73 + 0.68	0.92		
Soybean Isolate	-72.19 + 0.83	0.90		
Beef	-74.40 + 0.87	0.95		
Soybean-Beef	-79.90 + 0.87	0.89		
Figurines	-75.44 + 0.90	0.91		
Corn/Beans	-86.81 + 0.89	0.89		
Beans	-62.69 + 0.54	0.75		

Table 3. Nitrogen Balance Index Values Obtained in Adult Subjects at Incap

Protein Source	Short Assay NB = a + b (NA)		Conventional Assay NB = a + b (NA)	r
	a b	r	a b	r
Egg	-59.45 + 0.67	0.78	-39.65 + 0.57	0.81
	-51.58 + 1.02	0.95		
	-54.91 + 0.37	0.52		
Milk	-54.49 + 0.91	0.93	-57.21 + 0.93	0.97
	-47.08 + 0.97	0.91	-56.16 + 0.88	0.94
	-56.53 + 1.06	0.73		
	-41.89 + 0.88	0.84		
Casein	-48.42 + 0.70	0.92		
Soybean-TVP	-54.21 + 0.77	0.95		
Soybean Isolate	-56.29 + 0.91	0.93		
Beef	-54.88 + 0.86	0.97		
Soybean-Beef	-61.76 + 0.91	0.94		
Figurines	-52.05 + 0.83	0.91		
Corn-Beans	-63.81 + 0.95	0.92		
Beans	-57.44 + 0.81	0.82		

Various aspects of the results presented should be indicated, as shown in Table 4. The numerical experimental figures for total endogenous N excretion from the short method regression equations range from 42 to 64, with an average of 54. The average value as found in the literature is 50, of

Table 4. Summary of Values Derived From Regression Equations From the Short-Term Nitrogen Balance Index Assay

Equations: NB = Neo + K (NI) (1)
NB = Neo + K (NA) (2)

Parameters		*Short-Term*	*Coventional*	*Literature*
Neo (1)	Range	55.2 - 86.8	57.6 - 72.3	-
	Average	71	65	-
Neo (2)	Range	41.9 - 63.8	56.2 - 57.2	-
	Average	54	57	50

which 39 came from urine and 12 from feces. We believe that a good NBI assay should show a Neo value close to the theoretical figure.

The results obtained by the short assay also confirm results reported by other workers using the conventional approach as shown in Table 5. In this case the values of nitrogen intake and of nitrogen absorbed for nitrogen equilibrium are compared. The agreement is high between the results from the conventional and short-term assays as presently carried out within INCAP and also with values from other laboratories. Further evidence supporting the potential of the short-term method is presented in Table 6, showing protein intake values for nitrogen equilibrium for 4 protein sources assayed by the conventional and short method. Although the agreement is quite high, two points are of concern:

a) the short technique has a tendency to give higher values for efficiency of N utilization, and

b) the point for nitrogen equilibrium is lower.

The first situation shown in Figure 2 is due to the fact that when protein intake is increased and maintained at the same level, the NR during the adaptation period is more negative at below maintenance requirements and more positive above maintenance requirements than the NR for the balance period. Therefore, the slope using the initial retention values at a fixed N intake are somewhat higher than if the following NR values are used to calculate the regression equation. Therefore, the nitrogen equilibrium point also changes, becoming smaller. If efficiency of utilization (the slope) on nitrogen intake required to meet nitrogen balance of a test protein is expressed as percentage of a standard protein, there is probably no problem in interpretation, since the same situation will prevail for the reference protein at all nitrogen intakes.

Table 5. Some Comparative Values of Nitrogen Index (NI) and Nitrogen Absorption (NA) for Nitrogen Equilibrium (NE) for Various Proteins

Protein	*Method*	*Author*	*NI for NE* (mg/kg/day)	*MA for NE* (mg/kg/day)
Egg [1]	Conventional	Calloway & Margen	-	68
Egg	Conventional	Young et al '73	88	73
Egg	Conventional	Inoue et al '77	-	100
Egg	Conventional	INCAP	82	69-83
Egg	Short	INCAP	64-112	50-89
Milk	Conventional	Bricker et al '45	-	60
Milk	Conventional	INCAP	88	61-64
Milk	Short	INCAP	75-86	48-60
Casein	Short	INCAP	94	69
Soy TVP	Short	INCAP	97	70
Soy Isolate	Conventional	Young et al '73	107	-
Soy Isolate	Short	INCAP	87	62
Meat	Short	INCAP	85	64
Soy/Meat	Short	INCAP	92	68
Figurines	Short	INCAP	84	63
Corn/Beans	Short	INCAP	97	67
Beans	Short	INCAP	116	71

[1]*See Footnotes Table 2.*

Table 6. Protein Intake for Nitrogen Equilibrium, g/kg/day

Protein Source	*Conventional* [1,2]	*Short-Term*
Soy Isolate	0.67	0.54
Milk	0.63	0.62
50/50 Beef/Soy	0.59	0.57
Beef	0.64	0.53

[1]Scrimshaw and Young. This conference.
[2]Values corrected assuming 5 mg integumental and miscellaneous losses.

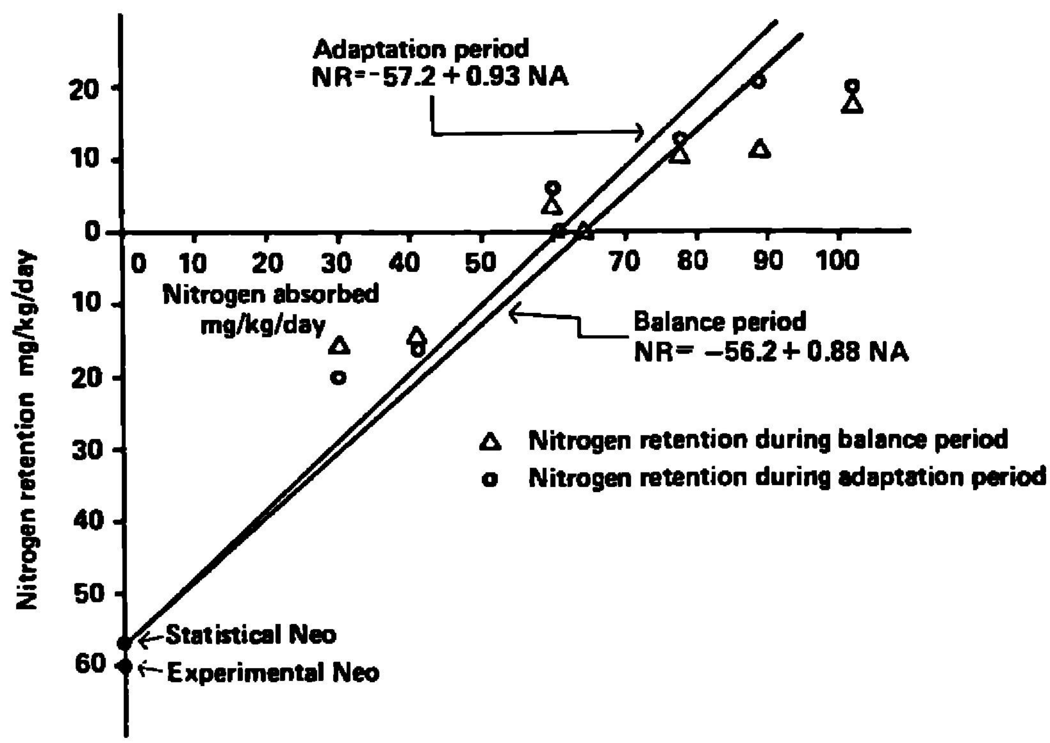

FIGURE 2. Nitrogen balance index of milk with adult human subjects. (Conventional Assay).

Up to the present time, only high quality proteins have been tested and it appears, from the data obtained, that there is rather good agreement between theory and actual results. It is of practical significance to discover if the same applies to protein of low quality with single or double amino acid deficiency. These are other aspects which are under study in our laboratories, as well as other aspects. These are:

a) The effect of protein level and length of intake during adaptation period before the test;
b) The length of the feeding period of a nitrogen-free diet (NFD) before protein feeding (no longer than four days);
c) The number of protein levels to be fed below and above the equilibrium point.

The results in experimental animals, although preliminary, so far indicate that previous dietary protein level before the test does affect the slope and intercept. The length of NFD feeding, which has a more significant influence, also influences the slope and intercept.

Further research is needed to establish the correct experimental conditions to assay protein quality in humans, and the short-term approach may be useful for such a purpose, as well as for establishing protein quality values and requirements.

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