Transamination of Leucine and Nitrogen Accretion in Human Pregnancy and the Newborn Infant

Satish C. Kalhan and Prabhu S. Parimi

ABSTRACT

Kinetics of leucine and its oxidation were determined in human pregnancy and in the newborn infant, using stable isotopic tracers, to quantify the dynamic aspects of protein metabolism. These data show that in human pregnancy there is a decrease in whole-body rate of leucine turnover compared with nonpregnant women. In addition, data in newborn infants show that leucine turnover expressed as per kg body weight is higher compared with adults. The administering of nutrients resulted in a suppression of the whole-body rate of proteolysis. Because nonessential amino nitrogen is an important component of nutritional nitrogen and can be limiting for growth under certain circumstances, and because BCAA are an important source of nonessential amino nitrogen, we have examined the relations among the transamination of leucine, leucine N kinetics, and urea synthesis and glutamine kinetics in human pregnancy and newborn infants. In human pregnancy, early in gestation, there is a significant decrease in urea synthesis in association with a decrease in the rate of transamination of leucine. A linear correlation was evident between the rate of leucine reamination and urea synthesis during fasting in pregnant and nonpregnant women. In healthy-term newborn and growing infants, although the reamination of leucine was positively related to glutamine flux, leucine reamination was negatively related to urea synthesis, suggesting a redirection of amino N toward protein accretion. The regulatory mechanism involved in this redirection of nitrogen from irreversible loss to accretion remains under investigation.

KEY WORDS: leucine, pregnancy, infants, nitrogen accretion
between various metabolic nitrogen pools, could play an important role in redistribution of nitrogen during states of protein catabolism and protein accretion. Pregnancy and growing infants provide unique opportunities to examine such interrelations. In this article, recent data describing changes in leucine transamination and its relation to urea synthesis and glutamine kinetics in pregnant women and newborn infants are described.

Pregnancy

Adaptation to pregnancy in humans involves a complex interaction between energy-yielding substrates and nitrogen metabolism in order to support the increasing requirements of the mother and the growing conceptus. Data from several studies show that as the rate of total energy consumption of the mother plus conceptus increases with advancing gestation, there is a parallel increase in the kinetics of energy yielding substrates, glucose and fatty acids, in the maternal compartment (14–16). There is also a strong correlation between the rate of glucose production by the mother and the fetal mass (weight) in the third trimester of human pregnancy (17). In addition, normal pregnancy in humans and in animals is associated with the development of insulin resistance. The magnitude of insulin resistance was shown to increase with advancing gestation (16). In contrast to the parallel changes in energy and substrate metabolism, the adaptive responses in nitrogen metabolism can be characterized as anticipatory and make it available for protein synthesis in the maternal and fetal compartment.

In relation to protein metabolism, a decrease in circulating α-amino nitrogen pool, decrease in plasma urea concentration and a lower rate of urea excretion during pregnancy in human and animal studies has been known for a long time (15,16). Stable isotopic tracer dilution studies and measurement of urinary urea excretion have confirmed that the rate of urea synthesis is decreased in pregnant women (18,19).

Estimates of the rate of protein turnover in the whole body using [1-13C]leucine tracer, [1-13C15N]leucine tracer, or [15N]glycine tracer suggest that the protein turnover and protein breakdown are either unchanged or decreased in human pregnancy with advancing gestation (14). These measurements are confounded by the difficulties in expression of the data because of the change in maternal lean body mass as well as changes in total body water related to pregnancy (20). We recently measured the kinetics of phenylalanine during human pregnancy as an estimate of whole-body protein breakdown (S. C. Kalhan, unpublished data). Because phenylalanine is not metabolized in skeletal muscle and is a major component of skeletal muscle protein, these measurements may represent a larger contribution of muscle protein when compared with leucine kinetics. We studied 14 pregnant subjects early in pregnancy and 9 were studied again late in the third trimester. Their data were compared with 6 nonpregnant women of similar age and body mass. As shown in Table 1, the rate of appearance of phenylalanine was significantly less than that of nonpregnant women, both early and late in gestation. A significantly lower rate of urea synthesis was also observed. These data suggest that during pregnancy, as a result of pregnancy-related hormones, cytokines or other mediators, changes in maternal nitrogen metabolism are aimed toward nitrogen conservation in order to make it available for protein synthesis in the maternal and fetal compartment.

Because BCAAs are the major source of nitrogen for glutamine and alanine synthesis, and ultimately for ureagenesis,

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Phenylalanine and urea kinetics in pregnancy</th>
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<tbody>
<tr>
<td></td>
<td>Nonpregnant</td>
</tr>
<tr>
<td>Phenylalanine Ra</td>
<td>41.1 ± 6.9 (6)</td>
</tr>
<tr>
<td>Urea Ra</td>
<td>258.8 ± 61.5 (6)</td>
</tr>
</tbody>
</table>

Mean ± SD; numbers in parentheses = n. Ra, Rate of appearance in μmol kg⁻¹ h⁻¹; Significantly different from nonpregnant control group using two-tailed t test: *P < 0.02, **P < 0.005. Data from S. C. Kalhan (unpublished).

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Leucine metabolism during fasting in pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonpregnant</td>
</tr>
<tr>
<td>Leucine nitrogen turnover</td>
<td>166 ± 35</td>
</tr>
<tr>
<td>Leucine carbon turnover</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>Rate of decarboxylation of leucine</td>
<td>18.2 ± 2.1</td>
</tr>
<tr>
<td>Rate of reamination of KIC</td>
<td>61.4 ± 29.9</td>
</tr>
<tr>
<td>Rate of deamination of leucine</td>
<td>79.6 ± 30.2</td>
</tr>
</tbody>
</table>

Mean ± SD; values are μmol kg⁻¹ h⁻¹; KIC, α-ketoisocaproic acid. *Significantly different from nonpregnant control group, Wilcoxon Signed Rank Test, P < 0.05. Data from Kalhan et al. (19).
we have quantified the changes in the rates of leucine N turnover, leucine transamination, and urea synthesis longitudinally, with advancing gestation (19). The isotopic tracer model described by Matthews et al. (21) using [1-13C,15N]leucine tracer was used for these studies. Rates of turnover of leucine nitrogen (QN) and leucine carbon (QC), reamination of α-keto isocaproic acid, and deamination of leucine were quantified (Table 2). As shown, the rate of turnover of leucine nitrogen was lower in pregnant women during the first and third trimester when compared with nonpregnant women. There was no significant change in leucine carbon flux or in the rate of decarboxylation of leucine. The rate of reamination of α-keto isocaproic acid was also lower in pregnant women. Since urea synthesis was significantly lower in the first trimester of pregnancy compared with nonpregnant women, these data also show that the quantification of the rate of leucine decarboxylation does not always provide a true estimate of oxidation of protein, at least in these short studies. As Figure 1 shows, there is a significant positive correlation between the rate of deamination of leucine and the rate of urea synthesis during fasting in pregnant and nonpregnant women. The correlation was not as significant during feeding.

Thus data from the studies in pregnancy show a down-regulation of the rate of transamination of leucine and urea synthesis early in gestation. In another series of studies we have also demonstrated that these changes are associated with a lower flux of other dispensable amino acids, that is, serine (22), glutamine, and alanine (S. C. Kalhan, unpublished data). The physiological mechanisms responsible for these adaptive responses have not been identified but may be related to the change in anaplerotic flux as a result of lower rate of proteolysis and due to redistribution of nitrogen for other synthetic processes.

The neonate

Data in newborn infants are confounded by the clinical state of the study population. Infants born at term gestation and studied during the immediate period after birth (<48 h) may show the variable and not the measurable impact of birth-related surges in a number of hormones and cytokines and, consequently, the extrauterine adaptation-related changes in metabolism (23). Studies in prematurely born infants are confounded by the acute illnesses associated with prematurity and the necessary clinical and nutritional support required for these infants (24). In addition, because neonates are fed at frequent intervals, that is, every 3–4 h, metabolic studies cannot be performed in a true postabsorptive or fasting state. Thus all so-called fasting data in the newborn infant should be considered to represent varying degrees of the postprandial state, except when the baby has not received enteral nutrition due to clinical reasons. In healthy term infants, a fasting state with demonstrable increase in plasma betahydroxybutyrate concentration was associated with nutrient deprivation of ~9 h (25).

Such a prolonged fast would not be acceptable for present-day research due to ethical considerations. Despite these limitations, some interesting data on changes in leucine catabolism and on transamination of leucine in relation to enteral and parenteral nutrient administration were obtained and are summarized below.

### TABLE 3

<table>
<thead>
<tr>
<th>Leucine Flux</th>
<th>Leucine Oxidation</th>
<th>% Leucine Flux Oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μmol kg⁻¹ h⁻¹</strong></td>
<td><strong>μmol kg⁻¹ 0.75 h⁻¹</strong></td>
<td></td>
</tr>
<tr>
<td>Infants (12)</td>
<td>164 ± 28*</td>
<td>219 ± 45</td>
</tr>
<tr>
<td>Adults (11)</td>
<td>87 ± 15</td>
<td>248 ± 46</td>
</tr>
</tbody>
</table>

Mean ± SD; numbers in parentheses = n.

*P < 0.01, **P < 0.05, when compared with data from adults. Leucine flux was calculated from 13C enrichment of plasma leucine during [1-13C]leucine tracer infusion.

Data from Denne and Kalhan (26).

### TABLE 4

**Effect of feeding on leucine C & N kinetics in full-term infants**

<table>
<thead>
<tr>
<th></th>
<th>Fast</th>
<th>Fed</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine N Ra (11)</td>
<td>337.5 ± 52.3</td>
<td>375.4 ± 71.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Leucine C Ra (11)</td>
<td>243.5 ± 42.2</td>
<td>237.7 ± 30.1</td>
<td>NS</td>
</tr>
<tr>
<td>Reamination of leucine (11)</td>
<td>93.8 ± 49.2</td>
<td>137.7 ± 54.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glutamine Ra (6)</td>
<td>545.6 ± 116.2</td>
<td>493.8 ± 100.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Urea Ra (11)</td>
<td>171.1 ± 66.7</td>
<td>159.2 ± 57.2</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

Mean ± SD; numbers in parentheses = n; Ra = Rate of appearance; values are μmol kg⁻¹ h⁻¹ and, P: significantly different from fast, paired t-test.

Leucine, glutamine, and urea kinetics were measured using [1-13C,15N]leucine, [5,15N]glutamine and [15N₂]urea tracers as prime constant rate infusion. Following a basal study, response to intermittent nutrient administration was examined over 3 h.

Data from Parimi et al. (33).
Studies using $[^{13}]$C-leucine tracer have shown that the rate of appearance of leucine and its rate of decarboxylation are higher in the neonate born at term gestation than those reported in adults, when expressed as per kg body weight (26) (Table 3). A similar, higher, weight-specific rate of appearance of leucine compared with adults was observed in a number of other studies (27–29). However, when the data were expressed per unit metabolic weight (wt$^{0.75}$), newborn infants and adults had similar rates of leucine flux and leucine oxidation. (Table 3). Energy expenditure (VO$_2$), when expressed also in relation to metabolic weight, showed no difference between adults and newborn infants (26). These data underscore the relation between energy consumption (metabolic weight) and protein turnover. Enteral and parenteral administering of mixed nutrients was shown to suppress proteolysis, as evidenced by a decrease in the rate of appearance of leucine in the blood (28). However, in contrast to adults, a significantly higher fraction (50% vs. 20%) of leucine is taken up by the splanchnic compartment during its first pass, presumably to support the high rate of protein synthesis in infants (30–32).

Recently we examined the relations between leucine nitrogen kinetics and glutamine and urea kinetics in healthy newborn infants (33). The rates of appearance of leucine N, leucine C, glutamine, and urea were quantified during fasting and during the administering of enteral nutrient (formula) using [1-$^{13}$C, $^{15}$N]leucine, [5-$^{15}$N]glutamine, and $^{15}$N$_2$urea tracers. As shown in Table 4, administering enteral nutrient caused a significant increase in leucine N Ra. Because total leucine C Ra remained unchanged in response to feeding, the estimated rate of reamination of leucine (difference between leucine N Ra and leucine C Ra) increased significantly in the fed state. Despite an increase in reamination (and therefore transamination) of leucine, there was a significant decrease in the rate of appearance of glutamine (P > 0.05) and a significant decrease in the rate of urea synthesis (P < 0.05). The decrease in the rate of appearance of glutamine may be the consequence of a decrease in the rate of protein breakdown in the peripheral (muscle) compartment. In contrast, the increase in reamination in response to feeding may represent the splanchnic response to enteral protein/nitrogen load.

The correlations between leucine nitrogen kinetics and glutamine and urea kinetics are of interest. During fasting, and as anticipated, there was a positive linear relation between leucine nitrogen flux and glutamine flux, since leucine N is an important contributor of glutamine N (Fig. 2). In contrast, the relation between glutamine flux and urea synthesis was negative (Fig. 3), and there was no significant correlation between leucine N flux and urea synthesis, underscoring 1) the response to enteral protein intake and 2) the contribution of leucine N and possibly glutamine N toward the synthesis of other nonessential amino acids in vivo. In response to feeding, when leucine N flux increased, the rate of appearance of glutamine decreased and the correlation between leucine N and glutamine became much weaker. These data suggest that the increase in leucine N Ra during enteral feeding represents

![Figure 3](image-url)

**FIGURE 3** Correlation between rate of appearance (flux) of glutamine and urea synthesis during fasting in newborn infants. Broken lines = 95% confidence interval ($y = 316.7 - 0.21x$; $R^2 = 0.39$, $P = 0.03$); solid circle = appropriate for gestational age; open circle = small for gestational age; shaded circle = infants of diabetic mothers. (Reproduced from Parimi et al. (33) with permission.)

<p>| TABLE 5 | Effect of parenteral glutamine on phenylalanine, glutamine, and urea kinetics |
|---------------------------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Endogenous Phenylalanine Ra</th>
<th>Endogenous Glutamine Ra</th>
<th>Urea N from Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol kg$^{-1}$ h$^{-1}$</td>
<td>µmol kg$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>C</td>
<td>97.7 ± 23.7</td>
<td>520.9 ± 97.7</td>
</tr>
<tr>
<td>G</td>
<td>62.2 ± 16.7</td>
<td>372.3 ± 65.6†</td>
</tr>
</tbody>
</table>

Mean ± SD; $n = 10$ in each group; Ra, Rate of appearance; C, Control; G, Glutamine supplemented group. Average parenteral phenylalanine infusion: C, 41.6 µmol kg$^{-1}$ h$^{-1}$; G, 33.8 µmol kg$^{-1}$ h$^{-1}$; and average glutamine infusion, C, 0; G, 195 µmol kg$^{-1}$ h$^{-1}$. Endogenous Ra = total exogenous rate of infusion in parenteral TPN. Significant difference between groups: *$P = 0.001$, †$P = 0.003$.

Data of Kalhan et al. (34).

![Figure 4](image-url)

**FIGURE 4** Relation between reamination ($X_N$) of leucine and urea synthesis (Su) in low birth weight babies receiving total parenteral nutrition. (Data from Kalhan et al. (34).)
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Effect of enteral nutrition in growing infants

The effect of growth and nitrogen accretion on whole-body nitrogen kinetics was examined in a group of low birth weight infants who had recovered from their acute illness and were growing at a normal rate (35). The infants were studied at 6 wk of age when their corrected gestational age was ~35 wk and weight ~1900 g. All infants were gaining weight at ~20 g kg\(^{-1}\) d\(^{-1}\). Their protein and calorie intake was 3.3 g kg\(^{-1}\) d\(^{-1}\) and 125 kcal kg\(^{-1}\) d\(^{-1}\), respectively. The data in Table 7 compares the effect of glutamine supplementation (0.6 g kg\(^{-1}\) d\(^{-1}\)) in enteral feeds on whole-body leucine N, glutamine, and urea kinetics. The kinetic data were obtained between 5 and 6 h after their last feeds. As suggested above, because of the variable rate of gastric emptying in the neonate, and because of the higher frequency of feeding, these data should not be considered a truly fasting data.

As shown, there was no significant difference in Ra leucine C in the control or glutamine supplemented groups (Table 7). Glutamine supplementation, by providing an additional source of glutamate, resulted in higher rate of reamination of leucine; however, no significant change in the systemic rate of appearance of glutamine was observed. Glutamine supplementation was associated with an equimolar increase in the rate of urea synthesis. These data suggest that enterally administered glutamine is metabolized entirely locally in the gut (and splanchnic compartment), and the majority of glutamine N appears in urea. The fate of glutamine C cannot be discerned from these data. The relation between leucine N Ra and urea synthesis are shown in Figure 6 and Figure 7. In the control group (i.e., growing infants who were enterally fed), there was a negative correlation between leucine N Ra and urea synthesis, whereas a positive correlation was observed in the glutamine-supplemented group. The correlation in the control group is similar to the one observed previously in the healthy term babies (Fig. 3) and in preterm infants (Fig. 5) who had received glutamine-supplemented parenteral nutrition and had shown a lower rate of protein breakdown. These data also show that provision of additional N by supplemental glutamine results in a higher rate of reamination or transamination of leucine aimed at disposal of nitrogen by directing toward urea synthesis, as seen between reamination of leucine and the rate of urea synthesis was evident\(^{\text{(a)}}\) (Fig. 5).

These data suggest that when proteolysis is suppressed and presumably there is greater nitrogen accretion (in this case by glutamine supplemented amino acid infusion), a large proportion of leucine N flux is directed toward other nonessential amino acids and toward protein synthesis, rather than protein oxidation.

Effect of parenteral amino acids with and without glutamine on leucine kinetics

The effect of parenteral amino acids, ~3 g kg\(^{-1}\) d\(^{-1}\) with or without supplemental glutamine 0.6 g kg\(^{-1}\) d\(^{-1}\), on leucine, phenylalanine, glutamine, and urea kinetics was examined in low birth weight infants 6–7 d after birth (34). Because these babies had not received any nutrients via the enteral route and were entirely parenterally fed, the observed changes in leucine metabolism and the effects of glutamine supplement may reflect, for the most part, changes in peripheral, most likely skeletal muscle, metabolism. As shown in Table 5, glutamine supplementation was associated with a lower rate of protein breakdown, as evidenced by the lower rate of appearance of phenylalanine N in the circulation. The lower rate of proteolysis in the glutamine-supplemented group was associated with a lower rate of leucine N Ra (Table 6) and a lower rate of appearance of endogenous glutamine (Table 5). No significant change in the rate of appearance of urea was seen. There was a significant correlation between leucine N Ra and glutamine Ra \((r^2 = 0.645, P < 0.001)\), consistent with other observations and with the evidence that leucine N is the major contributor of glutamine N. As was seen in fasting adults (Fig. 1), a positive linear relation was also observed between the rate of urea synthesis and the rate of reamination of leucine \((r^2 = 0.443)\) (Fig. 4) in the control group who were receiving parenteral amino acids only and were not enterally fed. In contrast, in the glutamine-supplemented group, although the rate of appearance of leucine N was decreased, a negative correlation between reamination of leucine and the rate of urea synthesis was evident \((r^2 = 0.536)\) (Fig. 5).

These data suggest that when proteolysis is suppressed and presumably there is greater nitrogen accretion (in this case by glutamine supplemented amino acid infusion), a large proportion of leucine N flux is directed toward other nonessential amino acids and toward protein synthesis, rather than protein oxidation.

FIGURE 5 Relation between reamination (X\text{N}) of leucine and urea synthesis (Su) in low birth weight babies receiving total parenteral nutrition supplemented with glutamine. (Data from Kalhan et al. (34).)

\[ R^2 = 0.54 \]

**TABLE 7**

Leucine kinetics in low birth weight infants in response to enteral glutamine

<table>
<thead>
<tr>
<th>Leucine N Ra</th>
<th>Leucine C Ra</th>
<th>Reamination of leucine</th>
<th>Glutamine Ra</th>
<th>Urea Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>495 ± 138 (7)</td>
<td>295 ± 92 (9)</td>
<td>247 ± 65 (7)</td>
<td>687 ± 117 (9)*</td>
</tr>
<tr>
<td>Control</td>
<td>434 ± 60 (7)</td>
<td>269 ± 41 (7)</td>
<td>201 ± 159 (7)</td>
<td>755 ± 152 (8)</td>
</tr>
</tbody>
</table>

Mean ± SD; numbers in parentheses = n; values are \(\mu\)mol kg\(^{-1}\) h\(^{-1}\).

\(^{\text{a}}P < 0.01\) compared with controls.

Data of Parimi et al. (35).
in adults during fasting and in preterm infants receiving parenteral amino acids without glutamine.

In summary, the study of leucine metabolism in combination with data on nitrogen kinetics relative to protein accretion in pregnancy and newborn infants has provided an important insight into the relation among transamination of leucine, glutamine turnover, and urea synthesis. These data show that states of protein accretion and positive nitrogen balance are associated with downregulation of the rate of transamination of leucine. Administering nutrient (protein) either enterally or parenterally, results in a higher rate of transamination of leucine and a higher rate of appearance of leucine N. The whole-body rate of transamination of leucine is positively correlated with the rate of appearance of glutamine and the rate of synthesis of urea during fasting and in states of negative nitrogen balance. In contrast, in response to suppression of proteolysis with parenteral glutamine in low birth weight infants and during the fed state in growing infants (i.e., states of nitrogen accretion), a negative relation among leucine N flux and its transamination and urea synthesis is evident. The regulatory paradigm for this shift in nitrogen flux toward urea during fasting and away from urea during protein accretion remains unknown. It could involve changes in the purine nucleotide cycle, regulation via aspartate aminotransferase, or changes in amino acid pools as a consequence of increased protein synthesis. The observed interrelations could be used for the examination of therapeutic strategies aimed at improving nitrogen balance and protein accretion in disease states.

ACKNOWLEDGMENT

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LITERATURE CITED