Cysteine supplementation to cysteine-free intravenous feeding regimens in newborn infants[1-3]

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ABSTRACT To determine if cysteine is an essential amino acid for the intravenously fed newborn infant, growth parameters, nitrogen balance, plasma sulfur amino acid levels, and urinary amino acid excretion of premature and term infants were measured in the presence or absence of infused cysteine. Control intravenous formulations provided amino acids, including adequate methionine, carbohydrate, lipid, minerals, vitamins, and trace elements to all infants. Group and pair-matched comparisons showed that nitrogen retention, weight change, and growth in length and head circumference were not affected by cysteine supplementation of 77 mg/kg/24 h. The failure of cysteine supplementation to alter nitrogen retention was independent of postnatal age or gestational age. Plasma ½ cystine concentration was increased by 60% in the supplemented group with a concomitant 3-fold increase in urinary excretion of ½ cystine and taurine, but not of urinary methionine or cystathionine. Cysteine-supplemented infants exhibited a small increase in 3-methylhistidine excretion compared to pair-matched controls, suggesting that either an increase in muscle protein catabolism or an increase in muscle mass may have occurred. *Am. J. Clin. Nutr.* 34: 914–923, 1981.

KEY WORDS cysteine supplementation, intravenous feeding, newborn infants, nitrogen balance, amino acid levels, 3-methylhistidine excretion

Introduction

Newborn infants, both full term and premature, are routinely fed via the oral route unless prohibited by grave medical or surgical emergencies. When intravenous nutrition is provided, the goal is to provide the appropriate amount and quality of nutrients to allow for optimal extrauterine growth and development. Although reasonable success has been achieved with parenteral nutrition support for newborns, it is recognized that the nutrient formulations available, i.e., amino acid mixtures (1, 2), lipid emulsions (3), and vitamin mixtures (4, 5) may be more suitable for meeting nutritional requirements of the adult or growing child than of the newborn, particularly the premature newborn. In this study we have examined the newborn's requirement for exogenous cysteine and hence the appropriateness of adding cysteine to parenteral amino acid formulations. All of the presently available intravenous amino acid formulations for pediatric use contain a mixture of nonessential and essential amino acids, yet only one manufactured product contains cysteine in significant quantity (Vamin, Pharmacia, Montreal, Quebec). The absence of cysteine in crystalline amino acid formulations (1, 14) or protein hydrolysates (2, 6) is primarily due to the poor solubility of cystine, the oxidized form of cysteine (7).

In older children and adults who are able to convert methionine to cysteine via the enzyme cystathionase, cysteine is appropriately called "nonessential", meaning that it can be endogenously produced from methi-

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onine (8). However, in the fetus and newborn human infant, reports that hepatic cystathionase, the enzyme that normally converts methionine to cysteine, was absent or in low concentration (9, 10) has led to a general acceptance that cysteine must be exogenously supplied and therefore is an "essential" amino acid. Cystathionase activity, however, increases with postnatal and possibly gestational age (11). Since the rate of maturation of this enzyme system is unknown in the human infant, the age at which methionine conversion to cysteine becomes adequate also remains unknown. From the pioneering work done by Rose in the 1940s to determine amino acid requirements in adults, and by Snyderman studying children, it has been established that abnormalities in growth and decreased retention of nitrogen occur when essential amino acids are excluded from the diet (12). Thus it would be predicted that if cysteine cannot be endogenously produced from methionine in newborn and premature infants, considerable improvement in nitrogen retention and growth would result if cysteine were added to intravenous amino acid mixtures. Based on this prediction, but not on clinical reports of its efficacy, cysteine is currently made available as an additive by one pharmaceutical manufacturer.

The purpose of the present study was to examine the hypothesis that cysteine is an essential amino acid for the intravenously fed newborn. A cysteine-free, methionine-adequate intravenous amino acid mixture was supplemented with cysteine, and growth, nitrogen retention, plasma amino acids, and urinary 3-methylhistidine responses measured in premature and term infants.

Methods

Subjects and experimental design

Twenty-eight infants were studied for a total of thirty-six 6-day feeding periods. Seventeen of the infants were premature with a mean gestational age of 31 wk (range 25 to 36) and 11 were full term. Gestational age was calculated from the mother's last menstrual period and by Dubowitz scoring (13). The average postnatal age at the beginning of the study was similar (range 2 to 46 days) in both groups. All infants were appropriate size for gestional age. The clinical diagnosis and description of the infants is shown in **Table 1**. All of the infants received total intravenous nutrition for at least 6 days because of medically or surgically related conditions which prevented oral feeding. The decision to start total parenteral nutrition (TPN) was made by the clinician on the ward independent of the research group. Infants receiving either plasma or blood transfusions were excluded from the study.

TABLE 1
Subject description*

	Cysteine supplemented† (n = 18)	Control‡ (n = 18)
Birth wt (g)	2231 ± 391	2141 ± 357
Gestational age (wk)	34.4 ± 2.2	34.2 ± 2.0
Postnatal age (days)	13.7 ± 5.5	15.0 ± 5.6
Diagnosis		
Necrotizing enterocolitis	8	9
Intestinal atresia	3	5
Diaphragmatic hernia	1	0
Tracheoesophageal atresia	2	0
Bowel perforation	2	0
Gastroschisis	2	4

* All values are expressed as mean ± SE.
† Supplemented with an average of 78 ± 2 mg/kg/day cysteine.
‡ No cysteine supplement. There is no cysteine in the control amino acid formulation (Aminosyn).

Infants chosen for the study were alternately assigned into a control or an experimental group (Table 1). Infants in both groups received a similar control formulation free of cysteine which consisted of a crystalline L-amino acid mixture (Aminosyn, Abbott Laboratories, Montreal, Quebec) in 10% dextrose and water, maintenance fluid and electrolytes (14), in addition to vitamins (2.5 ml of MVI, Arlington Laboratories, Montreal, Quebec alternating daily with 2.5 ml of Folbesyn, Lederle Products, Montreal, Quebec), minerals, and trace elements. This formulation was planned to provide an average fluid intake of 150 ml/kg/24 h which included 480 mg of nitrogen and 15 g of carbohydrate per kg/24 h. Additional energy was provided as lipid (Nutralipid, Pharmacia, Montreal, Quebec) at 2.5 g/kg/24 h with a planned total energy intake of 90 kcal/kg/24 h. Infusion was via a peripheral vein using a continous flow infusion pump. The experimental group received, in addition, a cysteine hydrochloride supplement (L-cysteine hydrochloride, 76.8% cysteine, Abbott Laboratories) of 77 mg of cysteine/kg/24 h. The control group did not receive a cysteine supplement, and therefore had zero cysteine intake. Informed parental consent was received before administering the cysteine supplement.

Within each group, eighteen 6-day study periods were completed. The first 3 days of each study period were considered to be a period of adaptation (1, 15). During the last 3 days, urine was collected and pooled along with any stool, nasogastric or other drainage (excluding dermal losses) for analysis of total nitrogen by the micro-Kjeldahl method (16). Detailed intake records were collected for the study period thereby allowing for the calculation of nitrogen retention. N intake was calculated on the basis of each 100 g of Aminosyn containing 15.84% nitrogen. During the study period routine hematological, biochemical, and clinical monitoring was

maintained as necessary. Infants were weighed and measured for length and head circumference immediately before entry into the study and at the end of the study period. Blood samples (300 μl) from heel puncture sites or a peripheral vein were obtained on the 6th day between 0900 to 1100 in order to negate the effects of diurnal variation on plasma amino acid levels.

Laboratory procedures

Each blood sample was immediately centrifuged (5 min at 8000 × *g*), the plasma decanted and immediately added in a 1:3 dilution to 10% sulfosalicylic acid. After centrifugation (5 min at 8000 × *g*) the supernatant was carefully drawn off and immediately frozen at −70°C to prevent alterations in cysteine content (17). At a later date the equivalent of 125 μl of plasma was applied to the column of a Beckman amino acid analyzer (Beckman Spinco amino acid analyzer, model 116/119).

Pooled urine was prepared for amino acid analysis by centrifugation (15 min at 3000 × *g*) in a 1:1 dilution with 15% sulfosalicylic acid. After centrifugation, the equivalent of 250 μl of urine was applied to the column or the supernatant was stored frozen at −20°C. Amino acids in plasma and urine were separated using a single column system with lithium citrate buffers. Amino acids were eluted off a UR-30 resin (59 cm) with lithium citrate buffers. The initial buffer (pH 2.77, 0.3 N) was changed for the second (pH 2.85, 0.3 N) 90 min after sample infusion. The second buffer was changed for a third (pH 4.13, 1.2 N) 250 min after the beginning of the run. The third buffer was changed for lithium hydroxide (0.3 N) 560 min after the beginning of the run for regeneration of the column. The total time required for a complete run, including regeneration was 610 min. The initial temperature of the column was 38°C, but beginning after 225 min the temperature was increased to 65°C for the remainder of the run. In this system, cysteine elutes between glutamine and proline, and is separate from ½ cystine.

Aliquots of pooled urine were also analyzed for 3-methylhistidine. Samples were made alkaline by adding saturated NaOH to pH 11 to 12, freeze dried, and placed in a dessicator with a beaker of concentration H_2SO_4 to decrease further sample ammonia concentration. Acid hydrolysis was not performed because six duplicate analysis of urine samples with and without acid hydrolysis showed no increase in total 3-methylhistidine, confirming that most 3-methylhistidine in human urine is in its free form, not peptide bound. The dried sample was then reconstituted to its original volume with 7% sulfosalicyclic acid. After centrifugation (15 min at 3000 × *g*) the superantant was decanted and the equivalent of 250μl of urine was applied to the column of an automated amino acid acid analyzer (Beckman Spinco amino acid analyzer, model 120 C, Beckman Instruments, Inc., Palo Alto, CA). 3-Methylhistidine was eluted off a PA-35 resin (21 cm) with sodium citrate buffer (pH 4.2, 0.3 N) at a constant temperature of 45°C. The total time required for the run, including regeneration time, was 235 min.

At various times during the infusion, samples of infusate were drawn off and analyzed for cysteine (18).

Calculation of cysteine intake

In order to investigate cysteine as an essential amino acid, the concentration of cysteine present in mature pooled human milk and milk from mothers giving birth prematurely was used as the reference. Pooled mature human milk contains 1.1 g of protein/dl and 20 mg of cysteine per g of protein (19). Assuming 2.2 g of protein/kg/day an infant receiving 200 ml/kg of pooled mature breast milk would receive 40 mg of cysteine/kg/day. Alternatively, the calculation could have been based on milk produced by the mother of a premature infant during the 1st postnatal month. This milk contains a mean concentration of 1.9 g of protein/dl (20). Assuming 20 mg of cysteine per g of protein and an intake of 3.8 g of protein/kg/day, which is possible if the infant tolerates 200 ml/kg, a premature infant fed his own mother's milk would receive 76 mg of cystiene/kg/day. Therefore, in the present study an amount equal to this figure was provided. In order to confirm that the infants were receiving the planned intake of cysteine, random samples of infusate were analyzed for total cysteine content. The cysteine HCl supplement was originally added to the amino acid formulation under a sterile laminar air flow hood in the hospital pharmacy and was given an expiry time of 72 h after which time the bag was discarded. Analysis of random samples of infusate at 24 and 72 h and one sample 2 months after the time of addition revealed no change in total cysteine content. The supplemented group of infants therefore received the planned intake of cysteine.

Statistical analysis

The outcomes compared between the two groups included change in weight and height, nitrogen retention, 3-methylhistidine excretion, and plasma and urine sulfur amino acid levels. In addition, the effect of gestational and postnatal age on nitrogen retention were determined. The data were evaluated for significance by the Student's *t* test for paired and nonpaired data. Regression analysis was also used where appropriate (21, 22).

Results

Nitrogen balance

Mean nitrogen, methionine, cysteine, taurine, and total energy intakes and nitrogen retention data are shown in **Table 2** for both groups. Neither nitrogen nor energy intakes were significantly different. In addition, both groups received similar intakes of total fluid, electrolytes, minerals and trace elements.

Both groups showed similar positive retention of nitrogen, 282 mg/kg/24 h, which was 56% of the infused nitrogen. These retentions are similar to those described in a previous study of infants fed an intravenous formulation containing similar quantities of nitrogen and total calories (23, 24) and also parallel the nitrogen retention expected in utero (25).

Plasma amino acid response

Plasma ½ cystine concentration, shown in **Table 3**, was greater in the supplemented group than in the unsupplemented group. Neither plasma methionine nor taurine were affected by cysteine additions. The plasma values for methionine and taurine were generally within what has been defined previously as normal limits but the ½ cystine value in the unsupplemented group may be low (2). No cysteine was found in the plasma samples. One infant in each group developed transient hypermethioninemia of unknown origin which was resolved with the termination of TPN. Data for one infant in each group suggested transient hypertaurinemia, but this observation may have been due to faulty plasma pipetting technique resulting in the sample being taken from the taurine-rich buffy layer (26).

Urinary amino acid response

The urinary excretion of amino acids is shown in **Figure 1**. Cysteine hydrochloride additions caused an increase in urinary ½ cystine and taurine, but had no affect on cystathionine or methionine. No cysteine was detected in the urine samples. Urinary 3-methylhistidine excretion was not statistically different between the groups (control = 3.3 ± 0.2; supplemented = 3.8 ± 0.2 μmol/kg/24 h).

Anthropometric data

Weight change was not different between the two groups (control = 10.2 ± 1.7 g/kg/24 h; supplemented = 6.1 ± 2.3). Similarly, there were no differences in head circumference (control = 0.6 ± 0.1 cm/6 days; supplemented = 0.9 ± 0.2) or length change (control = 0.6 ± 0.2 cm/6 days; supplemented = 0.6 ± 0.1) between the groups.

Effect of gestation and postnatal age

Postnatal age effect was examined in two ways. First, as shown in **Table 4**, the supplemented and control groups were subdivided into those ≤ 14 days versus those older than

TABLE 2
Actual nutrient intake and nitrogen retention in cysteine supplemented and control intravenously fed infants*

	Total energy	Intake†				Nitrogen	retention‡
		Nitrogen	Methionine	Cysteine	Taurine		
	kcal		*mg*			*mg*	%
Supplemented (n = 18)	87 ± 4	508 ± 13	125 ± 3	78 ± 2	0	280 ± 16	55.7
Control (n = 18)	87 ± 4	509 ± 15	127 ± 4	0	0	283 ± 11	55.6

* All values are expressed as mean ± SE/kg/24 h.

† When expressed on a per litre basis, the infused control solution supplied the following: glucose 100 g; nitrogen 3.2 g; Na 14 mmol; K 15 mmol; Cl 14 mmol; Ca 6.5 mmol; P 6.0 mmol; Mg 3.0 mmol; Zn 14 μmol; Cu 6.25 μmol; Mn 9.0 μmol; I 0.47 μmol; Se 0.38 μmol; Cr 0.048 μmol; vitamin B_1 27.5 mg; B_2 10 mg; niacinamide 87.5 mg; pantothenic acid 18 mg; B_6 13.5 mg; B_{12} 75 μg; folic acid 0.5 mg; vitamin C 650 mg; A 5000 IU; D 500 IU; E 5 IU. In addition lipid was provided from a 10% emulsion.

‡ Nitrogen retention = nitrogen intake (Aminosyn) − nitrogen loss (urine + stool + GI drainage) during 3-day balance period.

TABLE 3
Plasma sulfur amino acid concentration in cysteine supplemented and control intravenously fed infants* (μmol/dl)

	Methionine	½ Cysteine	Taurine
Supplemented	5.25 ± 0.41	5.22 ± 0.43†	4.81 ± 0.44
Control	5.16 ± 0.43	2.98 ± 0.23	4.42 ± 0.51

* All values are expressed as mean ± SE.

† Supplemented greater than control, $p < 0.05$.

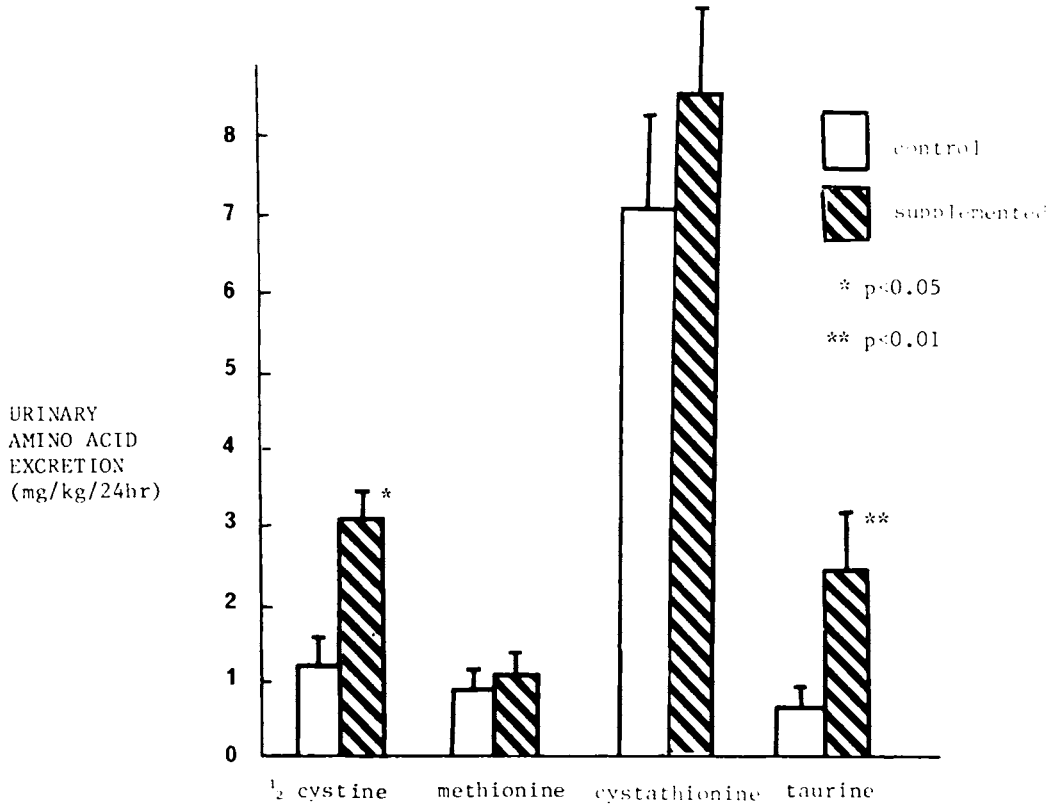

FIG. 1. The effect of intravenous cysteine supple- mentation on urinary sulfur amino acid excretion.

TABLE 4
Nitrogen retention and plasma ½ cystine concentration in cysteine supplemented and control intravenously fed infants of varying postnatal and gestational age

	Postantal age	n	Nitrogen retention*	Plasma ½ cystine†
	days		*mg/kg/24 h*	*μmol/dl*
Supplemented	≤14	12	283 ± 20‡	5.07 ± 0.4
	≥14	6	271 ± 29	5.56 ± 1.2
Control	≤14	10	293 ± 18	2.96 ± 0.5
	≥14	8	272 ± 13	3.0 ± 0.7
	Gestation age	n	Nitrogen retention	Plasma ½ cystine
	wk		*mg/kg/24 h*	*μmol/dl*
Supplemented	≤32	7	283 ± 22	5.08 ± 0.6
	≥37	8	290 ± 28	4.82 ± 0.7
Control	≤32	7	296 ± 22	3.53 ± 0.7
	≥37	8	304 ± 18	3.03 ± 0.7

* Nitrogen retention = nitrogen intake (Aminosyn) − nitrogen loss (urine + stool + GI drainage) during 3-day balance period.
† Blood sample drawn at end of 3-day balance period, between 0900 and 1100 h.
‡ Mean ± SEM.

14 days and by regression analysis of those ≤ 14 days. Neither nitrogen retention nor plasma ½ cystine were affected by postnatal age. Second, eight infants who received TPN during the 1st wk of life were pair matched. Cysteine supplementation was again found to increase plasma ½ cystine concentration (mean increase = 2.29 ± 0.5 μmol/dl; $p <$

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