Metabolism of threonine in newborn infants

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Metabolism of threonine in newborn infants. Am J Physiol Endocrinol Metab 289: E981–E985, 2005. First published July 19, 2005; doi:10.1152/ajpendo.00132.2005.—Threonine kinetics, threonine oxidative pathway, and the relationship between threonine and whole body protein turnover were quantified in 10 healthy term infants during the first 48 h after birth. The kinetic data were obtained 6 h after the last feed (fasting) and in response to formula feeding, using [U-13C6,15N2]threonine, [3H3]phenylalanine, and [15N]glycine tracers. The rate of carbon dioxide production (V˙CO2) and 13C enrichment of the expired CO2 were measured to quantify the rate of oxidation of threonine. The rate of appearance (Rα) of threonine (136 ± 37 μmol·kg⁻¹·h⁻¹) was higher in newborn infants than that reported in adults. Formula feeding resulted in a significant decrease in threonine Rα (P < 0.05). A significant positive correlation was seen between phenylalanine Rα and threonine Rα, both during fasting and after formula feeding (r² = 0.65). In contrast to a 1:1 ratio of threonine and phenylalanine in mixed muscle protein, threonine Rα relative to phenylalanine Rα was 2.2 ± 0.4. The fractional rate of threonine flux oxidized was 20% during fasting and 26% (P < 0.05) in response to nutrient administration. There was a significant correlation between plasma threonine concentration and threonine oxidation (r² = 0.75). No measurable incorporation of threonine in plasma glycine was seen. These data suggest that threonine is exclusively degraded by the glycine-independent serine/threonine dehydratase pathway. A higher flux of threonine relative to phenylalanine indicates higher turnover of threonine enriched proteins.

Threonine, a nontransaminating essential amino acid, participates in a number of metabolic processes. It is a major component of intestinal mucin constituting as much as 30% of its amino acid content (6). In addition, threonine comprises 12–14% of the amino acid content of 4E-binding protein-1, an important translational initiation pathway (6). Threonine also participates in 1-carbon metabolism by its conversion to glycine. Data from animal studies show that ~60% of threonine is extracted by the gut during first-pass metabolism (21). Limitation of threonine in the rat diet has been shown to impair mucin synthesis (11).

Degradation of threonine, primarily in the liver, involves a number of enzymes and cofactors. The oxidative catabolism of threonine follows either the glycine-independent or glycine-dependent pathway. The glycine-independent pathway is catalyzed by serine/threonine dehydratase (STDH), which irreversibly converts threonine to ketobutyrate. The ketobutyrate is subsequently decarboxylated to form propionyl-CoA (13). The activity of STDH is regulated by dietary protein, by its threonine content, and by insulin, glucagon, and cortisol (12). Glycine-dependent oxidation involves conversion of threonine to glycine and acetyl-CoA by coupled threonine dehydrogenase (TDH) and 2-amino-3-oxobutyrate-CoA ligase enzymatic reaction. Glycine thus formed has multiple fates, including cleavage to form CO2 and ammonia by the glycine cleavage system.

Hepatic threonine metabolism has been examined in human adults in response to oral administration of threonine (7, 23). These studies show that higher threonine content in the diet results in a higher rate of oxidation of threonine. In addition, the majority of the threonine is metabolized via the STDH pathway (glycine-independent), and only 7–10% of total threonine catabolism occurred via glycine-dependent oxidation (7). These data indicate that STDH is the major catabolic pathway in human adults.

The disposal of threonine in physiological states of protein accretion appears to be different from that reported in human adults. Studies in newborn pigs and cats (3, 14) have shown that ~80% of threonine is catabolized via the l-threonine 3-dehydrogenase (TDH) pathway. Darling et al. (8) estimated that, in growing premature infants, ~44% of threonine is catabolized by the TDH pathway. The hepatic STDH activity is low in the rat fetus, rapidly induced at birth, and stimulated by cAMP and glucagon (12). The enzymatic activity of STDH is higher in adult compared with newborn animals (12).

The rate of appearance (Rα) of threonine in the plasma and its relationship to the whole body rate of protein turnover in healthy newborn infants has not been examined. Using stable isotopic tracers, we have quantified the whole body kinetics of threonine, its contribution to glycine, and its rate of oxidation in healthy newborn infants. The observed data were compared with those previously published for human adults (7, 23) and during growth in prematurely born infants (8).

METHODS

Subjects. Full-term, appropriate-for-gestation infants (40 ± 1 wk, n = 10) were recruited from the newborn nursery after obtaining verbal assent from the attending pediatrician and written informed consent from the parent(s). Infants were studied at a mean age of 38 ± 10 h. Their weight on the day of the tracer study was 3,179 ± 288 g. All babies were healthy, had no antenatal or intrapartum problems, had an Apgar score >7 at 5 min of age, were not on antibiotics, and were being cared for in the normal-newborn nursery. All babies had been receiving formula (Similac; Ross Pharmaceuticals, Columbus, OH) ad libitum every 3 h from birth. Formula intake ranged between 68 and 92 ml·kg⁻¹·day⁻¹ the day before the study (day 1 after birth), with an average intake of 82 ± 14 ml·kg⁻¹·day⁻¹. According to the manufacturer, the ready-to-feed Similac formula contains 1.45 g/dl

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protein and 668 μmol/dl⁻¹ threonine as constituent of proteins. Breast-fed newborn infants were excluded from the study protocol because of the need for frequent feeding and difficulty in accurately determining the amount of milk ingested (threonine content) and in order to study a homogenous group. The study protocol was approved by the Institutional Review Board of MetroHealth Medical Center. All studies were performed in the General Clinical Research Center at MetroHealth Medical Center.

\[ [U^{13}C_3,^{15}N] \text{threonine (99% atom }^{13}\text{C, 99% atom }^{15}\text{N)} \text{ was obtained from Cambridge Isotopes Laboratories (Andover, MA), } [^{2H}_3] \text{phenylalanine (98% atom }^{2H}) \text{ and } [^{15}N] \text{glycine (98% atom }^{15}\text{N)} \text{ were obtained from Merck (Dorval, QC, Canada), and sodium } ^{13}\text{C} \text{ bicarbonate (99% atom }^{13}\text{C)} \text{ was obtained from Isotec (Miamisburg, OH). Each batch of the tracers was dissolved in 0.45 N saline, sterilized by Millipore filtration (0.22 μm), and tested for sterility and pyrogenicity.} \]

**General study design.** Three hours after their last feed, babies were transferred to the General Clinical Research Center. Two intravenous catheters were placed, one in the saphenous vein for blood sampling and the other in the dorsum of the hand for the infusion of tracer amino acid solution. After obtaining the basal (time 0) blood sample, the tracer amino acids were infused for 7 h. Three hours after the tracer amino acid solution was begun, babies were fed Similac amino acid solution. After obtaining the basal (time 0) blood sample, the other in the dorsum of the hand for the infusion of tracer was transferred to the General Clinical Research Center. Two intravenous sterilized by Millipore filtration (0.22 μm), and tested for sterility and pyrogenicity.

Analytical procedures. Blood glucose was measured by the glucose method on a Technicon AutoAnalyzer (Tarrytown, NY). The concentration of amino acids in the plasma and infusates was measured by high-performance liquid chromatography with a fluorescence detector. Precolumn derivatization of amino acids was performed, and an O-phthalaldehyde derivative was used for analysis (22).

Gas chromatography-mass spectroscopy analysis. Heparinized plasma samples were precipitated using 10% trichloroacetic acid. Amino acids were eluted by ion-exchange column chromatography. An N-acetyl-n-propyl ester derivative of threonine, phenylalanine, and glycine was prepared according to the method of Adams (1), with certain modifications. Methane chemical ionization was used, and the amino acids were separated on a Supelco Wax-10 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) on a Hewlett-Packard model 5973 gas chromatograph-mass spectrometer (GC-MS) system. The injector port temperature and auxiliary temperature were 250°C. The oven temperature ramp was set as follows: initial temperature of 85°C for 1.2 min, increased at 30°C/min to 200°C for 2 min and 220°C for 4 min, followed by an increase at 15°C/min to 240°C for 6 min. Glycine eluted at 8.5 min, threonine at 10.3 min, and phenylalanine at 16.4 min. The mass-to-charge ratios (m/z) were 160 and 161 (m + 0 and m + 1), 246 and 251 (m + 0 and m + 5), 250 and 255 (m + 0 and m + 5), representing unlabeled and labeled glycine, threonine, and phenylalanine, respectively, and monitored using the selective-ion monitoring software. In addition, the contribution of threonine to glycine was estimated by determining the tracer enrichment of glycine (m + 3). Standard solutions of known enrichments were run along with unknowns to correct for variations in analysis. Enrichment of 13C in the expired CO2 was measured by isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH).

Calculations. Threonine Ra, phenylalanine, and glycine in the plasma were calculated using the tracer dilution equation: $R_a = \frac{F \cdot [E_E/E_0] - 1}{E_0}$, where $R_a$ is the rate of appearance of the substrate, $E_I$ is the rate of infusion of the tracer isotope, and $E_0$ is the enrichment of the tracer infused, and $E_0$ is the enrichment of the substrate in the plasma.

The fractional rate of oxidation (F) of threonine was calculated as follows: $F = \frac{\Delta [^{13}\text{C}]O_2}{V_{CO_2}}$, where $V_{CO_2}$ is the rate of production of CO2 in μmol/kg⁻¹h⁻¹, 0.8 is the retention factor for $^{13}\text{C}$ enrichment of CO2 at steady state, and $I$ is the rate of infusion of [U-$^{13}$C$_3$, $^{15}$N]threonine (μmol/kg⁻¹h⁻¹).

The rate of oxidation of threonine was calculated by multiplying F by the $R_a$ of threonine. Contribution of threonine to glycine was calculated from the precursor-to-product relationship. No measurable m₁ enrichment of glycine was observed.

Statistical analysis. All data are reported as means ± SD. The data were analyzed using the Statistix 7.2 statistical package (Analytical Software, Tallahassee, FL). The kinetic data between the fasted and fed states were analyzed using a paired t-test and one-way analysis of variance. Pearson’s correlations were performed to examine the rela-
RESULTS

Newborn infants enrolled in the study were healthy, appropriate for gestational age, had adapted well to postnatal life, and had Apgar scores within the normal range. They had been receiving formula (Similac) ad libitum every 3 h from birth. The clinical course of infants before and after the study was uneventful. All babies were discharged to home within the prescribed time after birth.

Plasma amino acids. The plasma concentration of threonine during fasting was 154 ± 58 μmol/l and did not change significantly during feeding (170 ± 53 μmol/l). Compared with the basal data (fast), plasma threonine levels after feeding increased between 15 and 37% in six babies and did not change in the other babies. There was no significant difference in the plasma concentration of glycine, phenylalanine, and serine in the fasted and fed states. Formula feeding had no significant impact on the plasma levels of other amino acids.

Threonine kinetics. The Rₐ of threonine in newborn infants was 136 ± 79 μmol·kg⁻¹·h⁻¹ during the basal period (fast). A significant decline in threonine flux (126 ± 35 μmol·kg⁻¹·h⁻¹, P = 0.02) was observed after formula feeding containing 32 ± 4 μmol·kg⁻¹·h⁻¹ threonine.

The contribution of threonine carbon to expired CO₂ was estimated from the appearance of 13C in the CO₂. The data of VCO₂, Δ[13]CO₂, oxidation of threonine, and nonoxidative threonine disposal are displayed in Table 1. As shown, the 13C enrichment of respiratory CO₂ increased after feeding. The VCO₂ was unchanged. The fractional rate of oxidation of threonine was 21 ± 6% during fasting and increased to 26 ± 6% (P < 0.01) after feeding. The quantity of threonine oxidized was not different between fasting and feeding (fast, 29 ± 14; feed, 30 ± 13 μmol·kg⁻¹·h⁻¹). Nonoxidative disposal of threonine, the difference between Rₐ threonine and oxidation of threonine, was the major fate of threonine in both fast and fed states (Table 1).

Phenylalanine and glycine kinetics. The Rₐ of phenylalanine during fast was 65 ± 17 μmol·kg⁻¹·h⁻¹ (Table 2). Formula feeding had no effect on the kinetics of phenylalanine (66 ± 17 μmol·kg⁻¹·h⁻¹). Because protein breakdown contributes to the appearance of threonine and phenylalanine in the plasma during fasting, these amino acids are released in proportion to their composition in the mixed body proteins. Because skeletal muscle constitutes the largest protein pool in the body, it is of interest to compare the relative Rₐ of threonine and phenylalanine with the composition of skeletal muscle protein. The ratio between the Rₐ of threonine and Rₐ of phenylalanine was 2.2 ± 0.4 in newborn infants, in contrast to 1.3 reported in the mixed muscle protein (2).

Glycine flux declined significantly in response to feeding (fast, 280 ± 72 μmol·kg⁻¹·h⁻¹; fed, 227 ± 66 μmol·kg⁻¹·h⁻¹, P = 0.04). The incorporation of threonine into glycine delineates the oxidation of threonine by the glycine-dependent pathway. There was no measurable m + 3 enrichment of glycine in newborn infants both during fast and in response to feeding.

Correlations. There was a significant correlation between the Rₐ of threonine and plasma levels of threonine during both fasted and fed states (r² = 0.61, P = 0.007). There was a positive correlation between the Rₐ of threonine and Rₐ of phenylalanine (r² = 0.65, P = 0.005). The quantity of threonine oxidized was significantly correlated to plasma threonine concentration (P < 0.01) both in the fasted state and in response to nutrient administration (Fig. 2).

DISCUSSION

Our data show that the oxidative disposal of threonine in healthy newborn infants born at term gestation is predominantly via the STDH pathway. Enterally administered nutrients resulted in suppression of the systemic Rₐ of threonine with no significant change in phenylalanine Rₐ. Threonine Rₐ relative to the Rₐ of phenylalanine was higher (2.2) in newborn babies compared with the composition of these amino acids in the mixed muscle proteins (1.0) (2).

Oxidative catabolism of threonine in vivo occurs either via the STDH pathway or via the TDH pathway. The latter involves first conversion of threonine to glycine and then oxidation of glycine by the glycine cleavage system. By quantifying the appearance of 13C in CO₂ from labeled threonine and by

Table 1. Threonine kinetics and oxidation of threonine

<table>
<thead>
<tr>
<th></th>
<th>Threonine</th>
<th>13CO₂</th>
<th>VCO₂</th>
<th>Oxidation</th>
<th>NODT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intake*</td>
<td>Plasma level†</td>
<td>Rₐ*</td>
<td>ml/kg⁻¹·min⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>Fasted 10</td>
<td>154±58</td>
<td>136±37</td>
<td>0.008±0.001</td>
<td>6.03±1.26</td>
<td>21±6</td>
</tr>
<tr>
<td>Fed 10</td>
<td>34±2</td>
<td>170±53</td>
<td>126±35; 0.012±0.001; 5.90±1.23</td>
<td>26±6; 30±13</td>
<td>87±23</td>
</tr>
</tbody>
</table>

Values are means ± SD. VCO₂, CO₂ production rate; *μmol·kg⁻¹·h⁻¹; †μmol/l; APE: atoms% excess; NODT, nonoxidative disposal of threonine; †P < 0.05; fasted vs. fed, 2-tail paired analysis.

Table 2. Phenylalanine and glycine kinetics

<table>
<thead>
<tr>
<th>Phenylalanine (n = 10)</th>
<th>Glycine (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake*</td>
<td>Rₐ*</td>
</tr>
<tr>
<td>Fasted</td>
<td>67±11</td>
</tr>
<tr>
<td>Fed</td>
<td>24±11</td>
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</tbody>
</table>
quantifying the contribution of threonine to glycine, the relative contribution of each pathway to threonine catabolism can be quantified. However, such an analysis has a few problems. As shown by Darling and colleagues (7, 8), the contribution of threonine to glycine is small and requires sensitive isotope ratio mass spectrometry for its measurement. We could not detect any incorporation of threonine in glycine using the GC-MS methods. The low enrichment of plasma glycine could also be due to lack of equilibrium between various glycine pools, i.e., plasma, intracellular, and intramitochondrial compartments. However, we are not aware of any data suggesting such compartmentation of glycine pools. Thus a low incorporation of threonine C into glycine would suggest that the TDH pathway is not the predominant pathway of threonine catabolism.

House et al. (13) examined the disposal of 1-carbon of threonine to CO₂ in isolated rat hepatocytes using [1-14C]threonine and competitively inhibiting the transport of α-ketobutyrate into mitochondria (STDH-mediated disposal) or the glycine cleavage system. Their data show that ~65% of threonine oxidation occurs through the glycine-independent STDH pathway and that glucagon enhances the transport and oxidation of threonine (13). In vivo studies in animals documented species-specific differences in the relative predominance of threonine oxidative pathways. Data in pigs and cats show that TDH is the major oxidative pathway, although both pathways are equally operative in rats (3, 5, 14). Of the two enzymatic pathways, STDH is highly regulated by hormones, dietary protein intake, threonine content of diet, and metabolic intermediates (5, 16–18).

Only a few studies have examined the metabolism of threonine in vivo in humans (7, 8, 23). The metabolism of threonine, the rate of oxidation of threonine, and response to nutrient administration have not been examined in full-term newborn infants. Studies in human adults show that the Ra of threonine during the postabsorptive period was ~99 μmol·kg⁻¹·h⁻¹, STDH was the major oxidative pathway, and only 7–11% of threonine degradation occurred via the glycine-dependent TDH pathway. Approximately 16% of threonine was oxidized at intakes between 40 and 50 mg·kg⁻¹·day⁻¹, and the rate of threonine oxidation increased with higher intake of both free and protein-bound dietary threonine (7, 23), suggesting induction of threonine disposal by substrate (threonine). A previous study (8) in formula-fed, growing premature infants showed that both the plasma threonine concentration (419 μmol/l) and threonine flux (221 μmol·kg⁻¹·h⁻¹) were higher compared with those of adults. Even though the dietary intake of protein-bound threonine was significantly higher (179 mg·kg⁻¹·day⁻¹) compared with adult data, only 17% of threonine intake was oxidized in these premature infants.

Our data show that, in newborn infants, the Ra of threonine was ~136 μmol·kg⁻¹·h⁻¹ during fasting and decreased significantly in response to feeding. These data are higher compared with those reported in adults and lower than those reported by Darling et al. (8) in growing premature infants. A higher threonine flux in newborns is a consequence of a higher whole body rate of protein turnover (20). The difference in threonine flux between our study and that reported in growing, premature infants is likely related to the route of administration of threonine tracer. Whereas we infused the tracer intravenously, Darling et al. (8) administered the tracer via intragastric infusion. Enteral administration of amino acid tracers subjected to extensive first-pass splanchnic metabolism will result in lower enrichment of the tracer in the plasma compared with the enrichments achieved by intravenous tracer infusion (15, 21). Thus the higher threonine Ra reported in growing premature infants by Darling et al. (8) is probably due to the route of tracer administration.

We observed that threonine flux declined in response to feeding, even though formula feeding provided ~34 μmol·kg⁻¹·h⁻¹ threonine. A lower threonine Ra suggests suppression of proteolysis, resulting in a decreased rate of release of threonine. Because enterally administered protein-bound amino acids undergo first-pass splanchnic uptake, our data also suggest a significant uptake of threonine in the splanchnic compartment. The magnitude of the first-pass uptake of threonine would require administration of an additional threonine tracer via the enteral route.

Threonine and phenylalanine, being essential amino acids, are released in the same proportion as they are present in the mixed whole body protein, of which skeletal muscle is the largest constituent. Threonine and phenylalanine constitute 5.3 and 5%, respectively, of the skeletal muscle protein (2). If threonine is released exclusively from the skeletal muscle protein, the ratio between threonine and phenylalanine should be ~1. In our study, the plasma flux of threonine relative to the flux of phenylalanine was 2.2. These data suggest a significant contribution of threonine-enriched proteins to the whole body protein turnover. The nature and the site of these proteins cannot be discerned from the present data and could be glycoproteins and intestinal mucins, which contain ~30% of threonine (4).

In the present study, the fraction of threonine oxidized relative to threonine flux is higher than that reported in adults (7, 23) and in growing premature infants (8). In addition, we observed a significant relationship between plasma threonine concentration and threonine oxidation (Fig. 2). Although we have not quantified the rate of oxidation of threonine in response to graded threonine intakes, Zhao et al. (23) demon-
strated in human adults that the threonine oxidation was between 12 and 15% at threonine intakes between 30 and 100 mg·kg⁻¹·day⁻¹. Infants in the present study protocol received protein-bound dietary threonine at 34 μmol·kg⁻¹·h⁻¹, equivalent to 97 mg·kg⁻¹·day⁻¹ of threonine, and their rate of threonine oxidation was 26%. Because our studies were performed during the first 48 h after birth, the higher rate of oxidation of threonine may be related to the birth-related surge in hormones, specifically glucagon and cortisol, leading to an increased activity of STDH in the liver (12). The linear relationship between plasma threonine and threonine oxidation suggests substrate induction of STDH as demonstrated in other studies (5, 7, 23).

In contrast to data in animals (3, 14), glycine-dependent threonine oxidation is a minor degradative pathway in humans. Darling et al. (7) observed that only 10% of threonine catabolism proceeds via the glycine-dependent pathway in human adults. However, in growing premature infants, ~44% of total threonine degradation was estimated to occur via glycine-dependent threonine dehydrogenase pathway (8). TDH is expressed in all the tissues (9). The open reading frames (ORF) of TDH cDNAs encode full-length proteins of 373 residues (41.5 kDa) in mouse and pig (10). In contrast, instead of the expected 369 residue ORF, the 2 cDNA transcripts of human TDH encode truncated proteins of 151 and 230 residues (9). Our data of lack of incorporation of threonine in glycine agrees with nonfunctional TDH in human newborns. It should be underscored that the data of fasting as reported here, i.e., ~4–6 h after the last feeding, does not strictly represent a postabsorptive state. Because the infants are fed every 3 h and have varying gastrointestinal motility patterns, they remain in varying states of continuous feeding. Therefore, the response to feeding, as examined here, does not represent a response over a fasted state.

In conclusion, our data show that threonine disposal is mediated exclusively by SSTDH and induced by threonine. A higher threonine Rₜ relative to phenylalanine Rₜ suggests turnover of threonine-enriched glycoproteins, possibly in the splanchic compartment.

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GRANTS

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