Glycine Turnover Rates and Pool Sizes in Neonates as Determined by Gas Chromatography-Mass Spectrometry and Nitrogen 15

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Summary

This report makes use of a recent developed method with stable isotope and gas chromatography-mass spectrometry to determine the disappearance of labeled amino acids from plasma samples after iv administration of a single dose (93.3- μ moles/kg body weight) of [¹⁵N]glycine (92% N) in neonates. [¹⁵N]Glycine measurements were studied three times in each of six preterm infants at different gestational age and twice in two full-term infants. The first study was carried out in all infants 5 to 32 hr after delivery, the second study was performed on the third day of life, and the third study, included only the preterm infants, was at the age of 25 to 29 days. The isotope disappearance curves were linear within the first hr after [¹⁵N]glycine administration and represent mainly the hepatic uptake of glycine from the extracellular pool. The volume of glycine pool varied from the day of birth to 4 wk of life. Turnover rate constants of glycine ranging from 1.35 to 2.19 hr⁻¹ were observed in preterm and term infants during the first 32 hr. Significant increases in turnover rate constants were noted on the third day of life in most infants. At 3 to 4 wk of life, statistically significant differences in pool size and turnover rate constants were obtained. An increase of 2- to 3-fold in turnover rate constants was observed as compared to day of birth. Pool sizes declined by 50%, but the resulted fluxes remained almost unchanged during the neonatal period. At the age of 3 to 4 wk, all these infants showed similar kinetic data as in adults.

Speculation

The kinetic study of individual amino acids during the first month of life in newborn infants might help in determining the correct usage of intravenous amino-acids during this period in infants requiring parenteral nutrition.

In view of the increasing use of parenteral nutrition in premature and sick newborn infants, knowledge of the metabolism of infused amino acids is most important.

There are some known time-dependent changes in plasma amino acid levels during the first 10 hr and 3 days of life (2, 8, 24, 34) and changes according to dietary intake (23, 32, 33, 35, 40), but there are relatively few studies on the dynamic aspects of amino acid metabolism in the newborn (29, 30). Amino acid tracer studies in humans have provided a great deal of information on whole-body protein metabolism (25, 41, 46, 47), but practically no estimates of the rate of uptake and metabolism of individual amino acids. This is because it has not been technically possible to measure stable isotope enrichments of amino acids in small samples of plasma, and measurements have instead been made on the excretion of the isotope in the form of urinary urea. Inasmuch as the equilibration of labeled amino nitrogen with the body nitrogen is fast compared to formation and excretion of urea,

information is obtained on the behavior of metabolic nitrogen pool rather than on individual amino acids. The uptake of the amino acids into tissue is completed long before the conversion of the amino acids to the excretion products.

It has long been recognized that the rate of disappearance of a labeled amino acid from the circulation in the first 5 to 20 min after the IV administration reflects the transport of circulating amino acids from the extravascular-extracellular space to the intracellular pool (25). Radiolabeled amino acids were used in the early 1960's to follow the removal from circulation of glycine in children (27). This method was not further applied in studies in children due to health hazards associated with the use of radioactive tracers.

A rapid, convenient noninvasive stable isotope (and gas chromatography-mass spectrometry) method capable of measuring the rate of uptake of amino acid and the pools of extracellular amino acid in whole body was recently developed (14, 15, 18, 25) and applied to studies in humans (17, 19, 20).

MATERIALS AND METHODS

MATERIAL

[¹⁵N]NH₄Cl (92% ¹⁵N enriched) by the method of Schoenheimer and Ratner (9) recrystallized from ethanol and further purified by sublimation. Each preparation was at least 99% pure, measured by amino acid analyzer, mass spectrometry, and thin-layer chromatography. The [¹⁵N]glycine preparation was pyrogen-free by standard rabbit body temperature measurement. Standard solutions of the purified [¹⁵N]glycine (93.3 μ moles/ml) in distilled water, sterilized by passage through a Millipore filter (GSWP 02500), autoclaved, and administered as a single dose of 93.3 μ moles/kg body weight, dissolved in water.

SUBJECTS AND DIET

The subjects included six preterm and two full-term infants. All the infants had Apgar scores at 1 and 5 min between 7 to 10. Seven of the infants were born by vaginal vertex deliveries and one by cesarean section. Gestational age was assessed by the maternal obstetrical history, the Dubowitz scoring system (3), and examination of the anterior vascular capsule of the lens in the infants at less than 34 wk (13). All infants were appropriate for gestational age (45) and were well at the time of the studies. Pertinent biographic data of the infants is summarized in Table 1. All of the preterm infants and one of the term infants received no PO feedings before the first study. The preterm infants received only IV glucose in amounts between 5.2 to 5.7 mg/min/kg. The other term infant had two feeds before the study. In this infant as well as in those in the second and third studies, tests were performed 3 to 4 hr after a feeding. Two mothers (of H. A. and S. F. and S. S.) received Celestone Chronodose (betametasone disodium phosphate and betametasone acetate; Schering Co. Ken-

Table 1. *Subjects' biographic data and time schedule*

Subjects	Age and wt at time of investigation				Feeding regimes		
	Gestational age (wk)	First study (hr)	Second study (hr)	Third study (day)	First study	Second study	Third study
S. L.	29	10 (1140) ¹	79 (1040)	25 (1600)	175 (gl) ²	670 (gl)	185 (sm)
H. A.	31	9 (1400)	80 (1300)	29 (2300)	343 (gl)	166 (gl) 110 (sm)	174 (sm)
S. F.	33	5 (1600)	89 (1500)	29 (2100)	333 (gl)	160 (sm)	171 (sm)
S. S.	33	5 (1770)	89 (1600)	29 (2400)	333 (gl)	165 (sm)	200 (sm)
N. S.	34	17 (1600)	90 (1600)	29 (2400)	312 (gl)	135 (sm)	200 (sm)
A. D.	35	8 (1830)	75 (1720)	26 (2200)	328 (gl)	185 (sm)	240 (sm)
B. M. ³	39	13 (2550)	88 (2420)			130 (cm)	
Z. A.	41	32 (2830)	73 (2700)		62 (cm)	133 (cm)	

¹ Numbers in parentheses, weights at time of investigation.

² gl, glucose IV (mg/kg/hr); sm, simlac PO (ml/kg/day); cm, 66% cow's milk in water PO (ml/kg/day).

³ No feeding before first study.

ilworth, NJ) and Yutopar (ritodrin; Philips-Duphar B.V., Amsterdam, Netherlands) before delivery.

The nature of the investigation was explained to each mother, and babies were studied only after informed consent was obtained. The study received the approval of the Human Studies Committee of the Beilinson Medical Center, Petah Tikva.

EXPERIMENTAL DESIGN

[¹⁵N]Glycine kinetic measurements were studied three times in each preterm infant and twice in the full-term infants. Time schedules are detailed in Table 1. The first study was carried out in all infants between 5 and 17 hr after delivery, except in Z. A., a full-term infant in whom the [¹⁵N]glycine study was performed only at 32 hr of age. The second study was performed on the third day of life (73 to 90 hr). The third study, which included only the preterm infants, was at the age of 25 to 29 days.

PROTOCOL

A total of 22 studies were carried out on the 8 infants. An indwelling catheter was inserted into a peripheral vein for blood sampling. A single dose of [¹⁵N]glycine (92% ¹⁵N, 93.3 μ moles/kg body weight dissolved in 1 to 3 ml water) was administered via another peripheral vein over 10 to 20 sec. Blood samples were taken before (time zero) and at intervals of 5 min after IV administration. Between each sampling, the indwelling catheter was flushed with saline. Blood samples (0.8 to 1 ml) were collected in tubes containing 0.1 ml of 3.7% citrate solution and were iced before centrifugation. Urine was collected 1 to 3 hr before [¹⁵N]glycine administration and during the first 2 hr after administration.

SAMPLE PREPARATION

Immediately upon completion of the collection of the blood samples, plasma was isolated. The plasma proteins were precipitated with equal volume of 10% trichloroacetic acid. The supernatant was removed and extracted with five volumes of ether and was ready for derivatization.

GAS-CHROMATOGRAPHY MASS-SPECTROMETRY AND DATA ANALYSIS

The plasma amino acids obtained after delipidization were esterified for analysis by gas chromatography to either *n*-trifluoroacetyl or *n*-pentafluoropropionyl *n*-butyl derivatives. The amino acid mixture (0.1- to 0.3- μ mole aliquots) were separated by standard methods (7, 36, 38) using a Varian gas chromatography coupled by a metal splitter to a Finnigan quadrupole mass spectrometer (model 1015) and computer-averaging memory oscilloscope (19).

The isotope disappearance curves were linear in all studies

carried out. Hence, it was assumed (15, 26) that within the first hr after administration of the labeled amino acid, the disappearance of the [¹⁵N]glycine from the circulation can be described by unidirectional irreversible flux of glycine through a single compartment with a pool size (A), unidirectional influx (F), and efflux (E). The isotope enrichment (I) at time (t) following a single dose (w, μ equivalents ¹⁵N) of labeled compound is: $I_t = [W/(A + W)] \exp(kt) \times 100$, where k is the first-order rate constant for efflux ($E = k \cdot W$). The turnover rate constant of glycine in the pool is represented by k (hr⁻¹), which is obtained directly from the slope of the isotope enrichment time decay curve. The pool size, A (μ mole/100 g. body weight), is $A = d \times [I_t/I_0 - 1]$, where d is the dose of labeled glycine (μ mole/100 g body weight), I_t is the isotope enrichment of the administered glycine, and I_0 is the isotope enrichment of plasma glycine extrapolated to time zero. The errors in the slope and intercept were evaluated by standard statistical methods (9, 21). It was found (17, 19, 26) that the joint confidence region map (slope versus intercept) is a powerful and accurate method for visually evaluating the significance of differences in pool sizes, rate constants, and flux in a population of humans or an individual human in different metabolic states. Furthermore, certain areas on the joint confidence region maps can be associated with different metabolic and pathological states (17, 19, 26).

MEASUREMENT OF PLASMA GLYCINE LEVELS

A colorimetric-fluorometric method (37) was used to determine the plasma glycine levels in each blood sample with a volume of 0.1 cc of deproteinized plasma. Measurements were carried out using an Aminco-Bowman Spectrophotofluorometer (American Instrument Co. Inc., Silver Spring, MD).

DETERMINATION OF GLYCINE CONCENTRATIONS IN URINE

The method described above was used to measure glycine in urine before and after [¹⁵N]glycine administration.

RESULTS

PLASMA GLYCINE LEVELS

Plasma glycine concentrations were determined in each blood sample before and after [¹⁵N]glycine administered into the infants. As seen in Figure 1, during the first 3 to 5 min after [¹⁵N]glycine administration, glycine levels increase about 10 to 20% above the basal level. Significant differences in plasma glycine concentration were noted between the day of birth and the third day of life (Fig. 2) which corresponds to the findings of others (2, 24, 34).

URINE GLYCINE LEVELS

Table 2 summarizes the glycine concentrations in urine before and during the first and the second [¹⁵N]glycine administration in

Fig. 1. Plasma glycine levels after the injection of [^{15}N]glycine (9.3 $\mu\text{moles}/100\text{ g}$ body weight) in all infants studied at different ages. 1, day of birth; 2, third day of life; 3, 3 to 4 wk old.

Fig. 2. Plasma glycine levels of infants on day of birth and third day of life.

two preterm infants (A. D. and S. L.). The amount of glycine excreted in urine after glycine administration did not exceed 1.9% of the injected glycine dose and in most cases less than 1%. There is no indication that glycine could leak out as a result of a load of glycine.

DYNAMIC PARAMETER OF GLYCINE

[^{15}N]Glycine measurements were studied three times in each of the six preterm infants and twice in the two term infants. The isotope disappearance curves (Fig. 3) were linear within the first hr following [^{15}N]glycine administration. Mixing with the miscible

pool is completed within 5 min in most cases, allowing sufficient time for the measurement to be made before the isotope enrichments fall below the range which could not be measured. Delayed mixing does not appear to introduce errors into the determination of pool sizes from the zero time intercepts. The slopes and intercepts of the least squares lines describing the isotope enrichment time decay curves and the corresponding pool sizes and turnover rate constants are given in the joint confidence region map (Fig. 4; Table 3).

EXTRACELLULAR POOL SIZE

Statistically significant ($P < 0.01$) high glycine pool sizes ranging from 14.7 to 20.6 $\mu\text{moles}/100\text{ g}$ body weight were noted in all infants studied on the day of birth when compared with one month of life (Table 4; values of 7.7 to 11.5 $\mu\text{moles}/100\text{ g}$ body weight). A decrease in pool sizes was observed already on the third day of life in the preterm and term infants.

The volume of glycine pool (pool size: plasma glycine level) obtained in this study varied from day of birth to 3 days and 3 to 4 wk of life as depicted in Figure 5; 25 to 40% decline in pool volumes were observed in preterm infants at 3 to 4 wk of life. Mean values of 530 ml/kg body weight at day of birth and 360 ml/kg body weight at the age of 3 to 4 wk are compatible with the extracellular volumes at this age (5, 16).

RATE CONSTANTS OF GLYCINE DISAPPEARANCE

Turnover rate constants of glycine ranging from 1.35 to 2.19 hr^{-1} were observed in preterm and term infants on day of birth (Table 3; Fig. 4).

Significant increases ($P < 0.01$) in turnover rate constants were noted on the third day of life in most infants. In the most preterm infants, S. L. and H. A., turnover rate constants on the third day of life did not change significantly from day of birth, whereas at 3 to 4 wk of life, the turnover rate constants of glycine increased significantly ($P < 0.01$). The turnover rate constants at 3 to 4 wk of life range from 3.4 to 5.0 hr^{-1} , an increase of 2- to 3-fold as compared to day of birth. Glycine fluxes remained unchanged in most cases studied during the first month of life.

Table 4 and Figure 6 summarize the variation of plasma glycine dynamic parameters as a function of age of life and gestational age of the eight babies studied. Insignificant differences were found for all glycine dynamic parameters, pool size, and turnover rate constants between the different groups on the first day of life. Significant differences in rate constant of glycine disappearance were found between less and more mature preterm infants on the third day of life, although the pool sizes of the two groups were almost identical. Turnover rate constants were significantly ($P < 0.01$) elevated in the more mature infants and as a result of these the fluxes in this group were significantly higher. At 3 to 4 wk of life, statistically significant differences ($P < 0.01$) in pool size and turnover rate constants were obtained when compared to day of birth and 3 to 4 days of life. The pool size declined to 9.0 ± 2.2 $\mu\text{moles}/100\text{ g}$ body weight, whereas the turnover rate constant increased to $4.27 \pm 0.31\text{ hr}^{-1}$, and the resulted fluxes remained unchanged.

Table 2. Urine glycine levels before and after [^{15}N]glycine administration

Subject	Urinary glycine excretion ($\mu\text{moles}/\text{hr}$)		% excess excretion of administered glycine dose
	Before	After	
A. D.1 ¹	6.89	8.0	0.65
A. D.2	11.50	13.20	1.06
S. L.1	4.0	2.16	
S. L.2	3.84	5.69	1.9

¹ 1, first study; 2, second study.

Fig. 3. Plasma [^{15}N]glycine isotope decay curves (represented by nonweighted least squares lines) in infants on day of birth, after 29 to 41 weeks of gestation, (●); 3 days old (○); and 3 to 4 wk old (■).

Fig. 4. Joint confidence region maps of glycine pool size and turnover rate constants of infants studied; 1, on day of birth after 29 to 41 wk of gestation; 2, 3 days old; and 3, 3 to 4 wk old.

DISCUSSION

This study makes use of a recently developed method (15, 18, 26) with stable isotope and gas chromatography-mass spectrometry to determine the disappearance of labeled amino acids from plasma after IV administration of a single dose of [^{15}N]glycine in

neonates. Inasmuch as this method makes use of natural substances labeled with nonradioactive stable isotopes, it qualifies as a low risk, repeatable, and accurate kinetic method directly applicable to studies in humans.

Recent studies in human volunteers (17, 19) revealed that plasma glycine disappearance curves over the first hr following a

Table 3. Glycine kinetic parameters of preterm and full-term infants

Subjects and study number	Pool ($\mu\text{mole}/100\text{ g body wt}$)	Turnover rate constant (hr^{-1})	P^1	Flux ($\mu\text{moles hr}^{-1}/100\text{ g body wt}$)
S. L.1 ²	18.6 ± 3.15^4	1.56 ± 0.26		28.9 ± 3.24
S. L.2	15.6 ± 3.86	1.60 ± 0.52	N.S. ⁴	24.1 ± 7.99
S. L.3	7.95 ± 3.76	4.67 ± 0.56	<0.01	35.4 ± 18.8
H. A.1	14.7 ± 2.56	2.43 ± 0.26		35.4 ± 4.51
H. A.2	12.0 ± 2.43	2.25 ± 0.25	N.S.	26.5 ± 3.86
H. A.3	11.5 ± 2.22	3.74 ± 0.24	<0.02	42.7 ± 7.81
S. F.1	15.7 ± 2.54	1.51 ± 0.22		23.5 ± 2.10
S. F.2	14.2 ± 3.48	3.27 ± 0.36	<0.01	45.5 ± 9.58
S. F.3	9.86 ± 1.76	5.03 ± 0.22	<0.01	49.3 ± 9.34
S. S.1	20.5 ± 2.50	1.35 ± 0.20		27.5 ± 2.85
S. S.2	13.8 ± 3.17	3.45 ± 0.35	<0.01	47.1 ± 9.57
S. S.3	11.1 ± 2.07	4.25 ± 0.26	<0.01	46.6 ± 8.32
N. S.1	16.8 ± 5.81	2.10 ± 0.56		36.3 ± 11.8
N. S.2	12.4 ± 3.16	3.13 ± 0.29	<0.05	37.9 ± 9.88
N. S.3	9.05 ± 3.25	3.69 ± 0.47	<0.05	32.3 ± 11.70
A. D.1	18.6 ± 2.40	1.62 ± 0.90		30 ± 2.16
A. D.2	8.56 ± 2.53	3.65 ± 0.34	<0.01	30.5 ± 9.09
A. D.3	7.72 ± 1.57	3.85 ± 0.27	<0.01	29.4 ± 5.61
B. M.1	20.6 ± 3.78	2.00 ± 0.330		41.1 ± 4.62
B. M.2	9.94 ± 2.38	4.93 ± 0.36	<0.01	48.4 ± 11.5
Z. A.1	15.6 ± 1.92	1.55 ± 0.185		24.0 ± 1.6
Z. A.2	11.9 ± 2.06	3.36 ± 0.23	<0.01	39.5 ± 6.1

¹ P 's calculated by Student's t test using the day of birth as control experiment.

² 1, day of birth; 2, third day of life; 3, 3 to 4 wk old.

⁴ Mean $\pm$ S.D. given at 95% confidence limit.

³ N.S., not significant.

Fig. 5. Extracellular pool volumes (glycine pool size: glycine concentration) of preterm and full-term infants on day of birth, after 29 to 41 weeks of gestation (■), on third day of life (▨), and at 3 to 4 wk old (□).

single IV dose of the labeled amino acid represents mainly the hepatic uptake of glycine from the extracellular pool. This assumption is based on the recent finding in rabbits (26) showing that the amount of glycine removed from the circulation compares well with the rate of uptake of plasma glycine by the liver (12).

Flux (into the liver) was calculated in healthy volunteers (17) to be 21.8 to $33.5\ \mu\text{moles min}^{-1}/100\text{ g liver tissue}$ which is similar to those obtained by direct measurement in rabbits (26).

In this study, the volume of glycine pool varied from the day of birth to 4 wk of life. Mean values of $500\text{ ml/kg body weight}$ at day of birth to $350\text{ ml/kg body weight}$ at the age of 3 to 4 wk of life are compatible with the extracellular volumes found in neonates at this age (5, 16).

The equilibration of labeled amino acid with the extracellular

pool (Fig. 1) was found to be completed within 5 min and did not introduce any difficulties in steady-state kinetic analysis.

Although relatively large doses of [^{15}N]glycine were administered, no evidence for overloading or nonsteady state kinetics was found because disappearance curves were linear (Fig. 3) and plasma glycine levels were relatively constants (Fig. 1). No significant differences were observed in glycine kinetic parameters (pool sizes or rates of disappearance) when glycine dose was reduced to one-half ($46.6\ \mu\text{moles/kg}$) in human subjects studied (17) or in parallel tracer experiments in rabbits used [$^{14}\text{C}_2$] glycine, when [$^{14}\text{C}_2$]glycine was administered with and without an equal amount of nonlabeled glycine as in the ^{15}N tracer experiments (26).

No evidence of any trace of [^{15}N]glycine was found in the urine of the infants following the IV administration of [^{15}N]glycine.

The kinetic parameters observed in a group of preterm and term infants revealed significant differences in values obtained corresponding to days of life and gestational age. Pool size of 14.7 to $20.6\ \mu\text{moles}/100\text{ g body weight}$ found on day of birth were very significantly different ($P < 0.01$) from any pool size obtained at the age of 3 to 4 wk of life in preterm infants or 3 to 4 days after birth in term infants (7.7 to $11.9\ \mu\text{moles}/100\text{ g body weight}$). The latter values of pool sizes are similar to those found in adult humans (17).

The significant reduction in pool sizes during the first days of life is probably related to the diminished plasma glycine levels (2, 24, 34) and the decreased extracellular volume in preterm infants during their first days of life (5, 16).

The disappearance rate constants of glycine from the circulation in neonates change dramatically during the first month of life. A 2 to 3-fold increase in rate constants were found after 3 to 4 wk of life in comparison to values obtained on the day of birth. The most significant changes occur already after 3 to 4 days of life, except in the two smallest preterm infants (S. L. and A. L.). Rate constants at the age of 3 to 4 wk of life were similar to those obtained in normal adults (17).

The biochemical and physiologic explanation for the changes in glycine turnover rates during the neonatal period is speculative.

Table 4. Variations of glycine dynamic parameters as a function of age

Groups and numbers of subjects	Pool ($\mu\text{moles}/100\text{ g body wt}$)	Turnover rate constant (hr^{-1})	Flux ($\mu\text{moles hr}^{-1}/10\text{ g body wt}$)
A ¹ (n = 8)	18.0 $\pm$ 2.1 ²	1.68 $\pm$ 0.18	30.2 $\pm$ 2.0
B (n = 2)	13.3 $\pm$ 2.2	2.07 $\pm$ 0.24	27.4 $\pm$ 3.1
C (n = 4)	13.1 $\pm$ 2.5	3.43 $\pm$ 0.28	44.3 $\pm$ 7.2
D (n = 6)	9.0 $\pm$ 2.2	4.27 $\pm$ 0.31	37.8 $\pm$ 8.9

¹ A, all infants on day of birth; B, 3-day-old preterm infants after 29 to 31 wk of gestation; C, 3-day-old preterm infants after 33 to 35 wk of gestation; D, 3 to 4 wk old, all preterm infants.

² Mean $\pm$ S.D.

Fig. 6. Summary of glycine dynamics in preterm and full-term infants. A, all infants on day of birth; B, 3-day-old preterm infants after 29 to 31 wk of gestation; C, 3-day-old preterm infants after 33 to 35 wk of gestation; D, all preterm infants at 3 to 4 wk old.

Factors such as enzyme maturation, hormonal activities, and transport mechanisms probably affect the dynamic parameters of amino acids (glycine) metabolism after birth. It is well known that after birth some metabolic processes are impaired as a result of immaturity of enzyme systems (4, 10, 22, 35, 42). This might explain the reduced uptake of glycine into the liver. The hormonal activities of insulin, glucagon, and growth hormone were found to change rapidly after birth (1, 11, 34), which might explain their effect on amino acid transport and metabolism. It was found that hypoaaminoacidemic response to pharmacologic dose of glucagon in newborn infants on the third day of life resembles the response in adults but is attenuated on the day of birth (34). Inadequate liver transport mechanism which limits sulfobromophthalein sodium (28, 43) or bilirubin excretion (6, 44) may also effect glycine rates of uptake into the liver.

The study of the metabolism of individual amino acids in the neonatal period is most important in view of the markedly increased use of parenteral nutrition. We have shown in this study that there are significant differences in the dynamic parameters of IV administered labeled glycine during the first wk of life in both preterm and term infants. By the age of 3 to 4 wk, all these infants now showed similar kinetic data as in adults. Further studies are now in progress with other individual amino acids.

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