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Acute Effects of Intravenous Glutamine Supplementation on Protein Metabolism in Very Low Birth Weight Infants: A Stable Isotope Study

CLOTILDE DES ROBERT, OLIVIER LE BACQUER, HUGUES PILOQUET, JEAN-CHRISTOPHE ROZÉ, AND DOMINIQUE DARMAUN

Service de Néonatologie et Réanimation Pédiatrique, Hôpital Mère et Enfant, CHU de Nantes [C.D.R., H.P., J.-C.R.], and INSERM U.539, Centre de recherche en nutrition humaine [C.D.R., O.L.B., H.P., D.D.], Nantes, France

ABSTRACT

Although very low birth weight infants are subjected to severe stress and glutamine is now considered a conditionally essential amino acid that may attenuate stress-induced protein wasting in adults, current amino acid solutions designed for neonatal parenteral nutrition do not contain glutamine. To determine whether a short-term supplementation with i.v. glutamine would affect protein metabolism in very low birth weight infants, 13 preterm neonates (gestational age, 28–30 wk; birth weight, 820–1610 g) receiving parenteral nutrition supplying 1.5 g · kg^{-1} · d^{-1} amino acids and approximately 60 nonprotein kcal · kg^{-1} · d^{-1} were randomized to receive an i.v. supplement made of either *1*) natural L-glutamine (0.5 g · kg^{-1} · d^{-1}; glutamine group), or *2*) an isonitrogenous glutamine-free amino acid mixture (control group), for 24 h starting on the third day of life. On the fourth day of life, they received a 2-h infusion of $NaH^{13}CO_3$ to assess the recovery of ^{13}C in breath, immediately followed by a 3-h L-[1-^{13}C]leucine infusion. Plasma ammonia did not differ between the groups. Glutamine supplementation was associated with *1*) higher plasma glutamine (629 ± 94 *versus* 503 ± 83 μM, mean ± SD; $p < 0.05$, one-tailed unpaired *t* test), *2*) lower rates of leucine release from protein breakdown (−16%, $p < 0.05$) and leucine oxidation (−35%, $p < 0.05$), *3*) a lower rate of nonoxidative leucine disposal, an index of protein synthesis (−20%, $p < 0.05$), and *4*) no change in protein balance (nonoxidative leucine disposal − leucine release from protein breakdown, NS). We conclude that although parenteral glutamine failed to enhance rates of protein synthesis, glutamine may have an acute protein-sparing effect, as it suppressed leucine oxidation and protein breakdown, in parenterally fed very low birth weight infants. **(*Pediatr Res* 51: 87–93, 2002)**

Abbreviations

B_{Leu}, leucine release from protein breakdown
Eco_2, $^{13}CO_2$ enrichment
Ei_{Leu}, isotopic enrichment in infusate ^{13}C-leucine
Ep_{KIC}, isotopic enrichment in plasma KIC at steady state
$F^{13}co_2$, appearance of $^{13}CO_2$ in expired air
Fio_2, inspired air oxygen fraction
GC-IRMS, gas chromatography-isotope ratio mass spectrometry
GCMS, gas chromatography-mass spectrometry
i_{bicarb}, rate of [^{13}C]bicarbonate infusion
i_{Leu}, rate of [^{13}C]leucine infusion
KIC, α-keto-isocaproate
NOLD, nonoxidative leucine disposal
Ox_{Leu}, leucine oxidation
Ra_{Leu}, appearance rate of leucine into plasma
IUGR, intrauterine growth retardation
$\dot{V}co_2$, CO_2 production
VLBW, very low birth weight
PN_{Leu}, exogenous leucine from parenteral nutrition

During the last decade, several clinical trials have produced compelling evidence for a role of glutamine in improving nitrogen balance in adult patients undergoing elective gastrointestinal surgery (1, 2), cholecystectomy (3), or bone marrow transplantation (4). Other studies suggested glutamine might exert a trophic effect on gut as well (5–7).

Although preterm infants are exposed to stress-induced protein wasting and intestinal frailty, conventional amino acid solutions designed for neonatal i.v. nutrition do not contain glutamine, because glutamine is classified as nonessential, has relatively poor solubility, and cannot be heat sterilized. Yet Neu *et al.* (8) recently showed that, compared with control enteral regimens, a 20-d supplementation of enteral feeding

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Correspondence and reprint requests: Dominique Darmaun, M.D., Ph.D., Inserm U.539, Centre de Recherche en Nutrition Humaine, Hôtel-Dieu, 3ème étage aile nord, 44093 Nantes Cedex 1, France; e-mail: ddarmaun@nantes.inserm.fr
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with glutamine (0.3 g · kg^{-1} · d^{-1}) was safe, decreased the incidence of sepsis, and shortened hospital stay, as well as improved tolerance to subsequent enteral feeding, in preterm neonates. The regimens were, however, not isonitrogenous as the glutamine regimen provided more total nitrogen. Similarly, Lacey *et al.* (9) observed that i.v. glutamine facilitated weaning from parenteral nutrition and ventilatory support in a subgroup of infants with a birth weight <800 g. However, the decrease in the incidence of sepsis and hospital stay was not significant.

Because protein wasting impairs immune and respiratory function (10), one of the mechanisms for the putative clinical benefits of glutamine in VLBW infants could be through preservation of body protein. Enteral glutamine infusion was indeed associated with a drop in Ox in healthy adults—whether in the fasting state or made slightly catabolic by a glucocorticoid treatment (11, 12)—a rise in NOLD, an index of whole body protein synthesis in healthy adults (11), and a reduction in both Ox and protein breakdown in patients with Duchenne muscular dystrophy, a condition associated with severe, relentless muscle protein wasting (13).

The aim of this study was therefore to determine whether parenteral glutamine supplementation would acutely attenuate protein wasting in parenterally fed VLBW infants.

METHODS

Chemicals. L-[1-^{13}C]leucine, $NaH^{13}CO_3$ (both 99% ^{13}C), and L-[2-^{15}N]glutamine (99% ^{15}N) were purchased from Cambridge Isotope Laboratories, (Andover, MA, U.S.A.). L-[1-^{13}C]leucine and $NaH^{13}CO_3$ solutions were prepared in sterile 0.9% saline solution by the hospital pharmacy under a laminar flow hood and verified to be sterile (plate culture) and pyrogen-free by the Institut Départemental d'Analyze et de Conseil (IDAC, Nantes, France). Infusates were passed through a 0.22-μm Millipore filter (Bedford, MA, U.S.A.) and stored in sterile containers at 4°C until used. The concentrations of L-[1-^{13}C]leucine and $NaH^{13}CO_3$ in the infused solutions were found to remain stable for >6 mo, as assessed by GCMS and GC-IRMS, respectively.

Natural L-glutamine (tissue culture grade) was obtained from Sigma Chemical Co. (Saint Quentin Fallavier, France), and verified to be pyrogen-free (rabbit temperature). Solutions of natural L-glutamine were prepared under sterile conditions in a laminar flow hood by the hospital pharmacy, filtered through a 0.22-μm filter, and stored in sealed glass containers at 4°C until used. The concentration of natural L-glutamine in the infusates was proven to be stable for 4 wk at 4°C.

Ion-exchange resins were obtained from Touzart et Matignon (Temex 1X8, 100–200 mesh hydrogen form, Courtaboeuf, France) and Aldrich (Dowex 50WX8–200, Saint Quentin Fallavier, France). All chemicals and isotopic purities were verified by GCMS. All other chemical reagents were from Sigma Chemical Co.

Patients. Thirteen premature neonates weighting between 820 and 1650 g were recruited on the first day of life among babies admitted to the Neonatal Intensive Care Unit at the Hospital Mère et Enfant, Nantes, France. Written, informed consent was obtained from the parents before enrollment and after the purpose and potential risks of the study had been fully explained to them, according to procedures approved by the Ethical Committee of the University Hospital of Nantes, France (CCPPRB n°2, Région des Pays de la Loire). Inclusion criteria consisted of *1*) birth weight between 700 g and 1650 g, *2*) absence of sepsis, as judged from a C-reactive protein <20 mg/L, *3*) mild lung disease, as judged from an oxygen requirement Fio_2 <40%, and *4*) normal plasma ammonia. Patients were excluded if they received or had received a blood transfusion or albumin infusion, if they had major surgery, had an elevated C-reactive protein concentration, required an Fio_2 >40%, or if their parents refused participation in the study.

Nutritional regimens. In both groups, parenteral nutrition was started on the first day of life. Glucose was started at approximately 5 mg · kg^{-1} · min^{-1} (approximately 7 g · kg^{-1} · d^{-1}) and rapidly advanced as tolerated up to approximately 11 g · kg^{-1} · d^{-1}. Parenteral amino acids (Primène 10%; Baxter/Clintec, Maurepas, France) were started on the first day at 0.5 g · kg^{-1} · d^{-1} and increased by 0.5 g · kg^{-1} · d^{-1} to reach 1.5 g · kg^{-1} · d^{-1} by the third day. Composition of the amino acid mixture is given in Table 1. Parenteral long-chain triglyceride emulsions (Ivelip 20%; Baxter/Clintec) were started on the second day of life at 1 g · kg^{-1} · d^{-1} and increased by 1 g · kg^{-1} · d^{-1} to reach 2 g · kg^{-1} · d^{-1} by the third day of life. None of the infants received any enteral nutrition until completion of the isotope infusion study on the fourth day of life.

In a double-blind fashion, patients were randomly assigned to receive either a 0.5 g · kg^{-1} · d^{-1} L-glutamine supplementation, provided as a 2.6 g/dL solution of L-glutamine in water (Sigma Chemical Co., St. Louis, MO, U.S.A.), or an isonitrogenous amino acid solution (Primène 10%, diluted to provide 3.3 g amino acid/dL). The supplementation started at 1800 h the third day of life, and was infused continuously by means of a calibrated syringe pump, and piggybacked into the i.v. central catheter line at a constant rate until 1600 h the next day.

Protocol design. The isotopic study was performed on the fourth day of life in a total of 13 infants (six in the glutamine group, seven in the control group) while infants received continuous i.v. nutrition through a central venous catheter. The glutamine or Primène supplement was administered in a separate syringe, and piggybacked into the parenteral nutrition line, to be infused through the same central venous catheter.

Table 1. *Composition of amino acid mixture used for parenteral nutrition (Primène 10%; Baxter/Clintec parenteral SA, Maurepas, France)**

Leucine	1.000	Alanine	0.800
Isoleucine	0.670	Aspartate	0.600
Valine	0.760	Glutamate	1.000
Lysine	1.100	Glycine	0.400
Methionine	0.240	Proline	0.300
Phenylalanine	0.420	Serine	0.400
Tryptophan	0.200	Arginine	0.840
Threonine	0.370	Ornithine	0.318
Histidine	0.380	Cysteine	0.189
		Taurine	0.060
Total nitrogen	15 g/L		
Total amino acids	100 g/L		
Osmolarity	780 mosmol/L		

* Values for individual amino acids are given in grams per deciliter.

At 1000 h on the isotope study day, measurement of respiratory gas exchange was started and continued throughout the study until 1600 h by means of an indirect calorimeter as described previously (14). At 1030 h, a baseline venous or capillary blood sample (0.5 mL) was obtained for measurement of background isotopic enrichment in plasma KIC. Three aliquots of expired air were obtained at 10-min intervals for determination of background $^{13}CO_2$. For babies who were receiving ventilatory assistance, expired air was collected from the exhaust of the ventilator into a 10-L Douglas bag. For babies who were breathing spontaneously or with continuous positive airway pressure, expired air was collected from the outlet of the ventilated canopy. Triplicate aliquots of expired air from each sampling time were then immediately transferred with a syringe into airtight tubes (Exetainer system and gas testing vials, Labco, Bucks, U.K.) for later analysis.

Two stable isotope infusions were carried out consecutively on the same day in each infant through the central venous catheter (Fig. 1). First, a primed, continuous 2-h infusion of $NaH^{13}CO_3$ (7.5 μmol · kg^{-1} prime, 5 μmol · kg^{-1} · h^{-1} infusion) was performed from 1100 h to 1300 h. The purpose of this first isotope infusion was to estimate the rate of total $\dot{V}CO_2$. The labeled bicarbonate infusion was immediately followed by a primed, continuous 3-h infusion (approximately 48 μmol · kg^{-1} prime; approximately 48 μmol · kg^{-1} · h^{-1}) of L-[1-^{13}C]leucine from 1300 h to 1600 h, designed to assess leucine kinetics.

Venous or capillary blood samples (0.5 mL) were obtained to determine the concentration and ^{13}C-enrichment of plasma KIC, and plasma leucine and glutamine concentrations at 30-min intervals during the last hour of the labeled leucine infusion. The total volume of blood sampled was therefore approximately 2 mL, which represents <5% of blood volume in a 1000-g infant. Expired air samples were obtained at 15-min intervals between 1200 h and 1300 h and between 1500 h and 1600 h, during the last hour of the labeled bicarbonate and labeled leucine infusions, respectively.

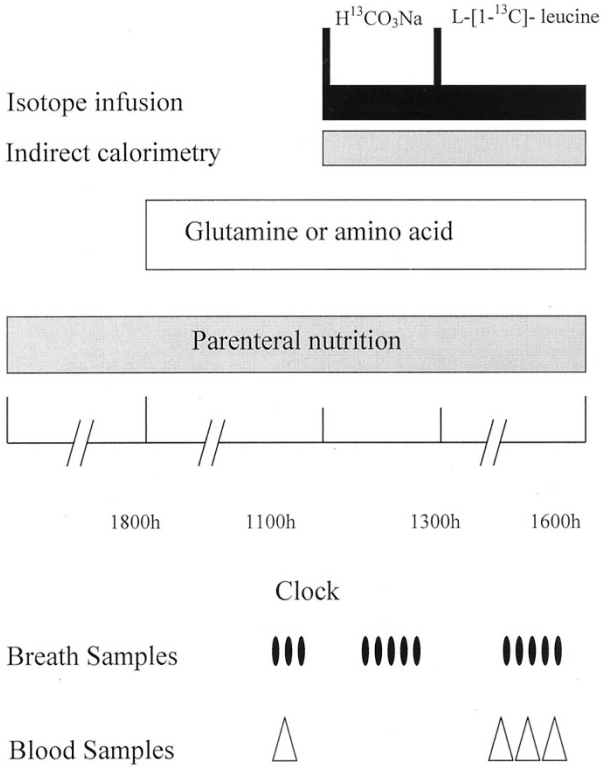

Figure 1. Protocol design.

Analytical methods. Known amounts of ketocaproic acid and L-[2-^{15}N]glutamine were spiked into a 100-μL aliquot of plasma to serve as internal standards for the measurement of KIC and glutamine concentrations, respectively, by reverse isotope dilution. Plasma glutamine was isolated and derivatized as described (15). KIC was extracted from 100 μL of plasma by passing the acidified plasma sample over an AG50 cation-exchange column, and converted to its oxime-*t*-butyldimethylsilyl KIC derivative as described (16, 17). After derivatization, samples were then measured by GCMS.

GCMS assays. The isotopic enrichment of KIC was determined using electron ionization GCMS on a 5890 series II gas chromatograph coupled with a 5971A mass selective detector (Hewlett Packard, Palo Alto, CA, U.S.A.) equipped with a capillary column (DB-1, 30 m × 0.25 mm ID, 0.25-mm film thickness, J&W Scientific, Courtaboeuf, France). Selected ion monitoring was used on ions of mass-to-charge ratio (m/z) 316 and 317, representing ions of the natural and [1-^{13}C]KIC, respectively. An m/z 316 ion was also measured for ketocaproic acid. For glutamine analysis, ions at m/z 340 and 341 representing natural and [^{15}N]-glutamine were selectively monitored.

Expired air assays. $E^{13}CO_2$ was measured by GC-IRMS (Finnigan-MAT, Bremen, Germany) using a Porapak column.

Steady state. A measurement was considered to be in steady state when its coefficient of variation was <10% during the period considered.

Calculations of leucine kinetics. As shown by earlier studies (18), ^{13}C-labeled bicarbonate infusion can be use to estimate the $RaCO_2$, which was calculated as follows:

Eq. 1

$$RaCO_2 = i_{bicarb} \times [(Ei_{bicarb}/E_{bicarbCO2}) - 1] \quad (1)$$

where i_{bicarb} is the labeled bicarbonate infusion rate (μmol · kg^{-1} · h^{-1}), and Ei_{bicarb} and $E_{bicarbCO2}$ are the ^{13}C enrichments in the labeled bicarbonate infusate and expired air during the last hour of labeled bicarbonate, respectively.

Leucine appearance (Ra_{Leu} in μmol · kg^{-1} · h^{-1}) into the plasma compartment was calculated as

Eq. 2

$$Ra_{Leu} = i_{Leu} \times [(Ei_{Leu}/Ep_{KIC}) - 1] \quad (2)$$

where i_{Leu} is the rate of ^{13}C-leucine infusion (μmol · kg^{-1} · h^{-1}), and Ei_{Leu} and Ep_{KIC} are the ^{13}C enrichments (mole percent excess) in the infusate leucine and in plasma KIC at steady state, respectively. Because leucine kinetics were measured under fed conditions, both exogenous leucine from parenteral nutrition (PN_{Leu}) and endogenous leucine contributed to the appearance rate of leucine. Furthermore, because leucine is an essential amino acid, release from protein breakdown

(B_{Leu}) is its only endogenous source. So, protein breakdown can be calculated as

Eq. 3

$$B_{Leu} = Ra_{Leu} - PN_{Leu} \quad (3)$$

In the control group, PN_{Leu} included the leucine supplied by the diluted amino acid supplement.

The appearance of $^{13}CO_2$ in expired air ($F^{13}_{CO_2}$, in μmol · $kg^{-1} \cdot h^{-1}$) is calculated as

Eq. 4

$$F^{13}_{CO_2} = (Ra_{CO_2} \times E_{LeuCO2})/100 \quad (4)$$

where E_{LeuCO2} is the $^{13}CO_2$ enrichment at steady state during the [^{13}C]leucine infusion. Leucine oxidation (Ox_{Leu}, in μmol · $kg^{-1} \cdot h^{-1}$) was determined by the following equation:

Eq. 5

$$Ox_{Leu} = F^{13}_{CO_2}/(Ep_{KIC} \times 100) \quad (5)$$

This rate of leucine oxidation represents total oxidation, and includes the oxidation of labeled leucine as well.

NOLD, an index of whole body protein synthesis, was calculated as

Eq. 6

$$NOLD = (Ra_{Leu} + i_{Leu}) - Ox_{Leu} \quad (6)$$

This rate of NOLD includes the nonoxidative disposal of labeled leucine as well.

Finally, net leucine balance, an index of the net protein leucine gain, was calculated as

Eq. 7

$$Balance_{Leu} = NOLD - B_{Leu} \quad (7)$$

Under conditions of steady state, leucine balance also equals the difference between total leucine intake—both natural leucine from parenteral nutrition and labeled leucine—and total leucine irreversible loss (Ox_{Leu}):

Eq. 8

$$Balance_{Leu} = (i_{Leu} + PN_{Leu}) - Ox_{Leu} \quad (8)$$

Sample size. In earlier studies in adults (11), enteral glutamine administration was associated with a 30–40% decline (δ) in Ox_{Leu}—19.0 *versus* 11.0 μmol · $kg^{-1} \cdot h^{-1}$—whereas the SD (σ) of Ox_{Leu} was 5.3 μmol · $kg^{-1} \cdot h^{-1}$, so that δ/σ = (19 − 11) / 5.3 = 1.5. Assuming a similar glutamine-induced change in Ox_{Leu} in the infants to be enrolled in the current study, the number of patients necessary was estimated as follows: $n = (Z\alpha + Z\beta)^2 / (\delta^2 / \sigma^2)$, where $Z\alpha = 1.96$, and $Z\beta = 0.84$ with an $\alpha < 0.05$, and a power $(1 - \beta)$ of 80% [using the tables in Friedman *et al.* (19)]. It follows that $n = (1.96 + 0.84)^2 / (1.5)^2 = 7.84 / 2.25 = 3.5$, for a one-tailed test. Our goal was therefore to recruit five to seven subjects in each group.

Statistics. Results are expressed as mean ± SD. Comparisons between groups were performed by using one-tailed unpaired *t* test; significance was established at $p < 0.05$.

RESULTS

Selected relevant clinical characteristics for the studied population, as well as the babies' nutritional intake on the day of the isotopic study (d 4), are given in Table 2. There was no significant difference between the two groups for birth weight or gestational age, even though the term was slightly, but not significantly greater in the control group, as there were more growth-retarded newborns in the control group (3 *versus*1). The average postnatal age at the time of the isotope infusion study was not statistically different between the groups (70 ± 4 h and 74 ± 7 h in the glutamine and control groups, respectively). Weight loss between birth and isotope infusion day was similar between groups (103 ± 34 g *versus* 105 ± 46 g, *i.e.* 9.7 ± 3.4% *versus* 8.8 ± 3.1% of birth weight; NS).

Three infants received caffeine in the glutamine group, one infant in the control group received insulin, and another one in the same group received midazolam (Hypnovel). None of these newborns received dopamine. With regard to antenatal corticoid treatment, two of the six infants in the glutamine group had received one or two courses of treatment, compared with five of seven in the control group.

Regarding ventilatory status, some infants were breathing spontaneously, whereas others received mechanical ventilation, including ventilation with nasal continuous positive airway pressure. None of the infants received an Fio_2 >40%. The average Fio_2 was 23 ± 3% in the glutamine group and 25 ± 2% in the control group.

Plasma glutamine concentration was higher in the glutamine group (629 ± 94 μM *versus* 503 ± 83 μM, $p < 0.05$, one-tail *t* test). Serum ammonia was not significantly different in the

Table 2. *Selected relevant clinical characteristics and parenteral nutrition intake of population studied*

Study group	Gestational age (wk)	Birth weight (g)	Ventilatory status*	Glucose intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Lipid intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Amino acid intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Nonprotein energy ($g \cdot kg^{-1} \cdot d^{-1}$)
Glutamine ($n = 6$)	28 ± 2	1085 ± 200	MV: 5 SO: 3 RA: 1	9.8 ± 1.7	1.7 ± 0.5	2.0 ± 0.1	54.8 ± 5
Control ($n = 7$)	30 ± 3	1130 ± 300	MV: 4 SO: 3 RA: 3	11.5 ± 2.6	1.8 ± 0.2	2.0 ± 0.3	62.1 ± 10.6
P†	NS	NS		NS	NS	NS	NS

* MV, mechanical ventilation (including continuous positive airway pressure); SO, supplemental oxygen (Fio_2 >21%); RA, room air with no supplemental support.

† Glutamine group *vs* control group as tested by unpaired one-tailed *t* test.

two groups: 79 ± 16 μM in the glutamine group *versus* 69 ± 31 μM in the control group.

Steady-state conditions, as defined by coefficients of variation <10%, were obtained during the last hour of tracer infusion for plasma KIC concentration, [^{13}C]KIC and [^{13}]CO_2 enrichments (Fig. 2), therefore allowing the use of steady-state equations for calculation of leucine kinetics. Plasma KIC concentrations were not different between the glutamine group and the control group (25 ± 10 μM *versus* 28 ± 6 μM). Ra_{CO_2}, measured by using labeled bicarbonate infusion, was not different between the two groups: 7.6 ± 2.0 mL · kg^{-1} · min^{-1} in the glutamine group *versus* 8.7 ± 2.5 mL · kg^{-1} · min^{-1} in the control group.

Ra_{Leu} was approximately 24% lower ($p < 0.01$) and B_{Leu} was reduced by approximately 17% in the glutamine group ($p < 0.05$; Table 3), compared with controls. Glutamine infusion was associated with an approximately 35% reduction in Ox_{Leu} as well ($p < 0.05$). Yet NOLD, an index of whole-body protein synthesis, was approximately 20% lower in the glutamine group, compared with the control group ($p < 0.05$). Net leucine balance, defined as the difference between total leucine intake and total leucine irreversible loss (Ox_{Leu}), was positive in five of six infants in each group, and was therefore significantly different from zero in each group. Net leucine balance did not differ between the two groups.

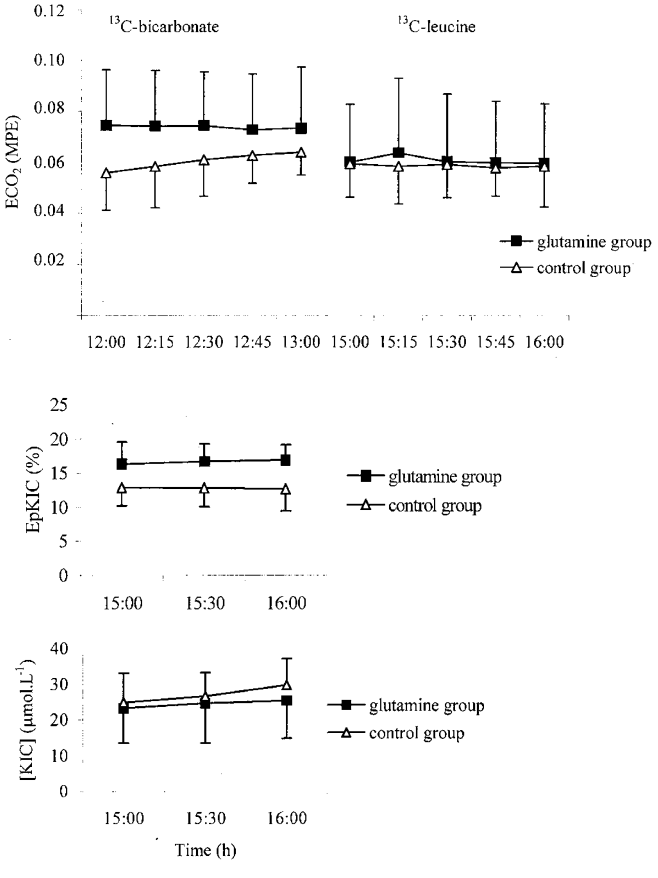

Figure 2. Time course of plasma [^{13}C]KIC enrichments, E[^{13}]$_{CO_2}$ enrichments, and plasma KIC concentrations, during [^{13}C]bicarbonate and [^{13}C]leucine perfusion in VLBW infants receiving an i.v. glutamine supplement, or an isonitrogenous glutamine-free amino acid supplement.

DISCUSSION

The specific aim of the current study was to determine whether a short, i.v. glutamine infusion, compared with an isonitrogenous amino acid mixture, was able to acutely affect protein metabolism in parenterally fed VLBW infants in the first few days of life. Our findings suggest that glutamine acutely suppressed rates of protein breakdown and leucine oxidation, but decreased rates of NOLD, an index of whole body protein synthesis, while both groups were receiving a total amino acid intake of 2 g · kg^{-1} · d^{-1}. Further studies would be required to determine whether more prolonged glutamine administration, along with higher protein intakes, or in more acutely ill preterm infants, would enhance overall protein accretion in VLBW infants.

Even though glutamine was only administered for <24 h, significant changes in leucine kinetics were detectable in the glutamine-supplemented group during the last 3 h of glutamine infusion, compared with the group receiving an isonitrogenous control amino acid mixture. The change observed in Ox_{Leu} (−35%) is of the same magnitude as reported in other populations. For instance, oral glutamine decreased Ox_{Leu} by approximately 37% in healthy adult volunteers (11), approximately 26% in adult volunteers made slightly catabolic by prednisone treatment (12), and approximately 35% in 8- to 13-y-old children suffering from Duchenne muscular dystrophy, a chronic disease characterized by chronic, relentless muscle protein wasting (13). Whereas the latter two studies were conducted in the fasted state, the current study is the first to demonstrate that glutamine can suppress Ox_{Leu} in the fed state as well.

The mechanism for the glutamine-induced decline in Ox_{Leu} remains to be elucidated. A decline in Ox_{Leu} may either be *1*) as a consequence of an increase in protein synthesis or *2*) as a consequence of direct inhibitory effect of glutamine on Ox_{Leu}.

A rise in protein synthesis was not detectable in the current study, as NOLD was in fact lower in the glutamine-supplemented group. Inasmuch as NOLD reflects the summation of the synthetic rates of a host of different proteins in various organs, this finding does not, however, rule out a putative stimulation of protein synthesis by glutamine in specific tissues. For instance, if skeletal muscle is a prime target of the putative protein anabolic effect of glutamine (1), the fact that skeletal muscle accounts for a much smaller fraction of body protein stores in VLBW infants, compared with adults, may explain why an increase in NOLD was not detected.

Alternatively, glutamine may primarily inhibit Ox_{Leu} rather than enhance protein synthesis. Although Ox_{Leu} is known to be tightly dependent on circulating leucine concentration (20), and although plasma leucine concentrations were not measured in the current study, the reduction in Ox_{Leu} is unlikely to result from a lower plasma leucine concentration: plasma KIC concentrations indeed were not different between groups (25 ± 10 μM *versus* 28 ± 6 μM), and plasma leucine usually correlates well with plasma KIC levels. Alternatively, glutamine can be readily used as a source of energy, because its conversion by glutaminase and glutamate dehydrogenase leads to α-ketoglutarate, which can, in turn, be oxidized in the tricarboxylic acid

Table 3. *Leucine metabolism in parenterally fed VLBW infants receiving an i.v. supplement made of natural L-glutamine (glutamine group) or of an isonitrogenous amino acid mixture (control group) on the fourth day of life**

Group, Patient No.	BW (g)	GA (wk)	Ra_{Leu}	PN_{Leu}	$i^{13}C_{Leu}$	B_{Leu}	Ox_{Leu}	NOLD	NOLD-B
Glutamine									
1	1320	28	168	24	38	144	65	141	−3
2	840	26	293	45	60	248	93	259	12
3	860	26	256	48	58	208	83	232	23
4	1220	28	225	42	41	183	53	212	29
5	1050	28	275	45	48	230	51	272	42
6	1220	31	221	49	41	171	64	199	28
Mean ± SD	1085 ± 202	28 ± 2	240 ± 45	42 ± 9	48 ± 9	197 ± 39	68 ± 17	219 ± 47	22 ± 15
Controls									
1	820	26	334	74	61	260	105	290	30
2	1350	33	290	76	37	214	ND	ND	ND
3	1060	28	341	79	47	262	100	289	26
4	850	28	339	102	59	237	162	236	−1
5	1350	33	257	73	37	184	74	220	36
6	880	27	279	73	57	206	87	249	43
7	1610	32	364	75	31	289	97	299	9
Mean ± SD	1131 ± 308	30 ± 3	315 ± 40	79 ± 10	47 ± 12	236 ± 37	104 ± 30	264 ± 33	24 ± 17
P†	NS	NS	0.004	0.00002	NS	0.047	0.014	0.044	NS

* All leucine kinetic variables are expressed in $\mu mol \cdot kg^{-1} \cdot h^{-1}$.

BW, birth weight; GA, gestational age; ND, not determined.

† Glutamine group *vs* control group, as tested by unpaired one-tail *t* test.

cycle. Glutamine is indeed known to be a preferred source of energy for rapidly dividing cells (21). We speculate that enhanced glutamine availability may increase glutamine oxidation and produce NADH, thus affecting cellular redox status. Changes in redox status are known to alter Ox_{Leu} through inhibition of branched-chain keto acid dehydrogenase, a key enzyme in leucine catabolism: for instance long-chain FFA, another prominent source of energy, are known to inhibit branched-chain keto acid dehydrogenase (22, 23).

In the current study, glutamine supplementation was associated with a significant reduction in B_{Leu} as well. This result is consistent with our earlier studies: in another group of VLBW infants, enteral glutamine delivery was associated with a trend toward a reduction in B_{Leu}; in that previous study, the approximately 16% decline, however, failed to reach statistical significance (24). Besides a type II statistical error, several differences in study design can, however, account for the difference: in that earlier study, glutamine was supplied via the enteral route, and the dose was only 40% of that used in the current study (0.2 *versus* 0.5 $g \cdot kg^{-1} \cdot d^{-1}$). In earlier studies in adults, glutamine did not alter rates of proteolysis (11), yet oral glutamine was associated with an approximately 8% decline in proteolysis in children with muscular dystrophy (13). The mechanism for this effect remains to be found. Because insulin inhibits proteolysis in a dose-dependent fashion in adult humans (25), glutamine may, in theory, suppress proteolysis through enhanced insulin secretion. This is, however, unlikely as *1*) proteolysis seems to be somehow insulin resistant in neonates (26), and amino acids may be the main factor regulating proteolysis in the neonatal period; and *2*) moreover, although insulin levels were not measured in the present study, we failed to observe any rise in insulin secretion in healthy adults given large oral doses of glutamine in previous studies [(11), and Darmaun *et al.* unpublished personal results]. In earlier studies, we found glutamine utilization rate and metabolic clearance rate (glutamine utilization rate divided by plasma glutamine concentration) to be much higher in VLBW infants than older children (24). The higher clearance rate of plasma glutamine may point to a greater glutamine requirement in VLBW infants. It is tempting to speculate that lack of sufficient glutamine availability in the VLBW infants receiving glutamine-free amino acid solutions may lead to enhanced rates of protein breakdown to produce the larger amounts of glutamine needed by fast-growing tissues. The acute reduction in rates of protein breakdown observed on i.v. glutamine infusion in the current study is compatible with the latter hypothesis.

Glutamine supplementation failed to enhance NOLD, an index of protein synthesis, in the current study, and was in fact even associated with a reduction in NOLD (Fig. 3). Although

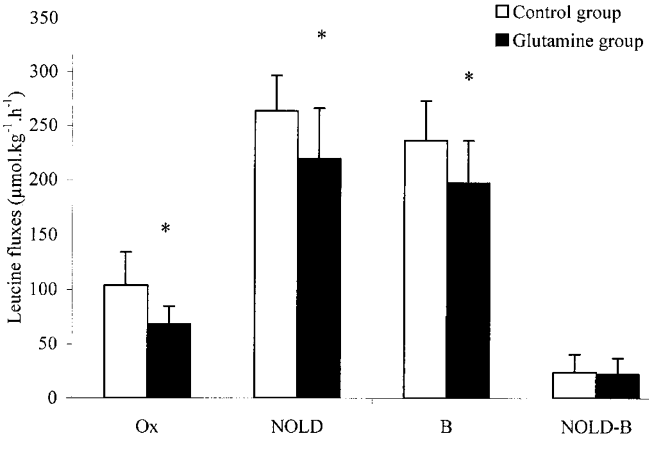

Figure 3. Leucine kinetics in VLBW neonates receiving i.v. supplementation with glutamine or with a control, glutamine-free, isonitrogenous amino acid mixture on the fourth day of life. $*p < 0.05$, glutamine group *versus* control group, as tested by unpaired, one-tail *t* test.

this contrasts with the protein anabolic effect of glutamine observed in healthy adults (11), failure of glutamine to enhance NOLD was, in fact, observed in prednisone-treated adults (12) and children with Duchenne muscular dystrophy (13) as well. In all three clinical situations, insufficient amino acid availability may preclude the protein anabolic effect of glutamine as *1*) our earlier studies (12, 13) were conducted in fasting subjects, and *2*) in the current study, our patients only received a total amino acid intake of 2 $g \cdot kg^{-1} \cdot d^{-1}$ on the fourth day of life, and would have reached their optimal intake of 3–3.5 $g \cdot kg^{-1} \cdot d^{-1}$ by d 6. This is because the introduction of amino acids has traditionally been prudent in the first day of life in VLBW infants, and amino acid intake gradually advanced thereafter, owing to concerns about the ability to metabolize amino acids with the putative risk of eliciting supraphysiologic elevations of plasma amino acid concentrations or blood urea nitrogen, and metabolic acidosis. Such a prudent approach may not be warranted as a recent study (27) suggests that 3 $g \cdot kg^{-1} \cdot d^{-1}$ parenteral amino acid intake from the first day of life may be well tolerated and associated with significantly greater rates of protein accretion. Further studies would be required to determine whether the same dose of glutamine, supplied for a longer time, and along with a more generous amino acid intake (*e.g.* 3 $g \cdot kg^{-1} \cdot d^{-1}$), would enhance rates of whole body protein synthesis.

In conclusion, although glutamine supplementation failed to enhance estimates of protein synthesis, i.v. glutamine may preserve body protein as it acutely suppressed rates of Ox_{Leu} and B_{Leu} in VLBW infants on the fourth day of life. Further studies would be warranted to determine whether prolonged glutamine infusions with a more generous protein intake would promote protein accretion in this patient population.

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