Effect of Quantitative Variation in Nonspecific Nitrogen Supplementation of Corn, Wheat, Rice, and Milk Diets for Adult Men[1]

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ABSTRACT

The influence of nonspecific nitrogen (NSN) additions on nitrogen (N) balance (protein nutriture) of men fed diets providing equal, but distinctly sub-optimal, amounts of food protein from several sources has been studied. The effect on N retention of ten men fed 4.0 g. N per subject per day from rice, corn, wheat, or milk plus, in each case, 0.0, 4.0, or 8.0 g. N per subject per day from an isonitrogenous mixture of glycine and diammonium citrate, was determined. Mean N balances achieved on these three levels of nonspecific supplementation with rice were −2.41, −0.78, and −0.19 g. N, respectively; with corn, −2.19, −1.00, and −0.17 g. N, respectively; with wheat, −2.38, −0.81, and −0.12 g. N, respectively; and with milk, −1.42, −0.50, and +0.66 g. N, respectively. On any given level of total N intake, significantly more N was retained when milk was the source of dietary protein than when rice, wheat, or corn served this purpose. However, the degree of N loss was significantly reduced at each level of total dietary N increase, regardless of the food source employed. Results confirm earlier findings that NSN supplementation of diets apparently has a sparing effect on protein requirements of humans.

Supplementation of milk, corn, and rice fed in slightly inadequate amounts with nonspecific nitrogen (NSN) results in increased nitrogen (N) retention by human adults (1–3) and infants (4). NSN is defined as N from any metabolically useable, nontoxic source, and may include N from protein, amino acids, and non-amino acid sources. Studies on corn protein indicate that the amount of corn protein necessary for maintenance of adequate protein nutrition of human adults can be substantially reduced when NSN supplements at high levels are used (5,6). NSN supplementation of corn diets seemingly reduces lysine requirement (first limiting amino acid in corn) and affects the order in which amino acids become second limiting (6,7). These results suggest that NSN supplementation of diets with such economic NSN sources as urea, glycine, and diammonium citrate may be used as a technique to make available protein resources to meet the protein needs of more people. However, before a generalization of this kind can be made, additional information is needed on the apparent protein-sparing effect of NSN on diets, presenting a variety of amino acid profiles.

The objective of the current study was to compare the effect on N retention of adult men by varying the level of total N intake while keeping the food protein intake at a constant, low level as provided by each of the following sources: milk, corn, rice, and wheat.

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[1] Supported by Public Health Grant number R01AM08846. Published with the approval of the Director as paper No. 2136, Journal Series, Nebraska Agricultural Experiment Station. Parts of the data in this paper pertaining to rice are taken from the dissertation submitted by Shirley Chii-Shya Chen in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE I. DIET PLAN

Period[a]	No. of Days	Dietary Protein[b] Source	Dietary Protein[b] Level g. N/day	DAC-Glycine Addition[c] g. N/day	Total N Intake[d] g. N/day
Part A					
Depletion	2	Rice	1.5	0	2.0
Adjustment	5	Varied[e]	4.0	0	4.5
Expt. 1	5	Corn	4.0	0	4.5
Expt. 2	5	Milk	4.0	0	4.5
Expt. 3	5	Wheat	4.0	0	4.5
Expt. 4	5	Rice	4.0	0	4.5
Part B					
Depletion	2	Rice	1.5	0	2.0
Adjustment	5	Varied[e]	4.0	4.0	8.5
Expt. 5	5	Corn	4.0	4.0	8.5
Expt. 6	5	Milk	4.0	4.0	8.5
Expt. 7	5	Wheat	4.0	4.0	8.5
Expt. 8	5	Rice	4.0	4.0	8.5
Part C					
Depletion	2	Rice	1.5	0	2.0
Adjustment	5	Varied[e]	4.0	8.0	12.5
Expt. 9	5	Corn	4.0	8.0	12.5
Expt. 10	5	Milk	4.0	8.0	12.5
Expt. 11	5	Wheat	4.0	8.0	12.5
Expt. 12	5	Rice	4.0	8.0	12.5

[a] Order of three sections and order of four experimental periods under each section were randomized for each subject.

[b] Dietary proteins used were characterized as follows: white, degerminated, unenriched corn meal having a N content of 0.0131 g. N per g. meal; dry, skim milk solids having a N content of 0.0542 g. N per g. solids; white, enriched wheat flour having a N content of 0.0184 g. N per g. flour; and long-grain, unenriched, polished rice having a N content of 0.010 g. N per g. rice. Caloric values of diets were maintained equal regardless of protein source through appropriate additions of corn starch.

[c] An isonitrogenous mixture of diammonium citrate and glycine was used as the source of NSN supplementation.

[d] Total N intake figures include 0.50 g. N per day provided by the basal diet. The basal diet consisted of 100 g. applesauce, 3.5 g. dry bouillon,30 g. cabbage, 100 g. peaches, 90 g. jelly, 100 g. pears, 50 g. tomato juice, 10 g. dry decaffeinated coffee. A vitamin supplement provided 5,000 I.U. of vitamin A; 500 I.U. of vitamin D; 2 mg. thiamine; 2.5 mg. riboflavin; 50 mg. ascorbic acid; 1 mg. pyridoxine; 1 γ vitamin B-12; and 20 mg. niacin. Mineral supplement provided the following (g. per subject per day): Ca, 1.00; P, 1.00; Mg, 0.199; Fe, 0.015; Cu, 0.002; I, 0.00015; Mn, 0.002; Zn, 0.0009.

[e] Source of dietary protein during adjustment periods was varied among the subjects so as to be the same as that which each received during the first experimental period of sections A, B, and C.

PROCEDURE

The experimental plan of the study is shown in Table I. The three 27-day sections which comprised the study were conducted immediately following one another. Each part was composed of an introductory 2-day N-depletion period, a 5-day N-adjustment period, and four experimental periods of 5 days each. Experimental periods of this length have been found in earlier studies to be sufficient length to give data for accurate comparative purposes (2,7). The sequence of the three sections as well as the order of the experimental periods within each section was randomized for each subject.

During the N-depletion periods in all three parts, N intake per subject per day totaled 2.0 g.; i.e., 1.5 g. N from rice and 0.5 g. N from the basal diet as described in Table I. Inclusion of an introductory period of low N intake has been found to speed the adjustment of subjects to later experimental diets[2].

During the adjustment period and experimental periods of Parts A, B, and C, N intake from food protein was maintained constant at 4.5 g. N per day–4.0 g. from the test food protein plus 0.5 g. from the basal diet. Dry skim milk solids, white degerminated corn meal, enriched white wheat flour, or unenriched polished rice was used as the chief dietary protein source during the adjustment period and four experimental periods of each part. An isonitrogenous mixture of glycine and diammonium citrate was used as a NSN supplement to provide 0.0, 4.0, and 8.0 g. N per subject per day during the adjustment and experimental periods of Parts A, B, and C, respectively. Thus, total N intake was varied from 4.5, to 8.5, to 12.5 g. N per day during Parts A, B, and C; but intake of food protein was maintained constant. A N-adjustment period was included in each part to allow time for subjects to reach a stabilized N output on the particular N-intake level involved before imposing the experimental variables.

Diets used in all parts of the study are described in Table I. Caloric intake for each subject was maintained constant at the level required for approximate weight maintenance (Table II) by varying the intake of corn starch and fat among the subjects; however, in all cases, fat provided 20% of the calories from either corn oil (subjects 91–95) or butter oil (subjects 96–100). Handbook values were used in determining amino acid intake provided by the four test protein resources (8) as shown in Table III.

Daily allotments of dry skim milk solids, wheat flour, or corn meal (as determined by the experimental design) were mixed with varying amounts of corn starch so that 2,000 kcal. was provided from these sources. These were then mixed

TABLE II. AGE, HEIGHT, WEIGHT, AND CALORIC INTAKE OF SUBJECTS

Subject Number	Age yrs.	Height cm.	Weight Beginning kg.	Weight Final kg.	Calories kcal./kg./body wt./day
91	22	180	77.6	77.6	35
92	23	183	75.3	74.4	36
93	29	185	74.4	73.9	36
94	22	183	73.0	73.5	37
95	30	178	68.9	68.0	51
96	27	178	84.4	85.3	32
97	22	178	73.5	69.9	48
98	29	180	69.9	69.4	50
99	22	180	81.6	82.1	33
100	23	173	65.3	64.9	41

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[2] Kies, Constance V., and Fox, Hazel Metz. Effect of "protein depletion" of humans on adjustment to low protein diets. (Abstr.) Fed. Proc. 29: 819 (1970).

TABLE III. ESSENTIAL AMINO ACIDS PROVIDED BY TEST FOODS

Essential Amino Acid	Minimum Requirement[a] g. AA/day	g. Amino Acids Provided by 4.0 g. N from[b]			
		Milk	Rice	Wheat	Corn
Tryptophan	0.25	0.360	0.256	0.280	0.152
Threonine	0.50	1.176	0.932	0.656	0.996
Isoleucine	0.70	1.628	1.116	1.048	1.156
Leucine	1.10	2.504	2.052	1.756	3.240
Lysine	0.80	1.984	0.940	0.520	0.720
Total sulfur-containing	1.01	0.852	0.752	0.756	0.788
Phenylalanine	1.10	1.236	1.196	1.252	1.136
Valine	0.80	1.752	1.664	0.984	1.276

[a] Minimum daily requirements of essential amino acids for young men as estimated by Rose (16).

[b] Estimated intake of essential amino acids as provided by the test protein sources. Values based on figures published by Orr and Watt (8).

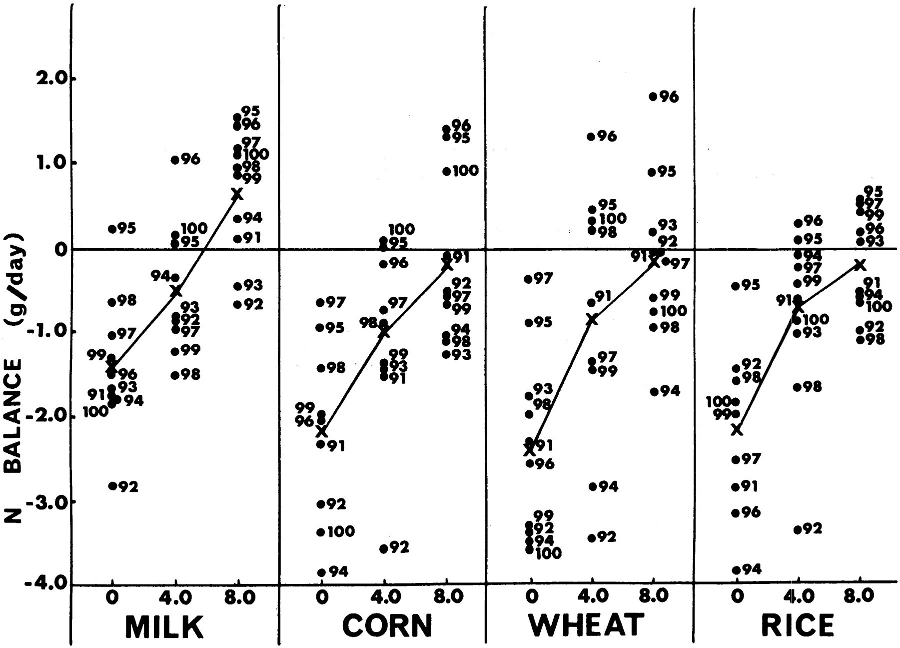

Fig. 1. Effect of diammonium citrate-glycine N additions (0.0, 4.0, or 8.0 g. N per subject per day) of diets containing 4.0 g. N from corn, wheat, rice, or milk on N balances of adult men. Dots represent N balances of individual subjects, and crosses represent mean N balances of all subjects on each dietary variation.

with a calcium phosphate baking powder, fat, mineral supplement, mucilose flakes, sodium chloride, and water by a basic muffin method. The batches were divided into thirds by weight for consumption at each of the three daily meals, and baked as drop biscuits. Rice was prepared by steaming, with the other items mentioned above being added to the diet via corn-starch wafers.

Glycine-diammonium citrate mixtures, when used, were given to the subjects in water solutions at each of the three meals in equal amounts.

A nonprotein, low-calorie carbonated beverage was allowed *ad libitum*. Other diet items, including vitamin and mineral supplements, were as described in Table I.

Details regarding the ten men, inmates of the Nebraska Penal and Correctional Complex who were subjects for the study, are listed in Table II. Institutional health records, as well as physical examinations conducted at the beginning and end of the project together with observations made by the institution's physician, indicated that all were in good health. Subjects were housed and ate meals together in a separate part of the institution; however, usual work assignments were maintained.

The N-balance technique was used as the principal method of evaluation. Urine, feces, and food were collected and prepared for analysis as previously described (9) and were analyzed for N content by the boric acid modification of the Kjeldahl method (10). Urine samples were preserved under toluene and were analyzed daily for 24-hr. excretion of N and creatinine (11). Daily fecal values were based on 5-day fecal composites. Statistical analyses of data, including analyses of variance and Duncan's Multiple Range Test (N-balance data), were done by the College of Agriculture Statistical Laboratory, University of Nebraska.

RESULTS

Individual mean N balances of the subjects achieved while receiving the various dietary treatments are shown in Fig. 1. N excretion partition data are given in Table

TABLE IV. NITROGEN EXCRETION PARTITION

	Mean Nitrogen Excretion (g. N/day)[b]	
Diet Type[a]	Urine	Feces
4.5 g. N intake/day		
Milk	5.34	0.58
Wheat	5.89	0.99
Rice	6.12	0.79
Corn	5.56	1.13
8.5 g. N intake/day		
Milk	7.80	1.20
Wheat	7.94	1.37
Rice	7.94	1.34
Corn	8.18	1.32
12.5 g. N intake/day		
Milk	10.50	1.34
Wheat	11.14	1.48
Rice	11.21	1.41
Corn	11.14	1.53

[a] Diets provided 4.0 g. N per subject per day from test protein; 0.5 g. N from basal diet; and 0, 4.0, or 8.0 g. N per day from glycine and diammonium citrate. Details given in Table I.

[b] Means based on 5-day excretion data from ten subjects for each period.

IV. Mean N balances of all subjects while receiving 4.0 g. N from milk plus 0.0, 4.0, or 8.0 g. N from glycine and diammonium citrate were -1.42, -0.50, and +0.66 g. N, respectively. On the same levels of NSN supplementation, mean N balances achieved on the rice diet were -2.41, -0.78, and -0.19 g. N, respectively; on the wheat diet, -2.38, -0.81, and -0.12 g. N, respectively; and on the corn diet, -2.19, -1.00, and -0.17 g. N, respectively. Analyses of variance indicated differences in data owing to treatment (>1%).

On any level of total N intake, significantly less N was lost when milk was the source of dietary protein than when wheat, rice, or corn served this purpose (Duncan's Multiple Range Test). However, the degree of N loss was significantly reduced at each level of total N increase regardless of the food source employed (Duncan's Multiple Range Test).

DISCUSSION

The failure of the unsupplemented milk, rice, corn, and wheat diets to support N equilibrium of adult men in the current study indicates that the protein intake supplied at the 4.0 g. N level from any of these sources is inadequate in one or more respects to meet the protein requirements of these individuals. Further evidence indicating probable protein insufficiencies in the above-mentioned dietaries is shown by the results of other N-balance studies in which higher minimal requirements for these proteins were found (1,3,12-14).

Assuming that body-N loss is proportional to degree of protein inadequacy and that reduction in N loss parallels improvement in protein source, the N-balance changes in the present study demonstrated that glycine and diammonium citrate compensated almost completely for the nitrogenous deficits in milk, and partially for those in the cereal proteins.

Earlier studies from this and other laboratories indicate that the negative balances observed in human subjects fed slightly inadequate amounts of corn or milk protein can be reversed by supplementation with such N sources as urea, glycine, and diammonium citrate (1-4). However, in these cases, the apparent essential amino acid intake as furnished by the dietary-protein sources was adequate or near-adequate as judged by the accepted standards of essential amino acid requirements (15). Comparison of the calculated intakes of the essential amino acids as supplied by 4.0 g. N from milk, corn, rice, and wheat (8) with the minimum requirements of the essential amino acids as determined by Rose (16) indicates quantitative deficits in one or more of the essential amino acids as shown in Table III. The following amino acids were supplied in less than listed requirement amounts: the sulfur-containing amino acids in all diets; lysine and the sulfur-containing amino acids in the case of the wheat diets; and tryptophan, lysine, and the sulfur-containing amino acids in the corn diets. Several previous studies indicate that improvement in N retention of human subjects fed similar amounts of these proteins can be obtained through purified amino acid supplementation (3,12,17-22). This further indicates quantitative essential amino acid inadequacies of the forementioned diets. Since NSN supplementation also resulted in increases in N retention, it seems possible that either the essential amino acid requirements of the subjects were lowered or the available essential amino acids were better utilized at the high levels of total N intake.

Much of the human work on minimum protein and essential amino acid requirements has been performed under conditions of variable total N intake (16,23,24). However, the results of the current study suggest that quantitative essential amino acid requirements are affected by the total level of N intake. Of course, while the N retention of subjects was significantly increased by NSN supplementation of the test diets in the current study, the possibility exists that this assumed improvement was statistically significant while not being biologically significant. In application of the N-balance technique to adult humans, there is a greater chance of over- rather than underestimating N intake and of under- rather than overestimating N excretion. These errors are additive and tend to overestimate the degree of N retention. Even so, additional investigation of essential amino acid requirement patterns as well as minimum protein requirements of humans under controlled conditions of both high and low total N intake seems indicated.

Acknowledgments

The authors wish to thank and to acknowledge the cooperation of Warden Maurice Sigler, Deputy Warden John B. Greenholtz, Associate Warden Ed Scarborough, Medical Officer Robert Moore, M.D., the staff of the Nebraska Penal and Correctional Complex–Reformatory Unit, and, particularly, the ten inmates who were subjects.

The technical assistance of the following individuals is also acknowledged: Mrs. Martha Dennis, Miss Ruth Diedrichsen, Mrs. Elsie Bishop, Mrs. Margie Kunzman, Mrs. Jean Lee, Mrs. Frances Mayfield, and Mrs. Elinor Kerrey.

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[Received June 24, 1970. Accepted July 17, 1971]