Protein and Amino Acid Metabolism in the Human Newborn

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Abstract
Birth and adaptation to extrauterine life involve major shifts in the protein and energy metabolism of the human newborn. These include a shift from a state of continuous supply of nutrients including amino acids from the mother to cyclic periodic oral intake, a change in the redox state of organs, thermogenesis, and a significant change in the mobilization and use of oxidative substrates. The development of safe, stable isotopic tracer methods has allowed the study of protein and amino acid metabolism not only in the healthy newborn but also in those born prematurely and of low birth weight. These studies have identified the unique and quantitative aspects of amino acid/protein metabolism in the neonate, thus contributing to rational nutritional care of these babies. The present review summarizes the contemporary data on some of the significant developments in essential and dispensable amino acids and their relationship to overall protein metabolism. Specifically, the recent data of kinetics of leucine, phenylalanine, glutamine, sulfur amino acid, and threonine and their relation to whole-body protein turnover are presented. Finally, the physiological rationale and the impact of nutrient (amino acids) interventions on the dynamics of protein metabolism are discussed.
INTRODUCTION
The transition from intrauterine environment resulting in a change from a state of total dependence upon the mother to an independent extrauterine environment is perhaps the most abrupt physiologic and metabolic event in life. It is associated with marked surges in several hormones, initiation of ventilation, regulation of temperature, initiation of endogenous glucose production, lipolysis, expression of several key enzymes, and a number of other, probably heretofore, unrecognized processes (for a review, see 61). Although a vast majority of neonates transition to the extrauterine environment uneventfully, a number of infants, in particular those born prematurely, develop significant clinical problems. Major advances in the care of these infants, such as ventilatory support, surfactant replacement therapy, and antepartum glucocorticoids, have markedly improved their survival yet at the same time made their nutritional care a challenge for the physician (28, 53). Additionally, because of their clinical problems, it is difficult to obtain true normative physiological data against which abnormal states can be compared. Thus, the physiological data obtained during the immediate neonatal period require careful evaluation, considering the numerous confounding variables present. In spite of these difficulties, a number of careful, controlled studies have been conducted using noninvasive stable isotopic tracers and body composition and energy balance measurements in newborn infants born prematurely and in those born at term gestation. In this review, we present the contemporary data on protein and amino acid metabolism and their clinical implications in the human newborn infant, focusing entirely on the human neonate with brief reference to animal data where appropriate. The present review complements several recent reviews on this subject (4, 15, 26, 57, 58, 89, 97, 99).

Since protein metabolism is closely related to energy metabolism, it is useful to examine it in the context of the fluxes of oxidative substrates and rates of energy consumption. A number of investigations have shown that newborn infants, soon after birth, are able to establish a steady rate of production of glucose, lipolysis, and energy consumption. Estimates of glucose kinetics, using stable isotopic tracers, reveal that healthy newborn infants produce glucose at the rate of 5–8 mg·kg\(^{-1}\)·min\(^{-1}\) (or 28–45 \(\mu\)moles·kg\(^{-1}\)·min\(^{-1}\)), of which \(\sim 50\%–70\%\) is contributed by gluconeogenesis (9, 54, 55, 59, 118). The rate of lipolysis quantified by the rate of appearance of glycerol or fatty acid corresponds to 6–12 \(\mu\)moles·kg\(^{-1}\)·min\(^{-1}\) (1, 12, 88, 115–117). All of these rates expressed on a weight-specific basis are significantly higher than those in healthy adults. The contribution of glucose to oxidative metabolism during fasting has been estimated to be \(\sim 50\%\) in these studies, corresponding to the estimated nonprotein respiratory quotient of 0.88 (54). The irreversible loss of protein or rate of oxidation of protein/amino acid, quantified from the rate of urea synthesis, corresponds to 0.8 g protein kg\(^{-1}\)·d\(^{-1}\) (56). Estimate of energy consumption using indirect calorimetry show that neonates consume oxygen at 6–9 ml·kg\(^{-1}\)·min\(^{-1}\), corresponding to 50–70 kcal·kg\(^{-1}\)·d\(^{-1}\) (3, 27, 32, 80, 126). The weight-specific rates for preterm infants are reported to be higher for all of the above parameters (4). Finally, the rates of oxygen consumption are reported to be lower during the first few days after birth, especially in prematurely born infants who have been on ventilatory...
support, possibly related to the lower work of breathing owing to assisted ventilation (27, 32, 126). In the following sections, data on metabolism of amino acids, for which new information is available, are summarized, followed by a brief discussion of their clinical applications.

**METABOLISM OF AMINO ACIDS**

**Leucine**

The branched-chain amino acids (BCAAs), leucine, isoleucine and valine, have been extensively studied in vivo and in vitro because of the unique role of leucine in protein synthesis in skeletal muscle. The pioneering studies of Matthews, Young, Bier and coworkers (73, 75), which quantified leucine C and N metabolism in vivo in humans using [1-13C]leucine and [1-13C,15N]leucine tracers, have led the way to a large literature on leucine metabolism in adults and in newborns in a variety of physiological and pathophysiological states. Leucine is unique because it promotes protein synthesis by regulating translation initiation, inhibits protein degradation, and stimulates the secretion of insulin (65, 109). BCAAs are metabolized via reversible transamination to produce corresponding branched-chain α-ketoacids, which in turn undergo irreversible decarboxylation catalyzed by branched-chain keto dehydrogenase complex (BCKDC) (13, 14). BCKD complex consists of multiple copies of BCKA decarboxylase (E1) dihydrolipoamide acyltransferase (E2) and dihydrolipoamide dehydrogenase (E3). The activity of BCKDC is regulated by both covalent and allosteric mechanisms. Harris and colleagues (39, 50) have suggested that the regulation of activity state of BCKDC by BCKD kinase and its phosphatase is critically important for growth, tissue repair, and maintenance of body protein. A developmental pattern for BCKDC expression has not been observed.

Hutson and colleagues (30, 46, 119, 121) have established that there are two isoforms of mammalian branched-chain amino transferase (BCAT): a mitochondrial form, expressed ubiquitously except in the liver in the rat, and a cytosolic form, expressed in large amounts in the brain, peripheral nerves, ovary, and placenta. BCAT catalyzes the transamination of BCAA with α-ketoglutarate and the formation of a number of nonessential amino acids and thus participates in the interorgan shuttle of nitrogen. Disruption of mitochondrial BCAT in mice resulted in elevated plasma BCAAs and decreased body weight and adiposity, along with increased protein turnover, energy expenditure, improved glucose and insulin tolerance, and protection against diet-induced obesity (107). During fetal and neonatal development, a decrease in BCAT activity in the skeletal muscle has been reported in sheep (30, 31). Such a change has not been observed in rats (51), and no such data are available for humans. The physiological significance of this developmental change is unclear.

BCAT, by shuttling nitrogen through various metabolic pools of nitrogen, plays an important role in the redistribution of nitrogen during states of protein catabolism and protein accretion such as growth. Studies have examined the relationship between transamination of leucine and synthesis of glutamine and urea in human pregnancy and in newborn infants during fasting and during enteral and parenteral nutrient administration (60, 63, 83). These data show that transamination of leucine is downregulated during states of protein accretion and positive nitrogen balance (60). Administration of nutrients, protein, or amino acids, either enterally or parenterally, is associated with an increase in the rate of transamination of leucine and with an increase in the flux of leucine N. During fasting, the whole-body rate of transamination of leucine is positively related with the rate of appearance of glutamine and with urea synthesis, underscoring the important role of transamination in interorgan shuttle of nitrogen from the periphery to the splanchnic tissues (63, 83). In contrast, during states of nitrogen accretion or positive nitrogen balance, such as during the fed state in growing infants or during suppression of proteolysis by parenteral nutrient administration...
in low-birth-weight babies, a negative relation between leucine N flux and urea synthesis is observed (52, 62, 84). The regulatory mechanism(s) for this shift in nitrogen flux toward urea during fasting and away from urea during protein accretion remains to be delineated.

Phenylalanine
Phenylalanine, an essential aromatic amino acid, has been used for quantification of protein kinetics in both adults and newborn infants. The primary routes of disposal of phenylalanine are its use for protein synthesis or its conversion to tyrosine by the phenylalanine hydroxylase system. In healthy human adults, the major sites of phenylalanine hydroxylation are the liver and the kidney (77, 122). Previous data from studies of human fetal liver in vitro had suggested a decreased ability of the human fetus to convert phenylalanine to tyrosine and thus provided a rationale for the addition of tyrosine to the parenteral amino acid mixtures. Kilani and colleagues (64), using [2H5]phenylalanine tracer, were the first to show that infants born at ∼29 weeks gestation were able to convert phenylalanine to tyrosine and that there was a substantial increase in the rate of conversion of phenylalanine to tyrosine following initiation of parenteral amino acid nutrition. These observations have since been confirmed by other investigators in studies of very-low-birth-weight babies (44) and in prematurely born infants studied at later times after birth and in response to varying parenteral amino acid mixtures (104). In this context, it is important to recognize that estimates of hydroxylation of phenylalanine were significantly less when determined using [3H3]phenylalanine tracer as compared with [1-13C]phenylalanine tracer, suggesting a possible isotopic effect with the deuterium-labeled tracer (21, 66, 72). A linear correlation between the whole body kinetics of leucine and phenylalanine has been observed in healthy newborn infants over a wide range ($r^2 = 0.66, p = 0.001$) (83). The relative ratio of leucine and phenylalanine rate of appearance (Ra) was higher than that observed in healthy adults and similar to that reported in low-birth-weight babies (124, 140). The higher ratio suggests a higher turnover rate of proteins enriched with leucine relative to phenylalanine in newborn infants.

Glutamine and Glutamate
Glutamine is the most abundant nonessential or dispensable amino acid in the blood and in the free amino acid pool in the body (148). It is synthesized by virtually every tissue in the body, although only certain tissues, e.g., skeletal muscle, brain, and lung, release it into the circulation in significant quantities. It plays an important role in the interorgan shuttle of nitrogen and carbon and is a primary oxidative fuel for enterocytes and lymphocytes. In addition, glutamine is a key substrate for ammonia production in the kidney, a precursor for purine and pyrimidine synthesis, and is suggested to play a role in the regulation of protein synthesis.

The importance of glutamine in whole-body metabolism is underscored by the recent report of congenital glutamine deficiency caused by mutation of glutamine synthase in two unrelated infants (36, 37). The infants were born with severe brain malformations and died in the immediate neonatal period as a result of multiorgan failure. Glutamine was largely absent from their serum, urine, and cerebrospinal fluid.

The rates of glutamine turnover and de novo synthesis of glutamine were quantified in healthy newborn infants by Parimi et al. (83). Whole-body rate of protein turnover and oxidation were measured simultaneously by quantifying phenylalanine and urea kinetics. These infants had not started to gain weight and accrete nitrogen, and possibly were in transition to the extrauterine environment. Therefore, these data may not be representative of growth and may have been influenced by the hormonal and other changes associated with birth and transition to the extrauterine environment. As shown in Table 1, the Ra of glutamine, measured using [5-15N]glutamine tracer, was similar in

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appropriate-for-gestational-age infants and in infants of diabetic mothers. Glutamine Ra was significantly higher in small-for-gestational-age infants. Expressed on a weight-specific basis, the Ra of glutamine and phenylalanine were higher than those reported in healthy adults and reflected the higher rate of weight-specific energy consumption by the neonate. In response to mixed-nutrient feeding, there was a significant decrease in the Ra of glutamine and urea in all infants. As shown in Table 1, de novo synthesis of glutamine was the major component of the glutamine turnover and contributed ~85% to the total Ra. Formula feeding resulted in a significant decrease in de novo synthesis of glutamine. The major source of glutamine carbon is the anaplerotic carbon flux (81) into the tricarboxylic acid (TCA) cycle as a consequence of high rate of protein breakdown, and therefore high amino acid flux, observed in newborn infants. Because transamination of BCAAs provides the major source of amino nitrogen for glutamine, the authors observed a significant linear relationship between simultaneously measured leucine N turnover and glutamine turnover ($r^2 = 0.59, p = 0.001$). In contrast, a negative correlation was seen between glutamine turnover and urea synthesis, underscoring the role of glutamine in nitrogen transport, not only for urea synthesis by the liver but also for other synthetic processes in the splanchnic compartment.

The key role of glutamine in the metabolism of gut was documented in a series of studies by Windmueller and colleagues in the late 1970s (147). Their data showed that glutamine is taken up in large quantities by the enterocytes in the jejunum and ileum from the arterial blood as well as from the gut lumen, in most laboratory animals studied. As much as 20%–30% of glutamine delivered via the arterial blood was taken up by the gut in the first pass, and the uptake was greatest in the intestinal epithelial cells. In the enterocytes, glutamine undergoes several metabolic transformations, resulting in the production of citrate, lactate, and carbon dioxide as the carbon end products and the formation of ammonia, alanine.
Table 2  Glutamine and phenylalanine kinetics in prematurely born infants receiving supplemental glutamine enterally

<table>
<thead>
<tr>
<th></th>
<th>Ra glutamine</th>
<th>Glutamine de novo synthesis</th>
<th>Phenylalanine–Ra</th>
<th>Urea Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>687 ± 117</td>
<td>632 ± 91*</td>
<td>599 ± 120</td>
<td>538 ± 92*</td>
</tr>
<tr>
<td>(9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>755 ± 152</td>
<td>644 ± 128*</td>
<td>655 ± 145</td>
<td>529 ± 117*</td>
</tr>
<tr>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Mean ± SD; Ra, rate of appearance.
* p < 0.001 compared with fasting, paired analysis.
* p < 0.05.
§ p < 0.001 compared with controls.
Data from Parimi et al. (84), reproduced with permission.

citrulline, and proline as the major nitrogen end products.

Studies in healthy adults showed that almost 75% of enterally administered glutamine was extracted in the splanchnic compartment and had no impact on whole-body rate of protein turnover (38). Data on the enteric uptake of glutamine in low-birth-weight prematurely born infants are qualitatively similar to those in healthy adults. There are no studies reporting the responses to enteral glutamine administration in healthy infants born at term gestation. Parimi et al. (84) examined the impact of enteral glutamine supplementation on whole-body leucine, phenylalanine, and urea kinetics in growing preterm infants (Table 2). Enteral glutamine did not have any effect on the tracer-measured whole-body rate of appearance of glutamine, neither in the fasting state nor in the fed state (when glutamine was being given with the feed), suggesting that enterally administered glutamine was entirely taken up and metabolized in the gut and did not enter the systemic circulation. In addition, enteral glutamine had no impact on the whole-body rate of protein turnover as measured by the rate of appearance of phenylalanine in the blood. Enterally nutrient administration did cause a decrease in de novo synthesis of glutamine, both in controls and in the glutamine-supplemented group. Of significance were the data on urea kinetics, which showed that enteral glutamine resulted in a marked and almost equimolar increase in urea synthesis (Table 2). As anticipated, enteral glutamine resulted in an increase in plasma concentration of arginine, citrulline, and ornithine. A lower dose of glutamine (0.2 g·kg⁻¹·d⁻¹) did not result in any change in plasma amino acids (105, 131). Darmaun et al. (23), using a lower enteral dose of glutamine (0.2 g·kg⁻¹·d⁻¹), also did not observe any change in systemic leucine and glutamine kinetics. Based upon these data, we speculate the following: Glutamine is rapidly taken up by the gut and converted to glutamate and ammonia by glutaminase. Glutamate serves as a respiratory fuel for the enterocytes and as a precursor for citrulline, ornithine, and alanine. Glutamate, in the liver, serves as a precursor for aspartate, which, along with ammonia, resulted in the observed increase in the production of urea following glutamine administration. The proposed schema is consistent with other data showing rapid first-pass uptake of glutamate by the gut in the neonate (40, 100), in adults (2, 74), and infant pigs (98).

**Response to parenteral amino acid.** Glutamine is synthesized from glutamate by glutamine synthase (EC 6.3.1.2). Catabolic efflux of carbons from the tricarboxylic acid (TCA) cycle via α-ketoglutarate with subsequent transamination with BCAAs leads to the synthesis of glutamate (81). Because there is an acute decrease in plasma glutamine concentration and in intramuscular pools of glutamine
Table 3  Effect of intravenous amino acid infusion on nitrogen kinetics in clinically stable low-birth-weight infants

<table>
<thead>
<tr>
<th>AA Load (g·kg−1·d−1)</th>
<th>Short study (n = 12)</th>
<th>Extended study (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 (20 h)</td>
<td>1.5 (20 h)</td>
</tr>
<tr>
<td></td>
<td>3.0 (5 h)</td>
<td>3.0 (20 h)</td>
</tr>
</tbody>
</table>

| Endogenous phenylalanine Ra | 71 ± 16               | 64 ± 14\*          |
| De novo glutamine synthesis | 378 ± 78              | 386 ± 67           |
| Urea Ra                     | 523 ± 153             | 480 ± 135\†        |

\*P < 0.001, \†P < 0.01 as compared with different amino acid load.

Values of rate of appearance (Ra) are μmol·kg−1·h−1, mean ± standard deviation. 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.

in the period immediately after birth or during acute illness, attempts have been made to enhance glutamine synthesis by infusing amino acids parenterally. An increase in anaplerotic influx by parenteral amino acid administration would be expected to increase the cataplerotic efflux and higher rate of de novo synthesis of glutamine. Data from studies in healthy adults show an increased de novo synthesis of glutamine across the forearm and leg in response to amino acid infusion (71). Supplementation of parenteral nutrition with analogs of α-ketoglutarate enhances the intracellular pools of glutamine in the skeletal muscle (142, 144).

The response to parenteral infusion of amino acids on glutamine turnover and urea synthesis in clinically stable low-birth-weight babies is displayed in Table 3 (86). Although an increase in amino acid infusion from 1.5 g to 3.0 g·kg−1·d−1 for a short duration (five hours) resulted in an increase in plasma glutamine concentration (from 338 ± 156 μmole/L to 437 ± 160 μmole/L), it had no effect on total rate of appearance of glutamine or on the rate of de novo synthesis of glutamine. In fact, an increase in amino acid infusion was associated with a decrease in the rate of urea synthesis, suggesting that acutely administered amino acid load resulted in an anabolic response so that the infused amino acids were utilized for protein synthesis. In contrast, when the amino acid infusion was prolonged for 20 hours, there was a significant increase in the rate of de novo synthesis of glutamine and an increase in the rate of urea synthesis. The changes in glutamine flux in relation to increased amino acid load are likely to be secondary to the changes in whole-body protein synthesis (discussed below). Because glutamine is a major nitrogen transporter from the periphery to the splanchnic compartment, an increase in glutamine turnover was associated with an anticipated increase in urea synthesis. Similar observations were made in acutely sick preterm infants by Kadrofske et al. (52), except that an acute increase in amino acid load in fact caused a decrease in the rate of de novo synthesis of glutamine. These data confirm the relationship between anaplerotic and cataplerotic fluxes and show that the rate of glutamine synthesis can be enhanced by increasing the influx of carbon into the TCA cycle in low-birth-weight infants.

SULFUR AMINO ACIDS

Methionine

Interest in the metabolism of sulfur-containing amino acids, i.e., methionine, cysteine, and taurine, in relation to development stems from the reports of Sturman, Gaull, and colleagues (33, 34, 114), which show the absence of transsulfuration pathway activity in the human fetal liver and thus the inability of the human fetus to synthesize cysteine from methionine. These authors suggested that cysteine is a “conditionally” essential amino acid for the fetus and for the neonate.
Methionine, an essential amino acid required for protein synthesis, is also a source of methyl groups for the methylation of nucleic acids (methylation of DNA in expression of various genes), protein, biogenic amines, and phospholipids (13). The metabolism of methionine is characterized by two components (Figure 1). The first is the ubiquitous transmethylation cycle, wherein methionine is transmethylated to homocysteine and remethylated back to methionine. The transmethylation cycle does not result in catabolism of methionine, but rather allows transfer of methyl groups in various methyltransferase reactions and recapture of the methyl group either from folate-dependent one-carbon pool (5-methyl tetrahydrofolate) or from (nonvitamin-dependent) betaine. The intermediates in the transmethylation cycle include the bioactive compound S-adenosyl methionine (SAM), which is also the universal methyl donor involved in multiple methyltransferase reactions. The various enzymes involved in methionine metabolism are identified in Figure 1; changes in their activity and expression during development or in the newborn infant are discussed below.

The catabolic (transsulfuration) pathway of methionine involves the condensation of homocysteine with serine to form cystathionine, catalyzed by cystathionine β synthase (CBS). Cystathionine is converted to cysteine, α-ketobutyrate, and ammonia by the pyridoxine-containing enzyme cystathionine γ lyase (CGL). The carbon skeleton of methionine is oxidized in the TCA cycle via α-ketobutyrate and propionyl CoA while the sulphydryl group condenses with serine to form cysteine. In the human adult, the transsulfuration pathway has a limited tissue distribution, being restricted to liver, kidney, intestine, and pancreas (13).

CGL (EC 4.4.1.1.) catalyzes the conversion of L-cystathionine into L-cysteine, α-ketobutyrate, and ammonia. Cysteine is required for the synthesis of glutathione and for synthesis of various proteins. CGL activity is not detectable or is absent in the liver of the human fetus throughout gestation (33, 96, 114). The activity is low, as compared with healthy adults, in prematurely born and full-term infants during the first few days after birth. Levonen et al. (96) observed that although the gene for CGL is expressed (its mRNA is present) in the liver of human fetuses as early as 15 weeks of gestation, the CGL protein or enzyme activity was not detected in the fetal liver through 42 weeks of gestation. In humans, two isoforms of mRNA from CGL have been characterized: a long and a short form (70). Expression studies show that it is the long isoform of CGL that causes an increase in the enzyme activity. Expression of the short isoform of CGL does not have any effect on enzyme activity. In the human fetus, the long isoform is more abundant in the liver throughout development (69). The mechanism of this post-transcription regulation of CGL remains unknown and is probably due to an inhibition of translation of CGL mRNA in the liver before birth or may be related to the stability of the protein.

In isolated liver explants from second-trimester human fetuses, the enzyme activity of CGL could be induced by dexamethasone, glucagon, or dibutylcyclic AMP (plus theophylline). Simultaneous treatment with cyclohexamide inhibited the increase in activity induced by various agents, which suggests that protein synthesis is required for the increase in activity (41, 42). In contrast, in vivo antepartum treatment of human mothers with pyridoxine or corticosteroids did not induce the activity of CGL (33, 150). Postnatally, there was an age-related increase in hepatic CGL activity, which reached a plateau by about three months of age (150). The postnatal data were obtained from autopsy studies, and the cause of death could have influenced the analyses.

In contrast to findings from studies in humans, significant cystathionase activity was present in the rat liver during development (33, 41). In adult rats, hepatic CGL activity was suppressed by insulin and increased by glucagon administration or by streptozotocin-induced diabetes (48, 49).
Examination of other tissues has demonstrated no CGL activity in the placenta and fetal brain, a significant enzyme activity in the kidneys and adrenals of prematurely born babies, and very low activity in the pancreas (150). Whether the CGL activity in the kidney can compensate for the low/absent enzyme activity in the liver remains unknown.

CBS (EC 4.2.1.22) irreversibly catalyzes the synthesis of cystathionine by condensing homocysteine with serine. Developmental expression studies show that CBS is present in human fetal liver and brain early in gestation, although the enzyme activity is much lower than that in adults (33, 114). In the rat, CBS activity is low or undetectable on day 14 of gestation and then rapidly increases by day 20, when it is 68% of the activity in maternal liver. In the placenta, CBS activity was noted to be high by day 10 followed by a marked decrease by day 16 (129).

Methionine-activating enzyme L-methionine S-adenosyl transferase (EC 5.6.1.6) is present in human fetal liver and brain early in gestation (33). In adult rats, treatment with glucagon does not affect the activity of this enzyme (49). SAH hydrolase activity is present in the rat embryo on day 9 of gestation and does not change significantly with advancing gestation (129). The activity of the two homocysteine methylating enzymes, N5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase) and betaine-homocysteine methyltransferase activity (BHMT), was measured by Gaull et al. (34) in human fetal liver and kidney. Their data showed that methionine synthase activity is higher in fetal liver and kidney and decreased to the levels in adults by term gestation. In contrast, BHMT enzyme activity is lower in the fetal liver and is similar to the levels in adults. BHMT in the fetal kidney increases with increasing gestation, although it is lower than that in adults at the time of birth. The activity of both of these enzymes is not effected by glucagon (49) or by streptozotocin-induced diabetes (48).

Data from the studies of expression and activities of various enzymes of methionine metabolism suggest that the transsulfuration pathway is absent in the fetal liver and appears for the first time after birth in the neonatal period. This appearance of transsulfuration is probably stimulated by the birth-associated decrease in plasma insulin, increase in glucagon and thyroid-stimulating hormone, and other changes (63). Since the transsulfuration pathway is also required for the synthesis of cysteine, the human fetus cannot synthesize cysteine from methionine and has been described as entirely dependent upon the mother for the supply of cysteine. For these reasons, cysteine has been considered a conditionally essential amino acid for newborn babies. However, clinical trials of cysteine supplementation have yielded negative results (151), and tracer studies do not show any limitations in cysteine synthesis from methionine in newborn babies (99). Recent data on healthy newborn infants and prematurely born low-birth-weight babies show that the human neonate develops the capacity to metabolize methionine via transsulfuration rapidly after birth (123a). In addition, these data show that in the presence of high methionine administration parenterally, in premature infants, there is a high rate of both transmethylation and transsulfuration compared with healthy adults. The high rate of transmethylation may be related to the high methylation demands for growth, cell proliferation, polyamine, and DNA synthesis in these babies, whereas the high transsulfuration may be for meeting the demands for cysteine and glutathione synthesis at the same time for the disposal of excess methionine. In the enterally fed piglet, the gastrointestinal tract has been shown to be a significant contributor to methionine metabolism (101, 108).

**Taurine.** Taurine (2-amino ethane sulfonic acid) is unique among amino acids in that it is not a constituent of any protein and is present in large concentrations in the free intracellular
amino acid pool. The intracellular concentration of taurine in many organs—heart, skeletal muscle, kidney, brain, and liver—is extremely high (16). Interest in the biological role during development was heightened in the 1970s, when taurine was found to be an essential amino acid for the cat. Taurine deficiency in the cat caused retinal degeneration, which could be reversed by taurine supplementation (112, 113). In addition, taurine deficiency was associated with reproductive failure, altered immune function, dilated cardiomyopathy, and myocardial failure (113). These changes were accompanied by decreased concentration of taurine throughout the body. It has been suggested that taurine participates in these events by osmotic regulation since it is present in such large concentrations in many tissues (47).

High concentrations of taurine have been reported in mature retina and in the developing brain. Data from studies in humans show that plasma taurine concentrations of formula-fed infants were lower than concentrations of infants fed human milk (95). This was attributed to the absence of taurine in the formulae and its presence in high concentration in human milk (19). Thus, taurine has been added routinely to most infant formulae since the mid 1980s. Only one randomized trial examined the impact of taurine supplementation in low-birthweight infants (128). Although this trial did not provide definitive clinical evidence of benefit (90), it did suggest that the infants assigned to the taurine supplemented formula had more mature auditory brainstem-evoked responses. There was no difference in the electroretinogram or the Brazelton scores among the unsupplemented and supplemented groups.

Wharton et al. (145), in a recent retrospective analysis, observed low plasma taurine concentration in premature infants fed nonenriched formula as compared with those fed mother’s milk or enriched preterm-infant formula. The low plasma taurine levels in the neonatal period were associated with lower scores on the Bayley Mental Developmental Index at 18 months and with the WISC-R arithmetic subset at 7 years. These data support the view that taurine is a conditionally essential nutrient, at least for the neonate, because a dietary supply was required for optimal outcome. As recognized by Wharton et al. (145) and discussed by Heird (43), the observed relationship between neonatal taurine levels and the neurodevelopmental outcome was not robust and was observed in a retrospective analysis. Nonetheless, they support the hypothesis that low neonatal taurine status can adversely affect later neurodevelopment of preterm infants and that the neurodevelopmental advantage of the human milk may be related, in part, to its taurine content (43). Such a hypothesis cannot be examined by randomized clinical trials in humans because of ethical considerations.

**Threonine**

Threonine, an indispensable amino acid, unlike BCAAs, does not participate in transamination reactions. It is a major component of intestinal mucin constituting as much as 30% of its amino acid content. In addition, it is a significant (12%–14%) component of 4E-binding protein-1, an important component of the translation initiation pathway. The liver and pancreas are the major sites of catabolism of threonine in vivo.

Threonine is metabolized in vivo via three independent pathways (Figure 2). It can either be converted to 2-amino-3-oxobutyrate by the mitochondrial enzyme threonine dehydrogenase (EC 1.1.1.103) and subsequently to glycine by 2-amino-3-oxobutyrate CoA ligase, or it can be directly converted to glycine by cytosolic threonine aldolase (EC 2.1.2.1). Glycine is then oxidized by the glycine cleavage system. Threonine can also be converted to 2-oxobutyrate by the cytosolic serine threonine dehydratase (EC 4.2.1.16). 2-Oxobutyrate enters the mitochondria, and its oxidation is catalyzed by either pyruvate dehydrogenase or by branched chain o xoacid dehydrogenase. The relative contribution of the various catabolic pathways of threonine was examined by House and colleagues (45) in isolated rat hepatocytes. Their
data show that ∼65% of threonine is oxidized via the glycine-independent threonine dehydratase pathway. Glucagon stimulated the oxidation of threonine in part by increasing the threonine uptake by the hepatocytes.

The metabolism of threonine has been examined in healthy adults and in neonates using isotopic tracer methods under varying dietary conditions (22, 85, 149). Darling and colleagues (22) examined the relative contribution of threonine dehydrogenase and threonine dehydratase in the catabolism of threonine in healthy adults. Using [1-13C]threonine tracers and sensitive isotope ratio combustion mass spectrometry, they measured the appearance of 13C in plasma glycine, urinary hippuric acid (a measure of hepatic intracellular glycine enrichment), and in expired carbon dioxide. Their data show that the contribution of threonine to glycine accounts for only ∼2% of threonine flux (threonine dehydrogenase pathway), and ∼10% of the threonine flux was oxidized. The major oxidative pathway in vivo in healthy adults was via threonine dehydratase and accounted for 90% of the threonine catabolism. Similarly, a minor contribution of the threonine dehydrogenase pathway was observed by Zhao et al. (149) in their studies of threonine and glycine kinetics in young healthy adults. In addition, increasing dietary threonine intake, either as free amino acid or as protein, results in increased oxidation of threonine and correlates with plasma concentration of threonine. However, at plasma threonine concentration beyond ∼250 μmole/L, there is no further increase in threonine oxidation, which suggests, as in studies in experimental animals (17, 68), that threonine dehydratase is not induced by its substrates.

Data from healthy newborn infants born at term gestation and studied during the first 48 hours after birth are displayed in Table 4 (85). As shown, the Ra of threonine is high during fasting when compared with that in adults [∼100 μmole·kg⁻¹·d⁻¹ (22)]. Formula feeding results in a significant suppression of threonine Ra. As reported in adults, there is a positive correlation between plasma concentration of threonine and its rate of oxidation (r² = 0.75, p < 0.001), which suggests a substrate regulation. The suppression of threonine Ra in response to feeding is in contrast to lack of any significant effect on phenylalanine Ra measured simultaneously in these infants and suggests an effect of feeding on threonine-enriched proteins, possibly in the splanchnic compartment. By quantifying the incorporation of threonine carbon

![Figure 2](image)

**Figure 2**
Hepatic metabolism of threonine. (1) Serine/threonine dehydratase, (2) threonine dehydrogenase, (3) threonine aldolase, (4) pyruvate dehydrogenase or branched chain oxo and dehydrogenase, (5) 2-amino-3-oxobutyrate CoA ligase, (6) glycine cleavage system. Enzymes 1 and 3 are cytosolic; the remaining enzymes are localized to the mitochondria. (Figure adapted from Reference 45.)

### Table 4  Threonine and phenylalanine kinetics in healthy full-term newborn infants

<table>
<thead>
<tr>
<th></th>
<th>Threonine</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake</td>
<td>Conc.</td>
</tr>
<tr>
<td>Fast</td>
<td>–</td>
<td>154 ± 58</td>
</tr>
<tr>
<td>Fed</td>
<td>34 ± 2</td>
<td>170 ± 53</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
Conc., plasma concentration μmole·L⁻¹; Ra, rate of appearance, μmole·kg⁻¹·h⁻¹; oxidation, μmole·kg⁻¹·h⁻¹ (% of Ra).
⁺p < 0.05, fast versus fed, two-tail paired analysis.
Data from Parimi et al. (85), reproduced with permission.
into CO₂ and into glycine, these authors (85) showed that threonine dehydratase was the major degradative pathway in the human neonate.

Studies in preterm infants and in newborn piglets also show that more than 80% of enterally administered threonine is taken up by the splanchnic compartment during its first pass (106, 135). Such high uptake of threonine by the splanchnic compartment cannot be reconciled with the linear dose-related increase in plasma concentration of threonine, shown by Rigo & Senterre (102). Increase in plasma concentration may be related to the limited maximal capacity of these babies to dispose of threonine. Estimates of threonine requirement were higher when the neonatal piglets were fed intragastrically as compared with when they were receiving an identical diet parenterally (17). The important role of threonine in splanchnic metabolism is further demonstrated by the increase in its utilization for the synthesis of acute-phase proteins, intestinal proteins, and mucins during sepsis in rats (29).

PROTEIN TURNOVER

Isotopic tracers of amino acids are the most commonly employed methods to quantify the dynamic aspects of protein metabolism in vivo. These methods have used either nonessential amino acids such as [¹⁵N]glycine to quantify whole-body nitrogen turnover, or labeled essential amino acids such as leucine and phenylalanine. Stochastic models are employed to examine the effects of various perturbations on rates of synthesis and breakdown of whole-body protein (8). By quantifying the rate of appearance of leucine or phenylalanine and from the respective leucine and phenylalanine composition of mixed proteins, the rate of protein breakdown can be computed. The rate of irreversible loss of protein can be estimated by the quantification of the irreversible loss of leucine by decarboxylation or of phenylalanine via hydroxylation to tyrosine. Finally, the rate of protein synthesis can be estimated from the difference between the rates of breakdown and irreversible loss of protein. The various assumptions and associated problems have been discussed by Bier (8).

Whole-body rate of protein turnover and its response to nutrient administration has been studied extensively, using labeled leucine, phenylalanine, or glycine, in both the healthy full-term infant and those born prematurely. Studies in healthy full-term infants show that the weight-specific rate of appearance of leucine and phenylalanine, and therefore protein breakdown, is significantly higher than rates in healthy adults during nutrient deprivation (24, 25, 44, 58). However, when the data were expressed in relation to metabolic weight (weight ⁰.₇₅), the rates of appearance of leucine and its decarboxylation were not significantly different between the adults and neonates (24). Similarly, energy expenditure when expressed in relation to metabolic weight was not different between adults and neonates, underscoring the close association between energy expenditure and protein turnover. Enteral and parenteral administration of mixed nutrients resulted in suppression of protein breakdown. In contrast to adults, a higher fraction of enterally administered amino acids appeared to be taken up by the splanchnic tissues during their first pass, presumably to support the high rate of protein synthesis and conversion to other amino acids in this compartment (110, 111, 134, 135, 138).

Response to parenteral amino acids. A number of studies have examined the response to parenteral amino acid administration on whole-body rate of protein turnover in healthy adults and newborn infants. These data should be examined in relation to the dose and duration of amino acids administered, plus the quality of amino acid mixture infused, and possibly the total amount of calories given. Most of these studies have examined the acute response to a short duration (3–5 hours) of amino acid infusion. Studies in healthy adults after an overnight (10- to -12-hour) fast show that intravenous infusion of an amino acid mixture for 3–5 hours results in suppression of the whole-body rate of protein breakdown (5, 6, 79, 82). Compartmental analysis combining isotope tracer infusion
and tissue-bed balance method showed that suppression of proteolysis was evident in both the splanchnic compartment and the skeletal muscle (6, 35, 79, 82). However, the inhibition of proteolysis in the skeletal muscle was not observed consistently in all studies (5, 143). Nygren & Nair (79), using a hepatic vein catheter and tracer balance technique, showed that acute infusion of amino acids to healthy adults had a profound inhibitory effect on protein breakdown in the splanchnic compartment, and this effect was independent of insulin. In this context, it is important to underscore that acute infusion of amino acids has not been shown to consistently stimulate insulin secretion, a key hormone responsible for the suppression of protein breakdown, nor does it cause an increase in plasma levels of other anabolic mediators, such as insulin-like growth factor (IGF)-1, IGF binding protein-1, or IGF binding protein-3 (120). Thus, the effect of amino acid infusion on protein breakdown in the splanchnic bed and in the skeletal muscle (if any) appears to be independent of insulin and the IGF system. Whether these acute responses to amino acid infusion are sustained during prolonged administration of amino acids experimentally or for clinical consideration has not been reported in adults.

In healthy newborn infants born at term gestation and studied at least 6–8 hours after their last feeding, a short 5-hour infusion of amino acids at 3 g·kg⁻¹·d⁻¹ resulted in suppression of whole-body protein breakdown as measured by the appearance of leucine or phenylalanine. By inference, there was an increase in the rate of synthesis of protein (25). Similar observations were made in preterm low-birthweight infants, although there were differences in the magnitude of suppression of proteolysis when compared with full-term infants (20, 92, 103, 127, 130, 137, 139). In contrast to these data, Poindexter et al. (93) did not observe any suppression of proteolysis in response to intravenous amino acid infusion in clinically stable preterm infants. However, these babies were older, were being fed enterally every 3–4 hours, and may not have been in a true fasting state. Rivera et al. (103) and van den Akker et al. (130) compared the rate of protein breakdown (leucine Ra) in premature infants receiving either glucose or amino acid for the first 48 hours after birth and noted no difference in the rate of appearance of leucine between the two groups, suggesting a lack of any effect of amino acids on protein breakdown. Van Lingen et al. (139), using [¹⁵N]glycine tracer, reported similar data. Collectively, these data show that acute infusion of amino acids for 3–5 hours in infants born at term gestation or those born prematurely results in suppression of whole-body protein breakdown. However, such an effect was not evident when the infants were examined following prolonged infusion of amino acids or soon after enteral nutrient administration.

Parimi et al. (86) have recently reported the effect of dose and duration of parenteral amino acids infusion on the rate of appearance of phenylalanine (protein breakdown), glutamine synthesis, and urea synthesis (protein oxidation) in clinically stable premature infants. As shown in Table 3, an acute increase (short study) in amino acid infusion from 1.5 to 3.0 g·kg⁻¹·d⁻¹ resulted in suppression of proteolysis (phenylalanine Ra) and a lower rate of urea synthesis and had no impact on de novo glutamine synthesis. In contrast, when the high rate of amino acids infusion was continued for an extended period, i.e., over 24 hours, the acute inhibitory effect of amino acids on protein breakdown had vanished. The high amino acid load was associated with higher rates of glutamine synthesis and amino acid/protein oxidation (urea synthesis). Kadrofske et al. (52) made similar observations in low-birth-weight babies studied within the first 48 hours after birth, when they were acutely sick and required significant clinical support.

Thus, the response to an acute parenteral administration of amino acids is a transient suppression of whole-body protein breakdown followed by a return to the basal state and oxidative disposal of the amino acid load. The mechanism of these responses remains unclear. Kalhan & Edmison (57) have speculated that the suppression of proteolysis by amino acids
may primarily involve the autophagic/lysome pathway in the splanchnic compartment and may be mediated by the activation of the mTOR (mammalian target of rapamycin) pathway.

Amino acid tracer incorporation into skeletal muscle protein suggests that amino acid infusion causes an increase in protein synthesis in humans and animals (6, 142). Bohé and colleagues (10, 11) showed that the stimulation of protein synthesis in skeletal muscle by amino acids starts after a latent period, reaches a peak by 2 hours, and then declines rapidly to the basal state. These data were interpreted to suggest that the accretion of skeletal muscle protein in response to continued availability of amino acids may be regulated by the amount of protein that can be stored in the muscle. The exact mechanism involved in such regulation remains unknown. Data cannot be gathered for newborn infants because of the invasive nature of the studies.

In relation to the neonate, two additional observations documenting variance from the above responses to nutrient/amino acid administration should be considered. In human newborns, glutamine supplementation of intravenous amino acids results in suppression of protein breakdown even after three days. The mechanism of this sustained inhibition of protein breakdown by supplementation with glutamine is unclear (62). Finally, in neonatal pigs, enteral nutrient administration resulted in increased rather than decreased rate of protein breakdown in the hind limb (123). Whether this increase in protein turnover is related to the high rate of protein accretion in the skeletal muscle of growing pig remains to be examined.

CLINICAL CONSIDERATIONS

A number of clinical strategies have been utilized to improve protein/nitrogen accretion in the newborn infant, in particular for the prematurely born low-birth-weight infant. These have been especially focused at the immediate newborn period, a time of the greatest attenuation of growth due to intercurrent illnesses. Data from multicenter studies show that, in spite of all efforts, the stated goal of achieving “fetal growth rate” has remained elusive so that at the time of discharge from the hospital, low-birth-weight babies weigh less than the corresponding fetal weight for gestational age (28). This may be attributed in part to changes associated with transition to extraterrestrial life such as change in oxygenation and redox state, change in substrate oxidation and energy expenditure, intercurrent illness, and inability to deliver adequate calories, fluids, and nutrients owing to cardiovascular decompensation and other confounding variables (87). Nevertheless, several clinical trials of amino acids and protein/amino acid supplementation have been performed in order to reduce the morbidity and mortality of newborn infants (6, 7, 76, 78, 91, 125, 131–133, 141, 146).

The physiological data discussed above for individual amino acids and their responses to enteral and parenteral supplementation provide the rationale for the conduct of these clinical trials. It should be underscored that, although physiological outcomes such as change in protein turnover and amino acid kinetics are easy to quantify in the short term, translating these data into clinical studies has not been easy. This is because the targeted clinical outcomes, e.g., change in mortality, rates of bacterial infection, feeding intolerance, length of hospital stay, etc., are complex consequences of a large number of variables and may not be affected by change in a single parameter. In addition, although the daily requirements of individual amino acids have been estimated using physiological and clinical methods, these estimates cannot be easily translated into clinical practices because of interactions with other metabolic processes.

Recent clinical trials of early amino acid administration and glutamine supplementation trials in the human newborn are briefly discussed below.

Glutamine. Because of its important role in the whole body, as the tissue and cellular metabolism nitrogen carrier in vivo, and as a major component of nitrogen transport for the mother and fetus, glutamine has been the
most frequently examined amino acid in clinical trials. Additional rationale include the demonstrated effect of glutamine on immune function in vivo and in vitro, on inhibition of bacterial translocation, on the barrier function of the gut, on skeletal muscle protein synthesis, and the marked decrease in plasma glutamine concentration in the neonate and in adults during acute illness and stress. The latter suggests an inability to synthesize glutamine to keep up with the increasing demands and thus is designated “conditionally essential.” However, the duration of the so-called conditionally essential requirement cannot be defined. This is important because the duration of this “imbalance” between synthesis and demand for glutamine may be an important determinant of glutamine requirement and may contribute to the negative outcomes of various clinical trials. The limited solubility of glutamine in aqueous solution and its relative instability have precluded its routine use in parenteral and enteral preparation. Because glutamine metabolism is strictly compartmentalized so that enterally administered glutamine appears to be entirely metabolized in the gut and the splanchnic compartment, the clinical outcome measures should be related to the route of glutamine administration.

Clinical studies of enteral glutamine supplementation have not shown any consistent reduction in incidence of sepsis, feeding intolerance, incidence of necrotizing enterocolitis, or any change in growth parameters in low-birthweight infants (78, 131–133, 141). Parenteral glutamine with amino acids also has not been shown to have a consistent impact on any measurable clinical parameters such as nosocomial infection or growth (67, 91, 125), although it did cause a decrease in the whole-body rate of protein breakdown (62).

**Early postnatal administration of intravenous amino acids.** The fetus in utero receives a continuous supply of nutrients including amino acids from the mother, which is discontinued at birth with the cutting of the umbilical cord. Therefore, newborn infants, in particular the low-birth-weight baby, require nutritional and other support soon after birth. Controversy had persisted regarding the amount and timing of this nutrient support because many of these babies were clinically unstable and would not tolerate parenterally administered amino acids, particularly the older preparations. Recent data, however, show that the early administration of amino acids immediately after birth, and in doses of 3–3.5 g·kg\(^{-1}·d\)^{-1}, are well tolerated and do not cause any untoward effects, such as metabolic acidosis. In addition, tracer isotopic and other studies (94, 103, 136, 139) suggest that such a regimen will place the baby into positive nitrogen balance. Whether such an intervention has any long-term advantage remains unknown (18), nonetheless, it may be critical for the nutritional support of these infants during acute illness.

**FUTURE ISSUES**

1. Evaluation is needed of splanchnic/enteral metabolism of amino acids using novel, non-invasive, in vivo methodologies.

2. Examination is needed of the mechanism of the transient effect of parenteral amino acids on whole-body protein synthesis and breakdown, and optimal methods must be developed for parenteral nutrition of low-birth-weight infants.

3. Development and validation are needed for optimal amino acid mixtures for the parenteral nutrition of the neonate in relation to protein/nitrogen accretion.
DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Figure 1
Metabolism of methionine in vivo. Abbreviations: BHM, betaine homocysteine methyl transferase; CγL, cystathionine γ lyase; CβS, cystathionine β synthase; GSH, glutathione; Hcy, homocysteine; SAH, S-adenosyl homocysteine; SAM, S-adenosyl methionine; THF, tetrahydrofolate.
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Errata

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