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The Effect of Prior Protein Nutriture and Nitrogen Retention on the Utilization of Nonessential Nitrogen in the Young Adult

Hans Fisher

From the amino acid requirement data for young adult women, ratios of essential to total amino acid N (E/T ratios) were calculated and shown to be of the order of magnitude of 3–5%. The E/T ratio for a good quality protein like egg is 35%, whereas that for wheat is only 20%. Evidence has been presented that wheat protein, nevertheless, meets the protein needs for maintenance of adult man and may even meet those of growing children. Depending upon the prior adequacy of the

diet in relation to specific essential amino acid or total protein intake, the subsequent ingestion of nonessential N can be either well retained or not utilized. Evidence is presented to show that muscle RNA and transaminase enzymes responded, following protein depletion, to dietary supplements of nonessential amino acids beyond the response obtained from a mixture of essential amino acids adequate for maintenance but inadequate in total N.

Proteins of high biological value for growth such as egg, milk, and others usually provide about 40% of the total nitrogen (N) in the form of essential amino acids, while relatively poor quality proteins may provide only about 25% of the total N in the form of essential amino acid N. In rapidly growing, young animals the need for essential amino acids approaches the proportions of essential to nonessential amino acids found in high quality proteins;

in fact, a high biological value is in itself an indication of such a balance between essential and nonessential amino acid N.

A rather different situation exists in relation to the essential amino acid as well as the total N requirements of adult man and animals. According to the available estimates for the essential amino acid requirements of adult men and women, the N that needs to be supplied by the essential amino acid portion of the total N requirement is very low. Table I shows ratios of essential to total amino acid N (E/T ratio) of 4.8 and 2.8%, respectively, for requirement data for young college women, determined in

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Table I. Essential Amino Acid Requirements of Young Women and Essential Amino Acid Content of Certain Food Proteins

	Requirements				Present in ^c			
	Leverson, Swendseid, and Jones, ^a mg/day		Fisher, ^b mg/day		Whole egg, g/16 g of N		Whole wheat, g/16 g of N	
Lysine	500	(96) ^d	50	(10)	6.9	(1.32)	2.5	(.48)
Tryptophan	160	(22)	50	(7)	1.6	(0.22)	1.2	(.16)
Phenylalanine + tyrosine	1120	(57)	400	(34)	5.8	(0.51)	4.4	(.37)
Threonine	310	(36)	200	(24)	5.0	(0.59)	3.9	(.46)
Methionine + cystine	550	(33)	150	(14)	3.3	(0.31)	1.2	(.11)
Leucine	620	(66)	150	(16)	9.4	(1.00)	6.9	(.74)
Isoleucine	450	(48)	100	(11)	6.9	(0.74)	4.4	(.47)
Valine	650	(78)	200	(24)	7.4	(0.89)	4.5	(.54)
	3710	(436)	1300	(140)	46.3	(5.58)	29.0	(3.33)
<i>E/T</i> ratio ^e		4.8		2.8		34.9		20.8

^a As summarized by Rose (1957). ^b Taken, in part, from Fisher *et al.* (1969, 1971, 1974). ^c Taken from Block and Weiss (1956). ^d Values in parentheses are nitrogen content. ^e Essential amino acid N as a percent of total amino acid N.

Table II. Nitrogen Balance of Male College Students Receiving Different Amounts of Dietary N and Tryptophan^a

Period ^a	Daily intake		
	Nitrogen, g	Tryptophan, mg	N balance, g/day
Experiment 1			
A	18.13 ^b	1100	2.58 ± 0.29 ^c
B	19.59 ^d	113	0.30 ± 0.10
C	19.59 ^d	213	-0.35 ± 0.53
Experiment 2			
A	9.47	213	-0.59 ± 0.32
B	19.47 ^d	113	1.78 ± 1.00
C	19.47 ^d	213	1.38 ± 0.82
Experiment 3			
A	9.47 ^e	113	-1.62 ± 0.31
B	19.47 ^d	113	3.78 ± 1.22
C	19.47 ^d	213	1.92 ± 0.52
Experiment 4			
A	19.47 ^d	113	-1.19 ± 0.49
B	19.47 ^d	113	0.42 ± 0.10
C	19.47 ^d	213	1.17 ± 0.38
D	19.47 ^d	213	2.35 ± 0.33
Experiment 5			
A	9.47 ^e	113	0.10 ± 0.19
B	19.47 ^f	113	1.66 ± 0.44

^a Each period was of 7 days' duration. ^b Mixed foods containing high quality protein. ^c Standard errors were calculated from the average values for four to five male college students; the average value for each student represents four 24-hr collections. ^d Hydrolyzed casein, 9 g of N + gelatin, 10 g of N + miscellaneous foods, 0.47-0.59 g of N. ^e Hydrolyzed casein, 9 g of N + miscellaneous foods, 0.47 g of N. ^f Hydrolyzed casein, 9 g of N + 10 g of N from glycine, alanine, and aspartic acid + miscellaneous foods, 0.47 g of N. ^g Taken in part from and with permission of: Fisher, H., Brush, M. K., Griminger, P., Sostman, E. R., *J. Nutr.* **87**, 306 (1965).

our laboratory (Fisher *et al.*, 1969, 1971, 1974) and by others (Leverson, Swendseid, Jones, and associates as summarized by Rose, 1957). Table I also gives the essential amino acid composition of egg and wheat protein. When the *E/T* ratio is calculated for these proteins based upon those amino acids that are essential for maintenance of adult man, egg protein supplies 35% and wheat 20% of its N as essential amino acids. In marked contrast to the needs for rapid growth, the *E/T* ratio for wheat does not appear to be out of line for the maintenance needs of

adult man. The data of Bricker *et al.* (1945) for adult women support these theoretical considerations, and recently Reddy (1971) has found wheat-based diets adequate in protein also for growing children.

Mitchell (1962) has referred to the amino acid requirements of the adult in terms of a *particulate* requirement in contrast to an *aggregate* requirement for the growing animal. By particulate he means that even single amino acids, essential or nonessential, can serve a useful function in the adult whereas in the young, growing animal all essential amino acids must be present simultaneously and in the right proportions in order for utilization to take place.

An interesting example of the validity for the low *E/T* ratios based upon the Leverson and Fisher requirements has been demonstrated by Kofronyi and Jekat (1965), as indicated in Figure 1. In this study the minimum N requirement for maintaining N equilibrium was measured using egg protein variously diluted with ammonium citrate. Egg protein could be diluted about 65% before the total N requirement showed an increase. Although egg protein has been shown even for the growing rat to supply an excess of essential amino acids when fed at the requirement level for total N (Bender, 1960), the magnitude of that excess is small compared to that demonstrated here for the adult.

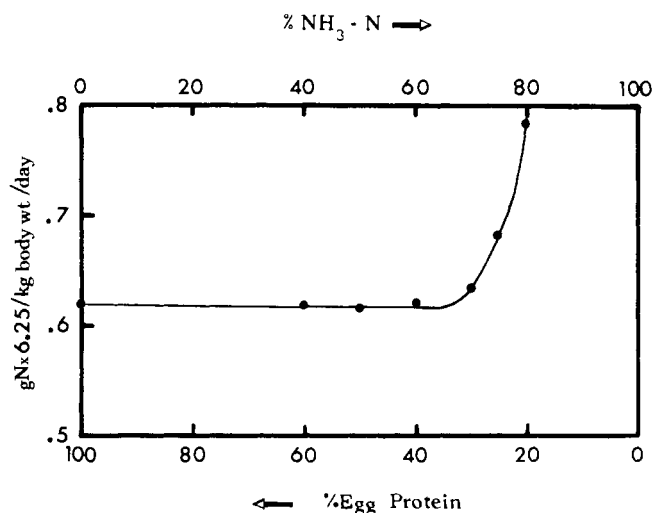


Figure 1. Change in protein requirement ($N \times 6.25$) of human subjects given different proportions of egg protein and ammonium citrate. Reprinted with permission of: Kofronyi, E., Jekat, F., *Z. Physiol. Chem.* **342**, 248 (1965).

Table III. Mean Values with Their Standard Errors for Body Weight Changes and Nitrogen Intakes and Losses of Leghorn Cocks during a 16-Day Repletion Period on Two Diets with Different Amounts and Source of Dietary Nitrogen

Measurement	Dietary nitrogen	
	Essential amino acids, 115 mg of N/kg of body weight daily (4) ^a	Essential amino acids + aspartic acid, 115 + 165 mg of N/kg of body weight daily (6) ^a
Body weight, g		
Initial	2188 ± 84	2153 ± 125
Final	1964 ± 80	2069 ± 122
Change	-224 ± 43	-84 ± 39
Nitrogen, g/bird		
Loss in excreta	4.46 ± 0.50	6.17 ± 0.32
Loss in feathers	1.86 ± 0.10	1.29 ± 0.10
In biopsy tissue ^b	0.15 ± 0.00	0.18 ± 0.01
Total loss	6.48 ± 0.50	7.64 ± 0.34
Total intake	3.42 ± 0.24	7.28 ± 0.90
Balance	-3.06 ± 0.55	-0.36 ± 0.10

^a Figures in parentheses are the numbers of birds. ^b All cocks underwent biopsy at the start of the repletion period. Reprinted with permission of: Ashley, J. H., Fisher, H., *Brit. J. Nutr.* **21**, 661 (1967).

The utilization of nonessential N by young men has been observed in our laboratory under conditions in which protein nutriture prior to the feeding of the nonessential N was inadequate. Table II summarizes a series of experiments in which adequate and inadequate levels of tryptophan were supplied followed by sharply increased N intake without improving the tryptophan supply. The results show the inverse relationship between N retention and protein nutriture prior to the ingestion of a diet high in N yet inadequate with respect to tryptophan. In experiment 2 the ingestion of an additional 10 g of N devoid of tryptophan caused a highly significant increase in N retention, which was also observed in experiments 3 and 5. In experiment 5 glycine, alanine, and aspartic acid made up the additional 10 g of N in place of gelatin, with the same high N retention as observed in experiment 2.

The considerable retention observed in these experiments is in line with animal studies that suggested that a portion of the so-called "labile N" or "protein reserve"

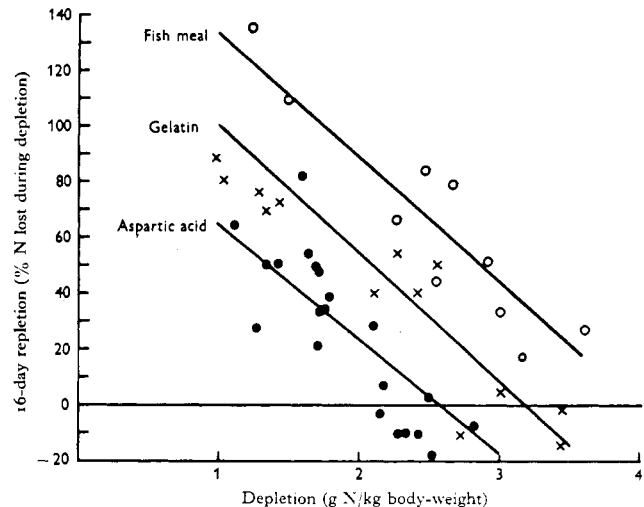


Figure 2. Nitrogen repletion as a function of prior N depletion in adult cocks fed on a maintenance mixture of amino acids and given aspartic acid, gelatin, or fish meal as the source of supplementary N. O, fish meal ($Y = 178.1 - 44.8 X$; $n = 10$); X, gelatin ($Y = 146.7 - 45.9 X$; $n = 13$); ●, aspartic acid ($Y = 104.9 - 40.8 X$; $n = 22$). Reprinted with permission of: Wessels, J. P. H., Fisher, H., *Brit. J. Nutr.* **19**, 57 (1965).

could be replenished following depletion with a nonessential N source. Wessels and Fisher (1965) depleted adult cocks of body protein to varying degrees and then repleted them for a 16-day period by giving them the maintenance needs for essential amino acids plus additional N in the form of either fish protein, gelatin, or aspartic acid. As shown in Figure 2, cocks that were depleted beyond a body N loss of 2.6 g of N/kg of body weight (equivalent to 6.9% total body N) could no longer be repleted if aspartic acid supplied the extra N above the maintenance needs. With gelatin as the supplemental N source, depletion up to a body N loss of 3.2 g of N/kg of body weight (equivalent to 8.6%) permitted repletion, whereas with fish protein no limitation occurred (as expected) in terms of the repletion potential following severe depletion.

It is interesting to note the regression lines for these three sets of repletion data. The lines appear parallel with nearly identical slopes; this permits a comparison of the point where the lines intersect the ordinate. If one subtracts the value of 100 respectively from the intersect

Table IV. Mean Muscle Nitrogen Components with Their Standard Errors of Leghorn Cocks before and after a 6-Day Protein Depletion Regimen and after a 16-Day Repletion Period with Different Amounts and Source of Dietary Nitrogen

Measurement ^{a,e}	Predepletion ^b (6) ^d	Postdepletion ^c				Postrepletion	
		(4) ^d		(6) ^d		Essential amino acids, 115 mg of N/kg of body weight daily (4) ^d	Essential amino acids + aspartic acid, 115 + 165 mg of N/kg of body weight daily (6) ^d
Muscle, dry tissue							
N, %	13.28 ± 0.08	12.94 ± 0.02	13.00 ± 0.10	12.52 ± 0.22	13.12 ± 0.07		
RNA, mg/g	1.90 ± 0.19	1.35 ± 0.10	1.25 ± 0.08	2.06 ± 0.24	2.51 ± 0.19		
DNA, mg/g	0.94 ± 0.10	0.98 ± 0.13	0.89 ± 0.09	0.95 ± 0.15	0.94 ± 0.11		
GOT, SF units × 10 ³ /g	117 ± 12	11 ± 7	25 ± 25	115 ± 8	180 ± 4		
GPT, SF units × 10 ³ /g	0.6 ± 0.2	0.6 ± 0.3	0.3 ± 0.4	0.6 ± 0.4	1.3 ± 0.4		

^a With the exception of muscle N, all analyses were carried out in duplicate on tissue from each cock; muscle N was determined in triplicate. ^b Predepletion biopsies were carried out on a separate group of cocks from those repleted on the two different diets. ^c The two subgroups under this heading represent the same birds as given for the postrepletion period. ^d Figures in parentheses are the numbers of birds. Reprinted with permission of: Ashley, J. H., Fisher, H., *Brit. J. Nutr.* **21**, 661 (1967). ^e RNA, ribonucleic acid; DNA, deoxyribonucleic acid; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; SF, Sigma-Frankel units (Sigma Chemical Co., St. Louis, Mo., 1959).

points of 104.9, 146.7, and 178.1, assuming that at 100 the cocks have been fully repleted to their starting status before N depletion began, the remainder appears to be equivalent to the biological values for the three N sources. In the case of aspartic acid this value was essentially zero, a not unexpected finding, since for tissue protein synthesis all the essential amino acids must be provided. In the case of gelatin the value (46.7) is not too different from the biological value that has been reported for the chick (Summers and Fisher, 1962) for this protein. Finally, the value of 78 for fish meal is also in line with the biological value of fish meal as obtained with the growing chick (Summers and Fisher, 1962).

Although these studies shed little light on the nature of the retained N, they do indicate that nonessential N can and does play an important role in intermediary N metabolism in the body. As a follow-up to the investigation just cited, additional adult cocks were N-depleted on a protein-free diet (Ashley and Fisher, 1967). One group was fed a maintenance mixture of essential amino acids only, which constitutes an inadequate amount of total N; a second group was given the same diet but with additional N in the form of aspartic acid to meet not only the maintenance needs for essential amino acids but also the total N needs to permit repletion. Table III shows the pertinent body weight and N loss measurements. Those cocks receiving the essential amino acids but an inadequate amount of total N were in severe negative N balance, whereas those receiving the additional aspartic acid N were in approximate N equilibrium. Table IV shows certain muscle N constituents, including RNA and transaminase enzyme concentrations. While the feeding of the essential amino acids alone repleted the RNA, GOT and GPT concentration in muscle, the further addition of as-

partic acid N to the essential amino acid mixture produced additional increases in RNA and both transaminase enzymes. This provides additional evidence for the direct utilization of nonessential N perhaps as a kind of reserve pool that may act as a buffer during periods of deprivation or inadequate protein intake. It may well be that the role played by such N sources is one of enzyme induction which may stimulate or decrease protein anabolism as well as catabolism, depending upon the dietary N intake.

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Nonessential Nitrogen Utilization by Children and Adolescents

Mary K. Korslund

Demands for growth may influence the quantity and quality of dietary protein needs. Thus, children and adolescents should be given special consideration in the discussion of utilization of nonessential nitrogen by human beings. Studies have shown, in general, that nonessential nitrogen can contribute significantly to the protein needs of children and adolescents. Nonspecific nitrogen was considered the first limiting nitrogenous factor in milk for human infants by Snyderman *et al.* (1962). In another laboratory, preadolescent girls responded as well to a supplement of nonessential nitrogen as to a mixture of essential amino acids considered the most limiting in a

diet simulating that of low-income southern families. Urea supplementation improved nitrogen retention in adolescent boys when opaque-2 corn supplied nearly the sole source of dietary protein. That nonessential nitrogen can be effectively utilized by children to meet protein needs of growth must be considered a tentative conclusion because of the dearth of studies on children. A better understanding of this aspect of human utilization of nonspecific nitrogen is needed if nonspecific nitrogen is to be considered as a potential means of expanding protein resources for human consumption.

Protein nutrition in young children is considered the major nutritional problem of the world by a joint FAO/WHO committee (1965). Protein demands during growth may be more stringent than for adult maintenance. Thus, study of human protein utilization is incomplete without

including young subjects from infancy through adolescence. Since chemical and genetic alterations of food materials are considered possible methods of improving the world's protein supply, there is a critical need for nutrition scientists to define human protein requirements as precisely as possible. More definitive information on human protein requirements throughout the life cycle is also needed for use in nutrition education programs. The role of nonspecific nitrogen in meeting human protein needs is not clear. Most of the work in this area has been

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