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Effect of Intravenous Amino Acids on Protein Kinetics in Preterm Infants

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Abstract

Purpose of review—To summarize recent findings of the effects of intravenous amino acids on protein kinetics in low birth weight infant, and to describe the potential cellular mechanism for these observations.

Recent findings—Intravenously administered amino acids for 3-5 hours in babies have been shown to suppress whole body proteolysis. Recent data in low birth weight babies show that an increase in the dose of amino acid caused a suppression of proteolysis and a decrease in the rate of glutamine and urea synthesis. However, these responses returned to basal state when the amino acid infusion continued for 20-24h. Supplementation with glutamine sustained the suppression of proteolysis after 3-5 days. Plasma insulin concentration did not change during the amino acid infusion. Data from studies in adults and from *in vitro* studies suggest that the amino acids impact protein breakdown and synthesis via the mTOR pathway, stimulating initiation of translation and suppressing autophagic proteolysis.

Summary—Intravenous amino acids, by increasing extracellular amino acid concentration, transiently stimulate protein synthesis and suppress protein breakdown. These effects return to basal state when the amino acid infusions are prolonged. The mechanism of this adaptive response remains to be determined.

Keywords

Amino acids; low birth weight; protein kinetics; phenylalanine; glutamine; urea

Introduction

Nutritional care of the prematurely born low birth weight infant, aimed at achieving intrauterine rate of growth and nitrogen accretion, continues to be the major challenge for the clinical neonatologist. Data from a number of studies show that despite the best efforts, a significant proportion of these babies fail to achieve the corresponding fetal rate of growth and are small for their conceptional age at the time of discharge from the Neonatal Intensive Care Unit (1-3).

Early enteral nutrient administration, although desirable for its known clinical and physiological benefits, is difficult to accomplish because of the functional immaturity and discordant motility of the gut. Therefore parenteral nutrition remains the major modality of nutritional administration, particularly for extremely low birth weight babies. Current clinical practice recommends that parenteral amino acids be initiated as early as possible after birth, although the dose of amino acids (protein) remains controversial (4,5). In addition, safety

concerns in relation to “tolerance” or the ability to metabolize the amino acids administered, specifically phenylalanine and methionine, caused clinicians to start parenteral amino acids in lower doses than the estimated requirements based upon fetal nitrogen accretion rates. However, studies by Denne and colleagues have demonstrated the low birthweight infant's ability to hydroxylate phenylalanine (6). In addition, Thomas and colleagues have shown the inducibility of methionine metabolizing enzymes in response to methionine load in low birth weight babies (7). Thus a high dose, $3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, parenteral amino acid initiated soon after birth has rapidly become part of the routine care of prematurely born infants. Although such a regimen has been shown to result in positive nitrogen balance, its impact on whole body protein dynamics has only been recently delineated. The purpose of the present review is to present recent physiological data examining the effect of parenteral amino acid administration on the whole body rates of protein and nitrogen metabolism in LBW infants. This has been made possible by our ability to study these infants in the neonatal period using stable isotopic labeled tracers. Advances in neonatal care have allowed these babies to rapidly transition to extrauterine environment and achieve a clinically stable state, thus permitting carefully conducted tracer isotope studies of protein metabolism.

Protein kinetics in infants

Data on protein kinetics in preterm infants are confounded by (a) the clinical characteristics of the study population, (b) the dose and duration of intravenous amino acid administered, and (c) the tracer amino acid used for the quantification of protein turnover. Since very preterm infants are sick during the immediate period after birth due to acute respiratory distress and vascular instability, require significant clinical support, and are often receiving vasopressor agents, they are in a “catabolic” state and negative nitrogen balance. Therefore the kinetic data obtained during this period after birth should be interpreted in this context when compared with clinically stable and growing full term infants, or with those preterm infants who have recovered from their acute illness and are growing and gaining weight. As discussed below, and also observed in adults, the duration and dose of amino acids administered, and possibly the amount of calories delivered, may have important impact on the measured rates of protein turnover. Finally, the tracer amino acid employed may also influence the measurement of protein turnover. Although conceptually any essential amino should give similar rates of protein turnover, each amino acid, because of its unique metabolic characteristics, may have different rates of oxidation and non-oxidative disposal so that quantification of rates of protein synthesis may not necessarily be the same with different amino acid tracers (8). In addition, the measurement of whole body rate of protein turnover will also be influenced by the relative rates of turnover and composition of several different body proteins.

Using the stochastic model and $[1-^{13}\text{C}]$ leucine tracer, a number of investigators have examined the effect of intravenous amino acid infusion on whole body rate of protein synthesis, breakdown and oxidation (9-16) in low birth weight infants. By design, and for clinical reasons, these studies involved the administration of amino acids at varying doses for variable *length of time (12 hours to 3 days)* prior to tracer isotope study. These data show that intravenous infusion of commercially available amino acid mixtures did not impact the rate of appearance (R_a) of leucine, was associated with a higher rate of oxidation or decarboxylation of leucine, and by inference, a higher rate of non-oxidative disposal of leucine. These data were interpreted to show that in preterm infants, amino acid infusion did not impact protein breakdown but did result in increased rate of protein synthesis and in improved nitrogen balance.

These data are in contrast to the studies in healthy full term or preterm infants. Since these infants did not require amino acid administration continuously for clinical considerations, the response to intravenous amino acids was examined following a *short 5-hour infusion*. These data showed that amino acid infusion suppressed whole body proteolysis, measured by leucine

or phenylalanine tracer, and increased protein synthesis in infants born prematurely and in those born at term gestation. The magnitude of decrease in proteolysis was noted to be less in extremely premature infants in one study (6). The only exceptions are the data by Poindexter *et al* (17) which showed a lack of suppression of proteolysis in premature neonates. However, these infants had been enterally fed and were studied 3-4 hours after their last meal.

These data suggest that acute increase in plasma amino acid in newborn infants, irrespective of their maturity (gestational age), results in suppression of whole body protein breakdown, an effect that is not evident when the studies are done after a prolonged amino acid infusion.

Two recent studies by our group examined the effect of dose and duration of parenteral amino acid infusion on the whole body rates of proteolysis (phenylalanine Ra), leucine nitrogen Ra, de novo glutamine synthesis and the rates of protein oxidation as measured by rates of urea synthesis (18,19). Low birth weight babies weighing less than 1500 g and ≤ 32 weeks gestational age were studied either within 48 hours after birth (during extrauterine transition) when they were acutely ill (Table 1), or when they had recovered and were on minimal support (Table 2). The kinetic parameters were measured by infusing various stable isotopic labeled tracers simultaneously. As shown, an increase in the dose of parenteral amino acids from 1.5 to 3.0 g.kg⁻¹.d⁻¹ resulted in a decrease in phenylalanine Ra (short study – 5 h). The suppression of proteolysis was associated with a lower rate of urea synthesis. An increase in the rate of amino acid infusion resulted in a higher rate of leucine N Ra, and probably an increase in the rate of leucine transamination. However, there was no change in the rate of de novo glutamine synthesis in the acutely sick infants, while glutamine synthesis decreased when the infants were studied during the recovery period. The quantitative differences in the rate of glutamine synthesis representing cataplerotic carbon efflux from the tricarboxylic acid cycle may be the consequence of higher energy demands and therefore higher rate of amino acid oxidation during acute illness.

In contrast to the effect of acute increase in the amount of amino acid administered, when the amino acid infusion was continued for an extended period (20-24 h), the inhibitory effect of the higher dose of amino acids on protein breakdown was not evident. As shown in Tables 1 and 2, the endogenous rate of appearance of phenylalanine was not different when amino acids were given for a prolonged period. This lack of suppression of proteolysis was associated with a higher rate of leucine N turnover, higher rate of de novo glutamine synthesis, and a higher rate of irreversible loss of nitrogen-urea synthesis. These data show that the responses to acute increase in parenteral amino acid administration are transient suppression of proteolysis and protein oxidation followed by adaptation, characterized by oxidative disposal of the higher amino acid load.

In contrast to the transient effect of intravenous amino acids on the rate of protein breakdown, glutamine supplemented total parenteral nutrition, when given for 3-5 days in clinically stable low birth weight infants, was associated with a lower rate of appearance of phenylalanine and glutamine (20) (Table 3). These data suggest that supplemental glutamine with parenteral amino acids resulted in a lower rate of whole-body protein breakdown and suppression of de novo glutamine synthesis. It is important to underscore that the infants in this study were clinically stable, however, their protein and energy intakes were similar (-70 kcal.kg⁻¹.d⁻¹) to those babies studied without supplemental glutamine (Tables 1 and 2).

Intravenous amino acids and protein breakdown

A number of studies have examined the effect of intravenous amino acid infusion on whole body rate of proteolysis in human adults by quantifying the rate of appearance of an essential amino acid such as leucine or phenylalanine. These data show that intravenously administered amino acids (for 3-5h) result in a lower rate of appearance of the traced essential amino acid

and, by inference, a lower rate of protein breakdown (21-23). Compartmental examination by combining organ balance with systemic tracer studies showed that amino acids caused suppression of protein breakdown in both leg (skeletal muscle) and in splanchnic tissues (21, 24). The suppression of proteolysis in skeletal muscle has not been consistently observed in other studies (25,26). Svanberg *et al* (27) showed that while amino acid infusion suppressed the degradation of globular proteins across the arm and leg in healthy adults, they did not effect the degradation of myofibrillar proteins, measured by 3-methylhistidine release. In addition, these investigators did not observe any change in plasma concentration of insulin, IGF-1, IGFBP-1 and IGFBP-3 in response to sequentially increasing dose of amino acid infusion (0.1 to 0.8 g N.kg⁻¹.d⁻¹). Administration of amino acids has not been shown to increase plasma insulin concentration consistently in studies by others (21,22,25). Finally, Nygren and Nair showed that amino acids had a profound inhibitory effect on splanchnic protein breakdown which was independent of insulin (24).

The response to a prolonged infusion of amino acids on whole body protein breakdown has not been reported in healthy adults. As discussed above, the data in preterm infants show that the suppressive response to amino acid infusion was transient and was not evident when the amino acid infusion was continued for a prolonged period (18,19), the only exception being when amino acids were supplemented with glutamine (20), where a prolonged infusion resulted in a sustained effect on protein breakdown.

The mechanism by which amino acid infusion causes suppression of proteolysis remains to be delineated. The major proteolytic pathways in the cell include the autophagic/lysosome pathway and the ubiquitin-proteasome pathway. The relative contribution of these two pathways in organ/tissue protein turnover has not been determined. Since in most visceral tissues, including the liver, the autophagic pathway is responsible for the bulk of proteolysis, and is most sensitive to amino acid regulation (reviewed in 28), it is hypothesized that acute suppression of proteolysis observed *in vivo* may be mediated through this mechanism. In addition, this pathway is not sensitive to insulin, and is consistent with Nygren and Nair's study of amino acid infusion and splanchnic protein kinetics *in vivo* in humans (24). Data from isolated organ and cell systems suggest that amino acids activate the mTOR pathway via a plasma membrane receptor or sensor, and mTOR in turn has negative influence on the lysosome/autophagy system, resulting in decrease in protein breakdown (Fig. 2). How this system responds to continued amino acid infusion or whether it is downregulated by sustained increase in extracellular amino acids remains to be determined.

In relation to the effects of glutamine, Häussinger *et al* (29) suggest that glutamine's antiproteolytic effects may be mediated through osmotic swelling and p38^{MAPK} pathway.

Amino acids and protein synthesis

Studies in healthy humans have demonstrated the stimulatory effect of amino acids on skeletal muscle protein synthesis by measuring the incorporation of tracer amino acids such as leucine or phenylalanine into mixed muscle protein or myofibrillar protein (23,26,30-33). These data show that amino acids, in particular branched chain amino acids and specifically leucine, stimulate skeletal muscle protein synthesis through an Akt-independent pathway. This has been further identified to be via activation of mTOR which, in turn, activates p70^{S6K} and dephosphorylates 4EBP-1, leading to translation initiation and protein synthesis (34-37). Bohé and colleagues have examined the time course of the response of human muscle protein synthesis to increase in availability of amino acids in plasma (38). Their data show that during mixed amino acid infusion the rate of muscle protein synthesis did not change from basal in the first 0.5h but then rose rapidly to a peak after 2 hours and then declined rapidly to the basal value (Fig. 2). The authors interpreted their data to suggest that the accretion of muscle protein in response to continued availability of amino acids may be modulated by a mechanism that

limits the amount of protein that can be stored in the muscle. After the initial requirement for protein synthesis are met, the excess amino acids are disposed via oxidation, ureagenesis and gluconeogenesis. In a companion study (39), the same group of investigators demonstrated that the skeletal muscle protein synthesis was modulated by the extracellular and not intracellular amino acid availability. The latter data are of interest in that they point to some plasma membrane receptor or sensor for the amino acids and intracellular transduction. The mechanism for the downregulation of protein synthesis in the presence of continued availability of amino acids remains to be elucidated.

Summary

Intravenous amino acid infusion in preterm babies *in vivo* causes a suppression of whole body rate of protein breakdown. However, such an effect is transient and reverts to basal state with prolonged infusion of amino acids, and the excess amino acids are disposed of via oxidation and ureagenesis. In addition, the stimulatory effect of amino acid infusion on skeletal muscle protein synthesis has also been shown to be transient. Thus stimulation of protein synthesis (in skeletal muscle) and suppression of protein breakdown (whole body) in response to increased amino acid supply are transient. Following a period of protein accretion (repletion) the excess amino acids are utilized for energy production or gluconeogenesis. Data from various studies in healthy adults show that these effects of amino acids on protein dynamics may be mediated via the mTOR pathway independent of insulin. These observations may have important clinical implications, and suggest that the maximal effect of amino acids on protein accretion may be best accomplished by intermittently increased doses rather than sustained continuous infusions.

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Figure 1.

Synthesis and breakdown of proteins by amino acids. The increased concentration of extracellular amino acids, via a plasma membrane signal or sensor, causes activation of mTOR which via eIF4E and p70^{S6K} stimulates protein synthesis and suppresses protein breakdown by the autophagy/lysosome pathway. Amino acids, by stimulating insulin secretion, could also induce these changes via the Akt pathway. However, the stimulatory effect of amino acid administration on insulin secretion has not been consistently documented (see text for details).

Figure 2.

Changes in protein synthesis and protein breakdown across arm and leg muscle bed in response to increasing doses of amino acid infusion in healthy volunteers. Mixed amino acids were infused in increasing doses sequentially as indicated on x axis. Changes in protein synthesis and breakdown were measured by A-V balance and stable isotope labeled phenylalanine (Reproduced with permission, Svanberg *et al*, ref. 27). * $p < 0.05$ vs. basal; † $p < 0.05$ vs. peak value.

Figure 3. Time course of rate of synthesis of mixed muscle protein in response to amino acid infusion in healthy humans. The rate of protein synthesis was quantified by the incorporation of deuterium labeled leucine into mixed muscle protein (Reproduced with permission, Bohé *et al*, ref. 38).

Table 1

Effect of dose and duration of amino acid infusion on nitrogen kinetics in low birth weight infants during the immediate neonatal period

	Short Study (n=7)		Extended Study (n=5)	
<i>AA Load (g.kg⁻¹.d⁻¹)</i>	<i>1.5 (19h)</i>	<i>3.0 (5h)</i>	<i>1.5 (24h)</i>	<i>3.0 (24h)</i>
Endogenous phenylalanine Ra	60.4 ± 13.9	51.2 ± 14.2 *	65.5 ± 14.3	61.0 ± 9.1
Leucine N Ra	340.7 ± 8.0	427.4 ± 35.4 †	390.7 ± 84.2	542.6 ± 170.8 §
<i>de novo</i> Glutamine synthesis	384.4 ± 38.0	368.9 ± 38.2 †	369.7 ± 92.6	483.4 ± 97.5 †
Urea Ra	506.0 ± 140.2	426.6 ± 110.3 †	474.2 ± 336.3	851.6 ± 427.4 *

Values of rates of appearance (Ra) are $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$; mean ± std dev

Short study indicates initial amino acid load given for 19 hours, then switched to higher amino acid load for the next 5 hours.

Extended study indicates initial amino acid load given for 24 hours, then switched to higher amino acid load for the next 24 hours

From Kadrofske et al (19)

* P < 0.01.

† P < 0.001

§ P < 0.05 as compared to different amino acid load.

Table 2

Effect of intravenous amino acid infusion on nitrogen kinetics in clinically stable low birth weight babies

	Short Study (n=12)		Extended Study (n=5)	
AA Load ($\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$)	1.5 (20h)	3.0 (5h)	1.5 (20h)	3.0 (20h)
Endogenous Phenylalanine Ra	71 $\pm$ 16	64 $\pm$ 14*	61 $\pm$ 17	74 $\pm$ 16
Leucine N Ra	419 $\pm$ 39	573 $\pm$ 77*	398 $\pm$ 57	657 $\pm$ 92*
de novo Glutamine Synthesis	378 $\pm$ 78	386 $\pm$ 67	411 $\pm$ 47	522 $\pm$ 53 [†]
Urea Ra	523 $\pm$ 153	480 $\pm$ 135 [†]	364 $\pm$ 107	560 $\pm$ 74 [†]

values of rates of appearance (Ra) are $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; mean $\pm$ std dev

Short study indicates initial amino acid load given for 20 hours, then switched to higher amino acid load for the next 5 hours.

Extended study indicates initial amino acid load given for 20 hours, then switched to higher amino acid load for the next 20 hours

From Parimi et al (18)

* $P < 0.001$,

[†] $P < 0.01$ as compared to different amino acid load.

Table 3
Effect of Parenteral Glutamine Supplement on Whole Body Nitrogen Metabolism

	Total amino acids	Ra Phenylalanine	Ra Glutamine	Ra Urea
	$\mu\text{mole.L}^{-1}$	$\mu\text{mole.kg}^{-1}.\text{h}^{-1}$	$\mu\text{mole.kg}^{-1}.\text{h}^{-1}$	$\mu\text{mole.kg}^{-1}.\text{h}^{-1}$
Control (n=10)	2422 $\pm$ 650	97.7 $\pm$ 23.7	520.9 $\pm$ 97.7	655.5 $\pm$ 259.6
Glutamine (n=10)	2127 $\pm$ 423	62.2 $\pm$ 16.7*	372.3 $\pm$ 65.6 [§]	756.4 $\pm$ 231.2

Data are mean $\pm$ std dev

Babies <32 weeks gestation were placed on parenteral amino acids between day 1 and 2 after birth, and tracer isotope studies were performed on day 6 or 7 when they had been on protein intake of 3.0 g.kg⁻¹.d⁻¹. The glutamine group was supplemented with 0.6 g.kg⁻¹.d⁻¹ intravenous glutamine. Energy intake: Controls 73.6 $\pm$ 24.8 kcal.kg⁻¹.d⁻¹; Glutamine 70.5 $\pm$ 24.4 kcal.kg⁻¹.d⁻¹. Ra = rate of appearance of phenylalanine or glutamine corrected for exogenous infusion.

From Kalhan et al (20)

*
p=0.001,

[§]
p=0.003 compared with controls.