Relation between transamination of branched-chain amino acids and urea synthesis: evidence from human pregnancy

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Kalhan, Satish C., Karen Q. Rossi, Lourdes L. Gruca, Dennis M. Super, and Samuel M. Savin. Relation between transamination of branched-chain amino acids and urea synthesis: evidence from human pregnancy. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E423–E431, 1998.—Protein and nitrogen (N) accretion by the mother is a major adaptive response to pregnancy in humans and animals to meet the demands of the growing conceptus. Quantitative changes in whole body N metabolism were examined during normal pregnancy by measuring the rates of leucine N (QN) and carbon (QC) kinetics with the use of [1-13C,15N]leucine. Rate of synthesis of urea was measured by [15N2]urea tracer. Pregnancy-related change in total body water was quantified by H2[18O] dilution, and respiratory calorimetry was performed to quantify substrate oxidation. A significant decrease in the rate of urea synthesis was evident in the 1st trimester (nonpregnant 4.69 ± 1.14 vs. pregnant 3.44 ± 1.11 μmol·kg⁻¹·min⁻¹; means ± SD, P < 0.05). The lower rate of urea synthesis was sustained through the 2nd and 3rd trimesters. QN was also lower in the 1st trimester during fasting; however, it reached a significant level only in the 3rd trimester (nonpregnant 166 ± 35 vs. 3rd trimester 135 ± 16 μmol·kg⁻¹·h⁻¹; P < 0.05). There was no significant change in QC during pregnancy. A significant decrease in the rate of transamination of leucine was evident in the 3rd trimester both during fasting and in response to nutrient administration (P < 0.05). The rate of deamination of leucine was correlated with the rate of urea synthesis during fasting (r = 0.59, P = 0.001) and during feeding (r = 0.407, P = 0.01). These data show that pregnancy-related adaptations in maternal N metabolism are evident early in gestation before any significant increase in fetal N accretion. It is speculated that the lower transamination of branched-chain amino acids may be due to decreased availability of N acceptors such as α-ketoglutarate as a consequence of resistance to insulin action evident in pregnancy.

leucine; stable isotopes; nitrogen accretion

THE METABOLIC ADAPTATIONS observed during pregnancy in humans and animals are aimed to provide the nutrient and energy requirements for both the mother and the growing conceptus. Because glucose is the primary source of energy for the fetus and is a significant contributor to maternal energy metabolism, changes in maternal glucose metabolism appear to occur in parallel with the increasing demands of pregnancy and conceptus (7, 26, 28, 34). Similarly, protein/nitrogen accretion by the mother for the synthesis of new maternal and fetal tissue necessitates changes in maternal protein turnover as well as nitrogen (N) excretion starting early in gestation. Previous studies using balance data in human pregnancy have shown that N retention by the mother throughout pregnancy occurs in excess of the theoretical protein cost, supporting the concept that maternal N gain as lean body mass is a significant component of protein accretion over and above that deposited in the fetus and products of conception (27). The physiological mechanisms associated with the N accretion of pregnancy have not been studied in detail.

Rates of urea synthesis and rates of excretion of urinary urea N have been employed to quantify catabolism and oxidation of protein late in gestation in humans and animals. These data show that in the latter part of pregnancy, in both humans and animals, the rate of synthesis of urea and its rate of excretion in urine are lower when compared with nonpregnant women, both during fasting and in response to amino acid load (4, 16, 29, 36). The mechanism for the lower rate of urea synthesis during pregnancy is not clear. It could be the consequence of decreased delivery of ureogenetic substrates to the liver or due to decreased activity of urea cycle (29). Although pregnancy hormones such as progesterone and estrogen have been shown to have suppressive effects on the activity of the urea cycle enzymes (39), a significant decrease in the plasma concentration of circulating amino acids has also been observed early in gestation, both in humans and animals (18, 29, 30). The decreased concentration of α-amino-N, by decreasing the hepatic delivery or ureogenic substrate, would be expected to result in decreased rate of urea synthesis. In the present study, we have quantified longitudinally the rate of urea synthesis through pregnancy in normal women, starting early in gestation with the use of stable isotopic tracer [15N2]urea. Because branched-chain amino acids are the major source of N for the ureogenic amino acids alanine and glutamine (21, 23), we have also quantified the rates of transamination of leucine and related these to changes in urea synthesis as gestation progresses. Direct carcass analysis and other indirect data suggest increased N accretion by the mother as lean body mass early in pregnancy, even before significant N accretion by the fetus (25, 44). Because N accretion has been associated with significant changes in rates of whole body turnover of protein, kinetics of leucine were also quantified as a measure of the dynamic aspects of whole body protein metabolism. In addition, changes in total body water (TBW) during pregnancy were measured with the use of H2[18O] to quantify changes in lean body mass. The studies were aimed at examining the hypothesis that conservation of N is an important component of N accretion and that it will manifest as decreased rate of urea synthesis and a decrease in the
rate of leucine transamination. Furthermore, these changes will be apparent early in gestation.

**MATERIALS AND METHODS**

Leucine and urea kinetics studies were performed during pregnancy in normal, healthy subjects. Ten subjects were studied in the 1st trimester, twelve in the 2nd trimester, and eight in the 3rd trimester. Seven subjects could be studied serially throughout the pregnancy. The reasons for attrition included premature delivery, lack of interest, and pregnancy-related problems. However, inclusion of their data as reported here did not have any significant impact on the conclusions of the study. The data on longitudinally studied subjects (n = 6) are displayed (see Figs. 2–4). The data on the seventh subject were outside the range of the others because of unexplained reasons (see RESULTS). In addition, she delivered a large-for-gestational-age infant at term gestation weighing 4,261 g. Seven nonpregnant women were studied as controls.

All subjects were healthy, were not receiving any medications other than vitamin supplements, and had no medical complications related to pregnancy. Their glucose tolerance tests at 26–28 wk of gestation were normal. The nonpregnant subjects were studied between day 8 and 15 of menstrual cycle. They were not receiving any medications, including oral contraceptives, and did not have a family history of diabetes. The clinical characteristics of the study subjects are presented in Table 1. The protocol was approved by the Institutional Review Board for Investigation in Humans. Written informed consent was obtained from each subject and her husband after the procedure was fully explained.

Subjects were asked to come to the General Clinical Research Center in the morning after an overnight fast of 10 h. Two 22-gauge cannulas were placed in superficial veins on the dorsum of the hands, one for the infusion of isotopic tracers and the other for obtaining blood samples. The sampling site was kept patent by infusing isotonic saline solution at 10 ml/h and was kept warm to obtain arterialized blood samples.

\[^{15}N\]urea, 99% \[^{13}C\]; \[^{13}C\]-leucine, 99% \[^{15}N\]; \[^{13}C\]-leucine, 99% \[^{13}C\]; and 99% \[^{15}N\]; NaH\[^{13}C\]O\(_3\), 99% 13C; and H\(_2\)\[^{18}O\], 99% 18O, were obtained from Merck (Dorval, PQ, Canada). The tracers for intravenous administration were dissolved in sterile isotonic saline, sterilized by Millipore filtration, and tested for sterility and pyrogenicity as previously described (29). After basal blood and breath samples were obtained, the tracers were infused according to prime constant rate infusion technique. For \[^{15}N\]urea, the prime was 2.0 mg/kg and the infusion rate was 0.2 mg·kg\(^{-1}\)·h\(^{-1}\). For \[^{13}C\]-leucine, the prime was 5 µmol/kg and the infusion rate was 5 µmol·kg\(^{-1}\)·h\(^{-1}\). To achieve an early steady state in the CO\(_2\)/bicarbonate pool, a priming dose of 10 mg of NaH\[^{13}C\]O\(_3\) was also administered at the start of the study. In addition, an accurately weighed dose (2–2.5 g) of H\(_2\)\[^{18}O\] was given orally to estimate the TBW. The subjects continued the fast for the next 165 min. The response to a mixed nutrient load was evaluated by giving oral Ensure Plus (Ross Laboratories, Columbus, OH) at a rate of 35 ml every 30 min for the next 3 h. This dose of Ensure Plus corresponds to 101 kcal and 3.7 g protein per hour or 2,442 kcal/day and 89 g protein/day. The study design is displayed in Fig. 1.

Arterialized blood samples were obtained at 30-min intervals during the 1st hour and every 15 min for the rest of the study. Blood samples were centrifuged immediately, and plasma was stored at –70°C until analysis. In addition, breath samples for \(^{13}C\) analysis were collected every 30 min by having the subject breathe through a one-way valve (3, 22). Respiratory calorimetry measurements were performed intermittently throughout the study using an open-circuit system described previously (3, 22). The rates of oxygen consumption (V\(_{\text{O}_2}\)) and CO\(_2\) production (V\(_{\text{CO}_2}\)) were measured at hourly intervals by placing a ventilated hood over the subject’s head. Recordings were obtained for a period of at least 15 min. The analyzer was calibrated using gravimetrically measured standard mixtures of oxygen and CO\(_2\). The accuracy and precision of the respiratory calorimetry system were checked by measuring the respiratory quotient of absolute alcohol and were within 2% of the expected value.

The effect of Ensure feeding without isotopic tracers on \(^{13}C\) enrichment of breath CO\(_2\) was examined in three nonpregnant and six pregnant women ranging from 12 to 34 wk of gestation. Feeding of Ensure Plus for 3 h resulted in an average increase in \(^{13}C\) enrichment of breath CO\(_2\) by 0.0016% (SD: ± 0.0009%). There was no significant difference between the pregnant and nonpregnant groups or within the pregnant group with advancing gestation. Because the change in \(^{13}C\) enrichment of CO\(_2\) was not significant, no correction was made for this change in calculating leucine oxidation during Ensure administration.

Analytic procedures. Plasma glucose and urea N concentrations were measured by glucose oxidase and urease methods, respectively, on respective commercial analyzers (Beckman Instruments, Fullerton, CA). Plasma insulin levels were measured by double-antibody RIA. The lowest detectable level by this assay is 2 µU/ml. These analytic techniques have been reported from our laboratory previously (26, 29).

Mass spectrometric analysis. The mass spectrometric methods for the measurement of isotopic enrichment of leucine, \(\alpha\)-ketosuccinic acid (KIC), and urea have been described from this laboratory (16, 19, 40, 42). Leucine and urea were

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Gestation, wk</th>
<th>Weight, kg</th>
<th>BMI, %</th>
<th>Weight Gain, kg</th>
<th>TBW, kg</th>
<th>TBW/WT, %</th>
<th>Calorie Intake, kcal·kg(^{-1})·day(^{-1})</th>
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<tr>
<td>Nonpregnant (n = 7)</td>
<td>28 ± 5</td>
<td>66.3 ± 14.6</td>
<td>23.7 ± 4.7</td>
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</tr>
<tr>
<td>1st Trimester (n = 10)</td>
<td>28 ± 5</td>
<td>64.4 ± 17.5</td>
<td>22.7 ± 5.0</td>
<td>35.7 ± 4.7</td>
<td>55.3 ± 8.0</td>
<td>32.8 ± 6.6</td>
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</tr>
<tr>
<td>2nd Trimester (n = 12)</td>
<td>28 ± 4</td>
<td>67.5 ± 15.9</td>
<td>23.0 ± 4.4</td>
<td>37.0 ± 4.7</td>
<td>56.0 ± 6.3</td>
<td>32.9 ± 7.8</td>
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</tr>
<tr>
<td>3rd Trimester (n = 8)</td>
<td>28 ± 3</td>
<td>75.7 ± 19.2</td>
<td>23.6 ± 5.6</td>
<td>41.4 ± 6.7</td>
<td>55.8 ± 6.2</td>
<td>30.5 ± 9.2</td>
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</tbody>
</table>

Values are means ± SD; n = no. of subjects. Body mass index (BMI) based on prepregnancy weight: Wt in kg/length in m\(^2\). Values for weight gain are during pregnancy. Total body water (TBW) measured by H\(_2\)\[^{18}O\] dilution.
The 15N enrichment of leucine, representing the mono- and di-labeled leucine, were monitored using mass spectrometry system. Methane chemical ionization method of Adams (1) with certain modifications (16). A 15N-derivative of leucine was prepared according to the method (not significantly) than that of [13C]KIC. Because [13C]KIC enrichment was measured directly and [13C]leucine enrichment was calculated as described previously (11, 35). Norleucine was used as internal standard to measure plasma leucine levels. A trifluoroacetol-hydroxypropimidine derivative of urea was prepared, and the 15N enrichment was quantified in the electron impact mode by monitoring of m/z 128 and m/z 129 were also monitored in the electron impact mode (35). From these data, the enrichments of [1-13C,15N]leucine and total [13C]leucine were calculated as described previously (11, 35). Norleucine was used as internal standard to measure plasma leucine levels. A trifluoroacetol-hydroxypropimidine derivative of urea was prepared, and the 15N enrichment was quantified in the electron impact mode by monitoring of m/z 153 and m/z 155 (42). The 13C enrichment of plasma KIC was measured using a tritiated derivative (19). Standard solutions of known enrichments were run along with the unknowns to correct for analytical variations. 13C enrichment of the CO2 in expired air was measured after separation of the CO2 by cryogenic distillation in vacuum, as previously described (22). 18O enrichment of the expired CO2 was also measured at the same time as 13C measurements with the use of an isotope ratio mass spectrometer (16).

Calculations. VO2 and VCO2 were calculated by multiplication of the gradients across the face with the flow rate of air and application of Haldane transformation (22). The measured VO2 and VCO2 were corrected to the standard temperature and pressure. The rate of appearance of leucine (Ra) was calculated from the tracer dilution during steady state (28). Ra = I × (Ei/Ep) − 1, where I is the rate of infusion of the tracer (µmol·kg⁻¹·min⁻¹) and Ei and Ep represent the enrichments of infused and of plasma leucine at steady state, respectively. Leucine carbon flux (Qc) was calculated using plasma [13C]KIC, and leucine N flux (Qn) was calculated using [1-13C,15N]leucine enrichment during isotopic steady state (11, 35). Isotopic steady state was determined by visual inspection of the data. Enrichment data between 135 and 160 min and between 315 and 360 min were used to calculate the turnover rate. The coefficient of variation for the enrichment data for leucine and KIC in individual subjects was between 3 and 5%, and the slope was not different from zero.

As discussed previously (11, 35, 37), during fasting, Qn measured by the dilution of [1-13C,15N]leucine tracer is the sum of leucine released by protein breakdown and that formed from reamination of KIC. Qc, in contrast, is predominantly due to protein breakdown, since leucine carboxyl-13C is not lost by leucine transamination to and from KIC. Hence, the subtraction Qn minus Qc provides an estimate of the rate of reamination (XN) of KIC. Because all of the labeled [13C]leucine is derived from [13C]KIC by reamination and because the rate of transamination of leucine is high relative to the Ra of leucine, the enrichment of [13C]KIC and [13C]leucine should be similar. The data in the present study (not reported) showed [13C]leucine enrichment to be slightly less (not significantly) than that of [13C]KIC. Because [13C]KIC enrichment was measured directly and [13C]leucine enrichment was calculated from two different measurements and had a high variance, we elected to use [13C]KIC enrichment to calculate Qc, as was done by other investigators (37). Inasmuch as [1-13C,15N]leucine enrichment is measured in plasma, the measured Qn is an underestimate, since the intracellular enrichment would be less than that in plasma.

Transamination (deamination) of leucine into KIC (XO) and reamination of KIC back to leucine (XN) were calculated as described by Matthews et al. (35): Qn − Qc = Xn + Xo − C, where C is the rate of decarboxylation of C-1 of leucine and KIC. Because Qn, Qc, and C are directly measured from the tracer data, Xn and Xo can be calculated. The fraction (F) of leucine turnover decarboxylated (oxidized) was calculated as follows: F = VCO2 × [13C]O2/I, where VCO2 is in µmol·kg⁻¹·min⁻¹, [13C]O2 is the 13C enrichment of CO2 during steady state, and I is the rate of infusion of [1-13N,13C]leucine in µmol·kg⁻¹·min⁻¹. The rate of oxidation of leucine was calculated by multiplication of F with leucine carbon turnover (Qc).

The TBW was measured by the dilution of H2[18O] tracer (16). Because 18O equilibrates rapidly between H2O and CO2, 18O enrichment was measured in the expired CO2. TBW (kg) = [dose of H2[18O] (g) × APE × f(E − Eo) × 1,000], where atom percent excess (APE) = 18O enrichment of H2[18O] administered, fractionation factor for the equilibrium of H2O and CO2 (f) = 1.039 at 37°C, and E1 and Eo are the 18O enrichments of expired CO2 at plateau and at time zero, respectively.

Statistics. All data are reported as means ± SD. The data were analyzed with the SPSS/PC+ version 4.0 Statistical Package for the Social Sciences (Chicago, IL). The Mann-Whitney U test and Wilcoxon's signed rank test were used for unpaired and paired interval level data, respectively.
The pregnant and nonpregnant women were similar in age, body weight, and body mass index (Table 1). All subjects gained weight appropriately during pregnancy. The serially studied subjects, those who completed all the studies, delivered at term gestation. Their infants were normal and appropriate for gestational age (birth weight 3,307 ± 564 g). With the increase in body weight through gestation, there was a corresponding increase in tracer-measured TBW. However, the fraction of body weight represented by body water did not change with advancing gestation (Fig. 2).

The plasma concentrations of glucose, urea N, leucine, and insulin during fasting and during feeding (Ensure Plus) are displayed in Table 2. As shown, the plasma glucose concentration during fasting was slightly lower in pregnant subjects compared with nonpregnant subjects. In response to feeding, there was a significant increase in plasma glucose concentration in all subjects. Plasma insulin concentration during fasting was slightly higher during pregnancy (not significant), particularly during the 3rd trimester, and there was a significant (P < 0.02) increase in insulin levels in response to feeding in all subjects. Plasma urea N concentration was significantly lower (P < 0.003) in early gestation compared with the nonpregnant group and remained significantly lower throughout pregnancy. In response to feeding, there was a significant decrease in plasma urea N concentration in the nonpregnant subjects. Plasma leucine levels, also displayed in Table 2, were slightly lower during pregnancy and increased in response to feeding.

Urea synthesis during pregnancy. The rate of urea synthesis during fasting in the 1st trimester of pregnancy was significantly less (P < 0.05) compared with nonpregnant subjects (Table 3). With advancing gestation, there was a further decrease in urea synthesis during fasting. Feeding did not result in any significant change in the rate of urea synthesis in all subjects.

Leucine carbon and N kinetics. The rate of appearance of leucine N (Q_N) was calculated from the enrichment of leucine (m+2) species, whereas Q_C was calculated from the enrichment of KIC (m+1) species in the plasma. During fasting, Q_N was less during pregnancy compared with nonpregnant state in the 1st and 3rd trimesters; however, the differences were statistically significant only in the 3rd trimester (P < 0.05). Q_N was higher during the 2nd trimester compared with the 1st and 3rd trimesters. In contrast to Q_N, Q_C was unchanged throughout pregnancy compared with nonpregnant controls. In addition, there was no difference in the fraction of leucine C-1 decarboxylated between nonpregnant or pregnant women nor was there any significant impact of advancing gestation on leucine oxidation. There was a trend toward decrease in fractional rate of oxidation with advancing gestation. The calculated rate of nonoxidative disposal of leucine carbon was not affected by pregnancy.

In the serially studied subjects (Fig. 3), there was no significant change in Q_N or Q_C between the 1st and 3rd trimesters during fasting. However, there was an increase in Q_N during the 2nd trimester when compared with the 1st trimester. There was a statistically significant decrease in the fraction of leucine carbon turnover oxidized (C/Q_C) between the 1st and 2nd trimesters (18.8 ± 2.9%, 1st trimester; 16.1 ± 1.6%, 2nd trimester; P < 0.05) and between the 1st and 3rd trimesters (16.2 ± 2.7%, 3rd trimester; P < 0.05). The changes in transamination of leucine in serially studied subjects

Table 2. Plasma substrate levels

<table>
<thead>
<tr>
<th></th>
<th>Glucose, mmol/l</th>
<th>Urea N, mmol/l</th>
<th>Leucine, µmol/l</th>
<th>Insulin, µU/ml</th>
</tr>
</thead>
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<td></td>
<td>Fasted</td>
<td>Fed</td>
<td>Fasted</td>
<td>Fed</td>
</tr>
<tr>
<td>Nonpregnant (n = 7)</td>
<td>4.5 ± 0.3</td>
<td>5.4 ± 0.4^a</td>
<td>5.1 ± 1.1</td>
<td>4.6 ± 1.0^b</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Trimester (n = 10)</td>
<td>4.1 ± 0.4^2</td>
<td>5.2 ± 0.4^d</td>
<td>3.3 ± 0.6^2</td>
<td>3.2 ± 0.7^2</td>
</tr>
<tr>
<td>2nd Trimester (n = 12)</td>
<td>4.0 ± 0.3^2</td>
<td>5.0 ± 0.4^c</td>
<td>2.9 ± 0.7^2</td>
<td>2.7 ± 0.7^2^3</td>
</tr>
<tr>
<td>3rd Trimester (n = 8)</td>
<td>4.1 ± 0.4^1</td>
<td>5.2 ± 0.5^e</td>
<td>2.9 ± 0.7^2</td>
<td>3.0 ± 0.7^2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. N, nitrogen. Significantly different from nonpregnant: ^1P < 0.05 and ^2P < 0.005. Significantly different from fasting state: ^3P < 0.05, ^4P < 0.01, and ^5P < 0.005.
are displayed in Fig. 4. As with the whole group, X₀ and X₇ were lower in the 1st trimester compared with nonpregnant subjects. There was no significant change in X₇ and X₀ from the 1st to the 3rd trimester. The fraction of leucine reaminated (X₇/X₀) was significantly reduced with advancing gestation when all the data were examined by Friedman two-way ANOVA (P < 0.05).

In response to feeding, although Q₇ in all groups, it was statistically significant only in the 2nd trimester of pregnancy (P < 0.05). In contrast, the Q₇ increased significantly at all times. On average, the increase in Q₇ was 15 µmol·kg⁻¹·h⁻¹ compared with leucine intake of 35–40 µmol·kg⁻¹·h⁻¹, suggesting ~50% uptake by the splanchnic tissues. In addition, the fractional as well as total rate of leucine C-1 decarboxylation increased in response to feeding. In the pregnant group, the rate of oxidation of leucine was significantly less in the 3rd trimester compared with the 1st trimester (Tables 3 and 4).

The six serially studied subjects showed a significant increase in Q₇ in response to feeding in the 1st and 3rd trimesters (1st trimester: fast 106.3 ± 21.3, fed 122.0 ± 25.7, P = 0.028 Wilcoxon’s signed rank test; 3rd trimester: fast 110.5 ± 21.2, fed 126.8 ± 18.1 µmol·kg⁻¹·h⁻¹, P = 0.028). In addition, there was a significant increase in fraction of leucine C-1 decarboxylated during the fed state in the 1st, 2nd, and 3rd trimesters (1st trimester: fast 18.8 ± 2.9 vs. fed 33.6 ± 6.5%; 2nd trimester: fast 16.1 ± 1.6 vs. fed 25.3 ± 2.6%; 3rd trimester: fast 16.2 ± 2.7 vs. fed 21.9 ± 5.0%; P = 0.028). It should be noted that the lowest value of P with Wilcoxon’s rank sum test would be 0.028 if all six subjects have the same trend.

On the basis of the Q₇ and Q₇ data, the X₀ and X₇ were calculated (Table 4). The X₀ (i.e., conversion to KIC) was less during pregnancy compared with non-pregnant controls. Similar decrease in X₇ (i.e., conversion of KIC to leucine) was also seen in pregnancy. The effect was statistically significant and most pronounced during the 3rd trimester. X₇ represented 50–75% of the deamination (X₇/X₀) of leucine. Qualitatively similar changes were seen in response to feeding.

Respiratory calorimetry. As described previously, there was no change in weight-specific V˙O₂ or V˙CO₂ values during pregnancy. The respiratory exchange ratio was less in the 1st trimester compared with nonpregnant subjects and was higher in the 3rd trimester compared with the 1st trimester and nonpregnant controls (Table 5). In response to feeding, there was an increase in V˙O₂ and V˙CO₂ as well as respiratory exchange ratio. All the observations were similar among the four groups in the fed state (data not shown).

Correlations. The X₀ of leucine was correlated with the rate of urea synthesis, both during fasting (r = 0.589, P = 0.0001, y = 93.6 + 1.78x; Fig. 5)
and in response to feeding (r = 0.407, P = 0.012). Removal of one outlier in the fasting data improved the correlation (r = 0.736). A significant correlation was also observed between the XN of leucine and synthesis of urea during fasting (r = 0.66, P = 0.0004) but not during feeding. Interestingly, there was no correlation between rate of decarboxylation (oxidation) of leucine (C) and rate of urea synthesis or between calculated rates of oxidation of protein using leucine or urea method.

One subject studied serially throughout pregnancy delivered a large-for-gestational-age infant (birth weight 4,261 g, K score 1.635). QN and QC during pregnancy (QN: 208, 157, and 178 µmol·kg⁻¹·h⁻¹; QC: 164, 144, and 153 µmol·kg⁻¹·h⁻¹) and rate of urea synthesis (7.8, 7.9, and 11.7 mg N·kg⁻¹·h⁻¹) were all higher than in other pregnant subjects.

**DISCUSSION**

The significant results of the present study can be summarized as follows: 1) commensurate with the increase in body weight during pregnancy, there is a proportional increase in TBW and hence lean body mass; 2) there is a decrease in the rate of synthesis of urea early in gestation that is sustained throughout the pregnancy; 3) a decrease in QN is evident early in gestation, whereas the QC does not change with pregnancy; 4) a significant correlation was evident between the XN of leucine and synthesis of urea. These data are significant in that, for the first time, they point to the mechanism of N conservation, i.e., lower rate of urea synthesis and lower rate of transamination of branched-chain amino acids during pregnancy, and that conservation of N occurs early in gestation before any significant growth in fetal mass. Thus these adaptive changes may be aimed at the accretion of N (protein) by the mother as lean body mass, which may be a necessary anticipatory adaptation to pregnancy and fetal N requirements.

Estimates of TBW have been used to quantify lean body mass to relate changes in protein and energy metabolism. Previous estimates of TBW in human pregnancy using either [³H]₂O or H₂¹⁸O are similar to those reported here (16, 25, 44). All of these show no significant change in the fraction of body weight represented by TBW during pregnancy. However, calculation of lean body mass from TBW alone using the conventional equations for nonpregnant adults may cause errors because of changes in hydration index of maternal tissue during pregnancy and because of the contribution of fetal tissues, with much higher content of water, to these measurements (8, 31, 44). Use of newer hydration index would have resulted in small change in estimated lean body mass. For these reasons, we elected not to calculate lean body mass from these data. Nevertheless, because there was no change in the fraction of body weight represented by TBW, expression of the kinetic data per unit lean body mass would have had minimal impact on the overall results. Finally, these changes in TBW during pregnancy do suggest that accretion of lean body mass (protein) is a significant component of increase in body weight by the mother during pregnancy and that this protein accretion starts early in gestation.

**Table 4. Effect of pregnancy on leucine metabolism**

<table>
<thead>
<tr>
<th></th>
<th>X₀, µmol·kg⁻¹·h⁻¹</th>
<th>Xₙ, µmol·kg⁻¹·h⁻¹</th>
<th>C, µmol·kg⁻¹·h⁻¹</th>
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<tr>
<td>Nonpregnant (n = 7)</td>
<td>79.6 ± 30.3</td>
<td>88.6 ± 32.7</td>
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<td>57.3 ± 31.3</td>
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</tr>
<tr>
<td>1st Trimester (n = 10)</td>
<td>56.1 ± 15.9</td>
<td>70.4 ± 20.8</td>
<td>37.8 ± 15.8</td>
<td>33.2 ± 17.2</td>
</tr>
<tr>
<td>2nd Trimester (n = 12)</td>
<td>61.8 ± 20.4</td>
<td>79.6 ± 23.4</td>
<td>44.1 ± 19.7</td>
<td>50.7 ± 23.8</td>
</tr>
<tr>
<td>3rd Trimester (n = 8)</td>
<td>43.7 ± 18.8</td>
<td>57.5 ± 25.0</td>
<td>26.4 ± 20.5</td>
<td>31.4 ± 22.5</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. X₀ and Xₙ, rate of deamination and of reamination of leucine, respectively; C, rate of decarboxylation (oxidation) of leucine. Significantly different from nonpregnant: ²P < 0.05 and ³P < 0.03. Significantly different from fasting state: ⁴P < 0.03 and ⁵P < 0.01. Significantly different from 1st trimester: ⁶P < 0.05.

**Table 5. Respiratory calorimetry during fasting**

<table>
<thead>
<tr>
<th></th>
<th>V₀₂, ml·kg⁻¹·min⁻¹</th>
<th>Vₐ₉, ml·kg⁻¹·min⁻¹</th>
<th>RER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (n = 7)</td>
<td>3.49 ± 0.47</td>
<td>2.80 ± 0.36</td>
<td>0.806 ± 0.06</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Trimester (n = 9)</td>
<td>3.43 ± 0.50</td>
<td>2.69 ± 0.47</td>
<td>0.773 ± 0.042</td>
</tr>
<tr>
<td>2nd Trimester (n = 12)</td>
<td>3.39 ± 0.48</td>
<td>2.72 ± 0.32</td>
<td>0.805 ± 0.053</td>
</tr>
<tr>
<td>3rd Trimester (n = 7)</td>
<td>3.42 ± 0.36</td>
<td>2.92 ± 0.55</td>
<td>0.853 ± 0.094</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. V₀₂, rate of O₂ consumption; Vₐ₉, rate of CO₂ production; RER, respiratory exchange ratio.

Fig. 5. Correlation between rate of deamination of leucine (X₀) and rate of synthesis of urea during fasting (y = 93.6 + 1.78x, r = 0.589, P = 0.0001). Correlation was better (r = 0.736) when 1 outlier was removed. Su, synthesis of urea.
Although a lower concentration of plasma urea N during pregnancy has been consistently reported in both human and animal studies, few studies have quantified the rates of urea synthesis in vivo. Studies in animals, either in vivo or of isolated perfused liver preparation, have shown a decreased rate of urea synthesis in response to an amino acid or protein load in pregnancy compared with nonpregnant state (4, 36). Data in human pregnancy show a decreased or unchanged rate of urea N synthesis and excretion during the 3rd trimester (16, 20, 29). Our previous data showed a lower rate of urea synthesis in both normal and diabetic women during fasting in the 3rd trimester compared with postpartum. Forrester et al. (20) have quantified rates of urea production in normal pregnant women in Jamaica throughout the day using $\text{[^{15}N_2]}$ urea tracer as intermittent intravenous dose. The dilution of the tracer was measured in urinary urea. In addition, the dietary protein and caloric intakes of their study subjects were higher throughout pregnancy compared with nonpregnant controls. These authors reported no change in urea production between pregnant and nonpregnant women. However, the rate of urea synthesis was significantly less during the 3rd trimester when compared with the 1st trimester. It should be underscored that their study represents a sum of both fasting and fed state, the data having been obtained throughout the day. In addition, the impact of intermittent tracer dose on calculated rates of urea synthesis remains unknown. In our study, we examined the responses during fasting and in response to nutrient intake after rigid dietary preparation and constant-rate tracer infusion. The protein and caloric intakes were those recommended for the pregnant and nonpregnant women. These data are the first to show a decreased rate of urea synthesis, or conservation of N, in the 1st trimester of pregnancy, much before there is any significant fetal N accretion. An almost 30% lower rate of urea synthesis, when compared with nonpregnant controls, was evident in the 1st trimester, and a 45% lower rate was evident in the 3rd trimester.

The dynamic aspects of protein turnover in pregnancy have been quantified using labeled leucine, phenylalanine, or glycine tracers to calculate change in rates of synthesis and breakdown of whole body protein. The impact of the use of different isotopically labeled amino acid tracers and related problems have been discussed (5). Studies in pregnant rats using $[1-\text{^{14}C}]$ leucine tracer showed a decrease in weight-specific rate of leucine turnover and an increase in oxidation of leucine between day 17 and 20 of gestation (33). However, there was no change in the amount of leucine incorporated into proteins. In contrast, studies in normal human subjects during the 3rd trimester of pregnancy, using $[1-\text{^{13}C}]$ leucine tracer, showed no significant change in leucine kinetics between 20 and 40 wk of gestation (16). When compared with nonpregnant women, there was a significant decrease in leucine turnover during pregnancy. However, these kinetics were calculated on the basis of enrichment of plasma leucine rather than that of KIC. Thompson and Halli-day (41) quantified protein turnover during pregnancy in six subjects with the use of $[\text{^{14}C}]$ leucine tracers and by measuring the dilution in plasma KIC. When expressed in relation to total body weight, the calculated rate of protein turnover did not change during pregnancy. All of these data are similar to those reported here, showing no change in the $Q_C$ during pregnancy. Of significance, these rates were similar to those in the nonpregnant women. In addition, the $Q_C$ values during feeding were also similar in pregnant and nonpregnant women (Table 3). There was a small decrease in the fraction of leucine decarboxylated (C/$Q_C$) during pregnancy, and it was statistically significant in the serially studied subjects (Fig. 3). These data suggest that, as with urea synthesis, N conservation by the mother is also evident in the lower rate of decarboxylation of leucine. However, the magnitude of change estimated by leucine decarboxylation was less than that measured by change in rate of urea synthesis, and there was no correlation between the rates of decarboxylation of leucine and urea synthesis.

Because deamination of branched-chain amino acids in the muscle is considered the major source for alanine and glutamine N (21, 23), which in turn are the sources for urea N, we hypothesized that the decreased rate of urea N synthesis will be associated with decreased $Q_N$. This hypothesis was confirmed by the lower $Q_N$ early in pregnancy. In addition, a strong correlation was observed between the rates of urea synthesis and rate of deamination of leucine (Fig 5). These are the first estimates of leucine N kinetics in human pregnancy and provide a mechanistic explanation for the decreased rate of urea N synthesis. A small decrease in glycine N turnover was also seen by DeBenost et al. (14) in pregnant women in late gestation.

Branched-chain $\alpha$-ketoadid dehydrogenase complex is the rate-limiting enzyme involved in the irreversible loss of leucine. No significant change in the rate of decarboxylation of leucine was observed in the present study or reported in other states of protein accretion (growth), such as puberty, growth hormone therapy, or newborn infant (2, 15, 38). A decreased rate of urea synthesis has also been observed after growth hormone therapy (12). Of interest, pregnancy, puberty, neonate, and growth hormone therapy are characterized as states of insulin resistance, specifically in relation to glucose uptake. The mechanism of the decrease in transamination and its impact on leucine metabolism and protein synthesis remain speculative. Because the equilibrium constant for most transaminase reactions is close to unity, transamination is a freely reversible process with no net transfer of N. However, the transfer of N may be regulated by the availability of the N acceptor, in this instance $\alpha$-ketoglutarate, which in turn may be regulated by changes in anaplerotic carbon flux into the tricarboxylic acid cycle induced by insulin resistance. Evidence presented by Hedden and Buse (24) in vitro muscle preparation suggests that an increase in N acceptors, e.g., by increasing pyruvate, results in decreased rate of protein synthesis, possibly by decreasing the intracellular leucine pool and increasing the
KIC pool. Such a situation should also lead to lower rates of glutamine and possibly alanine synthesis, as has been reported after growth hormone therapy (6). Whether such reduction in glutamine turnover also occurs in pregnancy needs to be examined.

In human studies, the contribution of the fetus to the overall whole body measurements in the mother, as described here, cannot be separated. The observed changes in maternal N/protein metabolism are likely to be minimally influenced by the quantitative N requirements of the fetus, since the estimated fetal N requirement for N accretion and energy (oxidation) cannot explain the observed changes in the N metabolism of the mother. First, the decrease in urea synthesis and in leucine N kinetics occurred before any significant fetal N accretion in the 1st trimester. Second, the changes in Q\textsubscript{\text{N}} during the 2nd and 3rd trimesters did not parallel changes in N accretion by the fetus. In fact, there was a small increase in Q\textsubscript{\text{N}} in the 2nd trimester (Table 3). Estimates of amino acid balance across the umbilical circulation and estimates of N accretion and amino acid oxidation by the fetus in human studies suggest fetal N uptake to be \(\sim 450 \text{mg N} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) (9, 32). Of this, only 120 mg N \cdot kg\textsuperscript{-1} \cdot day\textsuperscript{-1} represent N accretion by the human fetus (10, 45). In relation to the body weight of the mother, these represent an N uptake by the fetus of \(< 1 \text{mg N} \cdot \text{kg} \cdot \text{maternal body wt}^{-1} \cdot \text{h}^{-1}\) and a urea production rate by the fetus of 0.2 mg N \cdot kg\textsuperscript{-1} \cdot maternal body wt\textsuperscript{-1} \cdot h\textsuperscript{-1}, resulting in an almost negligible contribution to the maternal N metabolism.

In summary, data from the present study show that during human pregnancy, there is a significant reduction in the rate of urea synthesis early in gestation. The decrease in urea synthesis is correlated with a lower rate of transamination of leucine. It is speculated that the lower transamination of branched-chain amino acids may be the consequence of decreased availability of N acceptors (\(\alpha\)-ketoglutarate). The latter, in turn, may be related to the resistance to insulin action (glucose uptake) often manifest in states of N accretion and growth, e.g., puberty or after growth hormone therapy.

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