

## Ultra-Rapid Nitrogen Balances in Man<sup>1</sup> (35685)

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The dietary nitrogen balance method, (*i.e.*, determining the difference between intake and excretion of food nitrogen) has long been used to determine protein nutritional status and utilization of dietary protein in man. When the nitrogen source is given orally, digestability and absorption need to be considered and fecal excretion must be measured. To do this accurately, long balance periods (*e.g.*, days and weeks of study) are commonly used.

When the nitrogen source is given intravenously, questions of digestability and absorption are obviated. Moreover, fecal nitrogen excretion is minimal and can be neglected unless there is diarrhea or other gastrointestinal disease. In these circumstances, urinary nitrogen essentially comprises total nitrogen excretion and nitrogen balance becomes  $B = I - U$ , where "I" is amount of nitrogen infused and "U" is nitrogen excreted in urine.

We, therefore, report here ultrarapid urine nitrogen excretion and balances during intravenous administration of glucose (G) and glucose-amino acid (GAA) mixtures to healthy participants.

**Methods.** Healthy male volunteers aged 23 to 26 years old and consuming a mixed, nutritionally adequate diet were studied on a metabolic ward. Their history, a physical examination, hematogram, urinalysis, and other laboratory tests confirmed their health. These participants were studied after an overnight fast and thereafter took no food by

mouth except for noncaloric liquids *ad libitum*. All calories, electrolytes, and nitrogen were supplied intravenously.

Calories were supplied as 5 or 10% dextrose in water. The amount of calories was varied from 25 to 100 kcal/hr in different studies. Nitrogen was supplied as a 5% solution of fibrin hydrolysate in 5% dextrose in water (Aminosol).<sup>2</sup> The amount of nitrogen infused was kept constant at 840 mg of nitrogen/hr except in one study where twice this amount was infused hourly. The solutions of dextrose in water and the mixture of amino acids and dextrose were alternated consecutively in different studies at 8-, 4-, 2-, and 1-hr intervals over total infusion periods of from 8 to 48 hr. Urine collections were made by voluntary voiding in periods corresponding to infusion periods. Great care was taken to void with pressure at the end of each time period to empty the bladder.

Total nitrogen was determined by a modified Micro-Kjeldahl method (1). Free  $\alpha$ -amino nitrogen was determined by the colorimetric ninhydrin method, after extraction of ammonia (2).

**Results.** Eight subjects participated in 20 studies. During fasting all participants went immediately into negative nitrogen balance as apparent during the first timed collection period. Two participants fasting completely for a 48-hr period excreted 26.0 and 32.8 g of nitrogen, respectively. When 600 kcal/24 hr were supplied as a constant infusion of glucose, urine nitrogen excretion diminished by 34%. Increasing the glucose calories to 1200 and 2400 kcal/day did not further reduce significantly the urine nitrogen excretion.

The infusion of the amino acid mixture at the rate of 840 mg of nitrogen per hr (10–20

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mg N/kg/hr) to a fasting participant resulted in the nitrogen balance approaching equilibrium or, more commonly, clearly reversing from negative to positive balance. For example, a participant excreted 32 g of nitrogen during a 2-day complete fast. Given 1200 kcal/24 hr as a constant infusion of glucose, urine nitrogen was reduced to 20 g for the 2-day study. However, during a 24-hr constant infusion of amino acids, the urine nitrogen excretion was 15.3 g, and the overall nitrogen balance for 24 hr was a positive 4.8 g of nitrogen.

In two studies when glucose (25 kcal/hr) and the amino acid mixture (840 mg N/hr) were infused in consecutive alternating 8-hr periods over 2 days, nitrogen balance was strongly negative during each glucose infusion period and came into equilibrium or became positive during periods of amino acid infusion. When the alternating periods of infusion lasted 4 hr (one study during 24 hr) or 2 hr (two studies during 12 hr) or 1 hr (four studies during 8 hr) the patterns shown in Figs. 1 and 2 of negative nitrogen balance during glucose infusion and of reversals towards or achievement of positive nitrogen balance during amino acid infusions were evident in all studies.

As can be seen in Table III, urine nitrogen excretion during periods of amino acid infu-

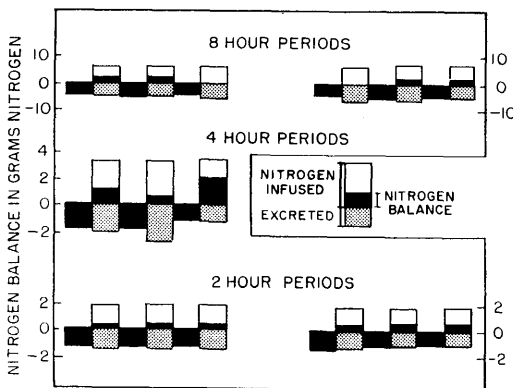


FIG. 1. Nitrogen balances in five studies made in healthy participants during 8, 4, or 2 hourly alternating infusions of glucose (odd periods), and infusions of amino acids and glucose (even periods). Bars above the zero line represent nitrogen infused. Bars below the zero line represent nitrogen excreted. During infusions of glucose alone, nitrogen excretion equals the nitrogen balance.

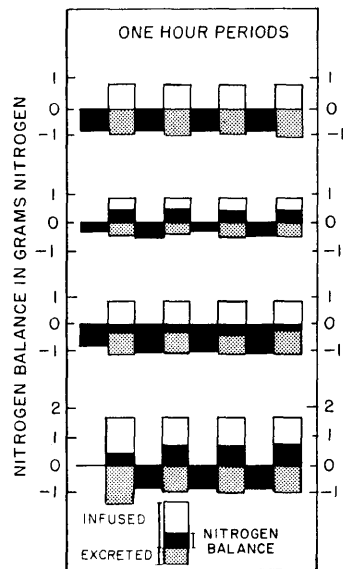


FIG. 2. Nitrogen balances in four studies made in healthy participants during hourly alternating infusions of glucose (odd periods), and infusions of mixtures of amino acids and glucose (even periods). Bars above the zero line represent nitrogen infused. Bars below the zero line represent nitrogen excreted. During infusions of glucose alone, nitrogen excretion equals the nitrogen balance.

sion were from 15.8 to 38.4% greater than nitrogen excretion during glucose periods. For example, during 8-hr infusion periods in W.J. (Table III), mean nitrogen excretion during glucose infusion periods was 4.90 g; mean nitrogen excretion during amino acid infusion periods was 6.13 g, an increase of 25.1% in nitrogen excretion during amino acid periods. The increase of 1.23 g of nitrogen excreted during the GAA periods represented 18.4% of the nitrogen infused during the GAA period.

In several studies, the urine nitrogen excretion during glucose infusion periods following an amino acid infusion was often less than the nitrogen excreted prior to giving amino acids (as seen in Figs. 1 and 2).

Urine-free  $\alpha$ -amino nitrogen excretion was also measured. During amino acid infusion periods, it was significantly greater than during glucose infusion periods as seen in Tables I and II. However, the absolute amounts excreted in the urine were very small in comparison to the amount infused so that free



TABLE II. Nitrogen and Free Alpha-Amino Nitrogen Intake, Excretion and Balance During Alternating Infusion of Glucose and Glucose-Amino Acid Mixtures.

Infusion Content: glucose (G), glucose- amino acid (GAA)	Subject: Infusion period (hr)		R.S.		T.C.		C.F.		G.M.			
	Intake	Excre- tion	Bal- ance	Intake	Excre- tion	Bal- ance	Intake	Excre- tion	Bal- ance	Intake	Excre- tion	Bal- ance
G	0.0	0.80	-	0.0	0.36	-0.36	0.0	0.75	-	0.00	- <sup>a</sup>	- <sup>a</sup>
GAA	0.84	0.87	-	0.84	0.51	+0.33	0.84	1.15	-	1.68	1.30	+ 0.38
G	0.0	0.80	-	0.0	0.38	-0.38	0.0	1.05	-	0.0	0.83	- 0.83
GAA	0.84	0.94	-	0.84	0.51	+0.33	0.84	1.06	-	1.68	0.97	+ 0.71
G	0.0	0.80	-	0.0	0.34	-0.34	0.0	0.92	-	0.0	0.86	- 0.86
GAA	0.84	0.88	-	0.84	0.55	+0.29	0.84	1.17	-	1.68	1.02	+ 0.66
G	0.0	0.76	-	0.0	0.48	-0.48	0.0	0.96	-	0.0	0.80	- 0.80
GAA	0.84	0.97	-	0.84	0.48	+0.36	0.84	1.04	-	1.68	0.92	+ 0.76
Amino N (mg)												
G	0	12	- 12	0	6	- 6	0	- <sup>a</sup>	- <sup>a</sup>	0	- <sup>a</sup>	- <sup>a</sup>
GAA	460	16	+444	460	11	+ 449	460	49	+411	920	85	+835
G	0	16	- 16	0	14	- 14	0	92	- 92	0	70	- 70
GAA	460	18	+442	460	13	+ 447	460	111	+349	920	102	+818
G	0	16	- 16	0	10	- 10	0	30	- 20	0	105	-105
GAA	460	19	+441	460	19	+ 441	460	- <sup>a</sup>	- <sup>a</sup>	920	127	+793
G	0	15	- 15	0	14	- 14	0	143	-143	0	58	- 58
GAA	460	23	+437	460	18	+ 442	460	108	+352	920	- <sup>a</sup>	- <sup>a</sup>

<sup>a</sup> Specimen lost.

TABLE III. Derived Data on Six Subjects in Nine Studies Receiving Alternating Infusions of Glucose and Glucose-Amino Acid Mixtures.

	H.S. <sup>a</sup> (8)	W.J. (8)	T.C. (4)	R.S. (2)	T.C. (2)	R.S. (1)	T.C. (1)	C.F. (1)	G.M. (1)
Mean N excreted during G infusion (g)	4.33 ± 0.58	4.90 ± 0.36	1.56 ± 0.26	1.17 ± 0.05	1.20 ± 0.16	0.79 ± 0.02	0.39 ± 0.05	0.92 ± 0.11	0.83 ± 0.02
Mean N excreted during GAA infusion (g)	5.50 ± 0.92	6.13 ± 0.77	2.16 ± 0.53	1.40 ± 0.00	1.20 ± 0.08	0.92 ± 0.04	0.51 ± 0.02	1.11 ± 0.06	0.97 ± 0.04
Difference mean N excreted (GAA-G periods) (g)	1.17	1.23	0.60	0.34	0.00	0.125	0.124	0.180	0.140
Increase N excreted during GAA infusion (%)	27.0	25.1	38.4	20.7	0.00	15.8	31.8	19.6	16.9
Percentage of infused N excreted during GAA infusion	17.5	18.4	17.9	20.2	0.00	14.9	14.8	21.4	8.3
Mean amino-N excreted during G infusion (mg)	220 ± 106	181 ± 49	36 ± 11	33 ± 2.9	33 ± 1.9	15 ± 1.6	11 ± 3.3	88 ± 46	77 ± 20
Mean amino-N excreted during GAA infusion (mg)	290 ± 80	317 ± 26	89 ± 23	41 ± 1.7	48 ± 1.4	19 ± 2.5	15 ± 3.3	89 ± 29	105 ± 17
Difference amino N excreted (GAA-G periods) (mg)	70	136	53	8	15	4	4	1	28
Increased amino N excreted during GAA infusions (%)	31.8	75.1	147.2	24.2	45.5	26.7	36.4	1.13	36.4
Percentage of infused amino N excreted during GAA infusion	1.89	3.67	2.88	0.86	1.63	0.86	0.86	0.21	3.04

<sup>a</sup> Subject initials with duration of infusion period in parentheses.

$\alpha$ -amino nitrogen loss per period was always less than 4% of the amount of  $\alpha$ -amino nitrogen infused in any one of these studies.

*Discussion.* Thus far there has not been available a method which could be used to rapidly evaluate amino acid and protein requirements in man or to determine the efficiency of various amino acids in diets to maintain an adequate nutritional state in health and disease. The understanding of protein metabolism and the unique position of essential amino acids has been largely the result of long-term classical oral nitrogen balance studies (3-5). Traditionally, however, such balance studies were laborious, difficult, and expensive to carry out as they took days to weeks in the hospital for completion. In recent years, there has been a reemergence of enthusiasm for provision of essential nutrients by parenteral administration to patients who are unable to maintain an adequate nutritional state by the normal route. Dudrick (6) and others (7, 8) have demonstrated that some intravenous amino acid mixtures can maintain a positive nitrogen balance in such patients for a long period of time.

Our results confirm these observations and further show that when amino acid-glucose mixtures are given intravenously to healthy participants one can detect changes in nitrogen balance in as short as 1 hr.

Eckhardt *et al.* (9, 10) and Doolan *et al.* (11) have previously shown that very little free amino acid is recoverable in urine following either a rapid infusion of amino acids or a constant infusion of amino acids. In acute amino acid load studies, Eckhardt *et al.* (10) showed that of the small amount of amino acids lost from the body and recovered in the urine, virtually all appeared within 4 hr, with more than 90% of this recoverable within the first postinfusion hour. There was no delayed excretion of amino acids. In other studies with patients given 50 to 100 g of amino acids intravenously daily for several days, they showed there was no cumulative increase in urinary amino acid excretion (9). The excretion of nitrogenous end products of amino acid metabolism have similarly been shown to occur rapidly following amino acid infusions. In studies on dogs given a rapid

amino acid infusion, Allison (12) has shown that urinary ammonia and urea excretion increase abruptly, but are back to basal levels within 4 hr.

In the studies reported here, urinary total nitrogen and free  $\alpha$ -amino nitrogen excretion were increased during periods of amino acid infusion. With constant infusion of the amino acid mixture there was no evidence of significant delayed excretion of nitrogenous end products. In several studies, the nitrogen excreted was less in the period following amino acid infusion than in the initial timed period when glucose alone was infused.

It is unlikely that the infused amino acids are being lost into the gastrointestinal tract. The amino acid content of fasting succus entericus is minimal (13). The transport of amino acids across the gut wall favors absorption by greater than 90 to 1, so that only a negligible amount of amino acids could be sequestered in the gut lumen.

Whether or not the positive nitrogen balances evident in these studies during short infusion periods of amino acids are correlated with incorporation of the amino acids into tissue and plasma proteins remains to be determined by radioactively labelled amino acid studies. Data from isolated perfused liver studies and *in vivo* animal studies, however, indicate that incorporation of amino acids into proteins does indeed occur very rapidly, measurable within 20 min (14, 15).

The technique of ultrarapid nitrogen balance studies with intravenous administration of amino acid mixtures would appear to have wide application in the study of utilization of various amino acid mixtures, the effects of drugs and hormones thereon, the study of amino acid requirements in health and disease and in the study of mechanisms which control amino acid metabolism.

*Summary.* Fasting healthy participants were in negative nitrogen balance when given glucose calories alone intravenously. Reversal of nitrogen balance from negative to positive occurred during the intravenous administration of an amino acid-glucose mixture. During alternating infusions of glucose and amino acid-glucose mixtures to fasting healthy participants, the pattern of negative ni-

trogen balance during glucose infusions alone and positive nitrogen balance during amino acid-glucose infusions remained evident as infusion periods varied from 8 to 4 to 2 to 1 hr.

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