

Methionine metabolism in human pregnancy^{1–3}

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ABSTRACT

Background: Hyperhomocysteinemia during pregnancy, which is a consequence of perturbations in methionine and/or folate metabolism, has been implicated in adverse outcomes such as neural tube defects, preeclampsia, spontaneous abortion, and premature delivery. The adaptive changes in methionine metabolism during pregnancy in humans have not been determined.

Objective: Our objective was to examine the kinetics of methionine and its rate of transsulfuration and transmethylation in healthy women with advancing gestation.

Design: The whole-body rate of appearance (Ra) of methionine and phenylalanine was measured in healthy pregnant women during the first ($n = 10$), second ($n = 5$), and third ($n = 10$) trimesters of pregnancy. These data were compared with those for nonpregnant women ($n = 8$). Tracers [$1-^{13}\text{C}$]methionine, [C^2H_3]methionine, and [$^2\text{H}_5$]phenylalanine were administered as prime-constant rate infusions. The effect of enteral high-protein, mixed-nutrient load on tracer-determined variables was also examined.

Results: In pregnant women, the Ra of phenylalanine was significantly ($P < 0.05$) lower in the first trimester than in the second and third trimesters and was significantly lower than that in nonpregnant women. A linear positive correlation was evident between gestational age and phenylalanine Ra. The fractional rate and total rate of transsulfuration of methionine was significantly ($P < 0.05$) higher during the first trimester, whereas the rate of transmethylation was higher during the third trimester. Plasma concentrations of total cysteine and homocysteine were lower during pregnancy.

Conclusions: Uncomplicated pregnancy in humans is associated with a higher rate of transsulfuration early in gestation and a higher rate of transmethylation of methionine in late gestation. These data may have implications for understanding the role of methionine and homocysteine in complications of pregnancy and for the nutritional care of pregnant women. *Am J Clin Nutr* 2010;91:357–65.

INTRODUCTION

The integrated metabolism of methionine and folate, which is aimed at the sustenance of the one-carbon pool, is critical for the provision of methyl groups required to meet the demands of purine synthesis and for the numerous methylation reactions such as methylation of DNA and epigenetics, proteins, biogenic amines, and phospholipids during development (reviewed in references 1 and 2). Interest in folate metabolism during early gestation is underscored by the documented relation between folate supplementation and reduction in neural tube defects (NTDs) in the

fetus (3). The mechanism of reduction in NTDs is not clear. Methionine is a key component of the one-carbon metabolism required for the transfer of methyl groups from folate to *S*-adenosylmethionine (SAM), the ubiquitous methyl donor (**Figure 1**). Additionally, data in humans have implicated homocysteine, an intermediate of methionine metabolism, to pregnancy-related disorders such as preeclampsia, spontaneous abortion, placental abruption, and premature delivery (4–7). Studies in humans also show absence of transsulfuration activity in the fetal liver and therefore the inability of the fetus to synthesize cysteine, a key component of glutathione (3). Therefore, transsulfuration in the maternal compartment becomes an important source, other than protein breakdown and diet, of cysteine for the fetus.

Methionine, which is an indispensable amino acid required for protein synthesis, is also a key source of methyl groups for methylation reactions. During its metabolism, methionine is converted to its active form, SAM. After SAM-dependent transmethylation, the product *S*-adenosylhomocysteine (SAH) is metabolized to homocysteine and adenosine (Figure 1). Homocysteine can be remethylated to form methionine, either by methionine synthase or by betaine-homocysteine methyltransferase (BHMT). 5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the one-carbon donor for the methylation of homocysteine to methionine. Homozygosity for the genetic polymorphism ($677\text{C} \rightarrow \text{T}$) in *MTHFR* results in reduced enzyme activity, which may impair methylation of homocysteine and contribute to perturbations in methionine metabolism (8–10). The process of conversion of methionine to homocysteine and back to methionine, termed the *methionine* (or transmethylation) *cycle*, is ubiquitous in vivo. Homocysteine can also be metabolized via the transsulfuration pathway to cystathionine and then to cysteine and

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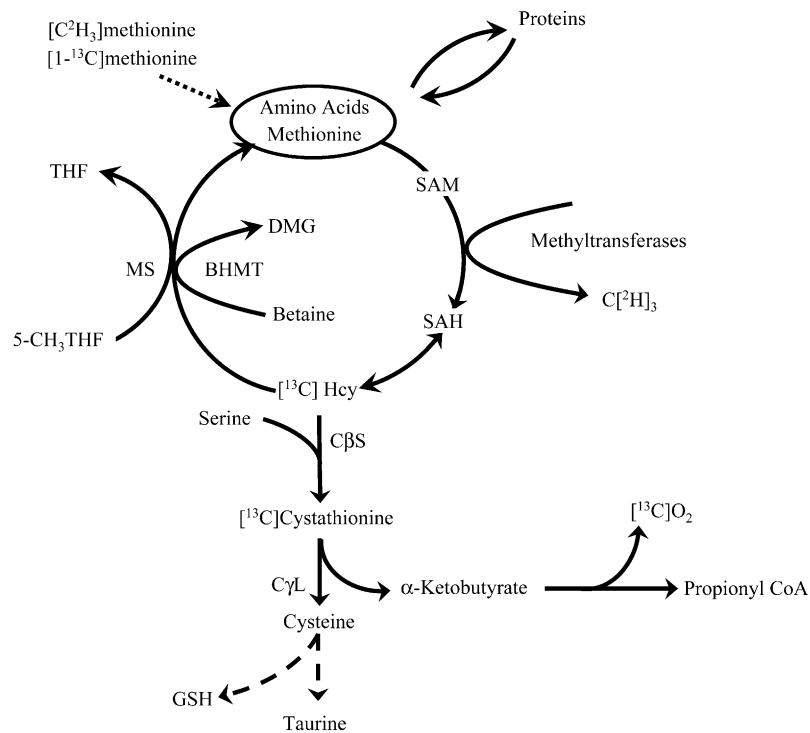


FIGURE 1. Methionine metabolism in vivo. Methionine, released into the amino acid pool as a result of protein breakdown, is converted to *S*-adenosylmethionine (SAM), the universal methyl donor. After loss of the methyl group in various methyltransferase reactions, SAM is converted to *S*-adenosylhomocysteine (SAH), which in turn is converted to homocysteine (Hcy) via a reversible reaction. Homocysteine can be remethylated either by methionine synthase (MS) or by betaine-homocysteine methyltransferase (BHMT) to methionine and thus complete the methionine cycle. Homocysteine may also be metabolized via the transsulfuration pathway to form cystathionine, cysteine, and other downstream products. Also shown is the fate of [^{1-13}C]methionine and [C^2H_3]methionine tracers. As shown, the [C^2H_3] tracer is lost in the methyltransferase reactions, so that homocysteine will only be labeled with ^{13}C , which is lost when $^{13}\text{CO}_2$ is formed during the conversion of α -ketobutyrate to propionyl coenzyme A (CoA). During fasting, the rate of appearance of methionine measured by [^{1-13}C]methionine tracer represents methionine entering the amino acid pool due to protein breakdown, whereas that measured by [C^2H_3]tracer includes protein breakdown plus that formed from methylation of homocysteine. *C* β *S*, cystathionine β synthase; *C* γ *L*, cystathionine γ lyase; GSH, glutathione; DMG, dimethylglycine; THF, tetrahydrofolate.

α -ketobutyrate. The changes in methionine metabolism—ie, transmethylation and transsulfuration—during human pregnancy have not been examined.

In the present study, we have quantified the kinetics of methionine and its rate of transmethylation and transsulfuration through advancing gestation in humans and compared these measurements with those for healthy nonpregnant women. In addition, changes in whole-body protein turnover were quantified by using phenylalanine tracer for comparison with methionine tracer. Because transmethylation may be affected by *MTHFR* polymorphism, we also examined the methionine kinetics data in relation to *MTHFR* zygosity.

SUBJECTS AND METHODS

Ten healthy, nonobese women were recruited during the first trimester of pregnancy. Repeat studies were performed on 4 subjects each in the second and third trimester. Seven additional subjects (1 at 20 wk gestation and 6 at 29–30 wk gestation) were studied later in pregnancy. Eight healthy, nonobese, nonpregnant women were recruited as controls. The clinical characteristics of the study subjects are shown in **Table 1**. None of the subjects had a family history of diabetes, prepregnancy medical illness, or pregnancy-related illness, and none were receiving any medications other than routine prenatal vitamins containing folate (800 μg) and vitamin B-12 (12 μg). Their glucose tolerance tests at 26–30 wk gestation were within the normal range. The non-

pregnant subjects were not taking any medications, including oral contraceptives. The *MTHFR* polymorphism data are also shown in Table 1. All subjects were placed on a diet containing ≥ 75 g protein/d for 7 d before the tracer study. Actual intake and compliance were monitored by interview by the General Clinical Research Center (GCRC) nutritionist and estimated by self-recorded diaries. Each subject consumed an average of 200–220 kcal and 75–100 g protein/d. There was no difference in the weight-specific calorie intake between pregnant and nonpregnant subjects. Written informed consent was obtained from each subject and her spouse (when available) after fully explaining the procedure. The protocol was approved by the Institutional Review Board of MetroHealth Medical Center in October 2005, and the first subject was enrolled in March 2006. All studies were performed in the GCRC.

Subjects reported to the GCRC on the morning of the tracer study after an overnight fast of ≈ 10 h. After a physical examination, including measurement of weight and height, they were asked to lie down on the bed. Two indwelling cannula were inserted, one in each superficial vein on the dorsum of the hand, one for the infusion of isotopic tracer and the other for obtaining blood samples. The sampling site was kept warm (by a thermostatically controlled heating pad) to obtain arterialized blood samples and kept patent by infusing isotonic saline solution.

L-[^{1-13}C]Methionine, L-[C^2H_3]methionine, L-ring[$^2\text{H}_5$]phenylalanine, and [^{13}C]sodium bicarbonate were purchased from



TABLE 1
Characteristics of the study population¹

	Nonpregnant (n = 8)	First trimester (n = 10)	Second trimester (n = 5)	Third trimester (n = 10)
Age (y)	23.25 ± 3.99 (18–31)	25.4 ± 3.66 (19–32)	23.40 ± 3.81 (19–32)	23.07 ± 4.65 (18–32)
Weight (kg)	63.13 ± 7.20 (55–74)	58.50 ± 7.76 (44–70)	64.0 ± 4.12 (57–68)	70.10 ± 10.05 (50–87)
BMI (kg/m ²)	23.98 ± 1.27 (21.99–25.61)	22.21 ± 2.81 (17.19–26.03)	25.03 ± 1.99 (22.27–26.71)	23.52 ± 3.72 (17.01–27.89)
GA at study (wk)	—	11.80 ± 1.50 (9–14)	20.80 ± 1.92 (19–24)	30.20 ± 1.03 (29–32)
<i>MTHFR 677C→T</i>				
N	7	7	4	5
HTZ	—	3	—	4
HZ	1	—	1	1

¹ All values are means ± SDs; ranges in parentheses. GA, gestational age; N, normal; HTZ, heterozygous; HZ, homozygous.

Cambridge Laboratories (Boston, MA). The tracers had been tested for purity, pyrogenicity, and sterility by the manufacturer. A weighed amount of the tracers was dissolved in 0.45% saline solution and administered as prime-constant rate infusion. The doses of the respective tracers were as follows: L-[1-¹³C]methionine (prime: 3 μmol/kg; infusion rate: 3 μmol · kg⁻¹ · h⁻¹), [C²H₃]methionine (prime: 4.5 μmol/kg; infusion rate: 3 μmol · kg⁻¹ · h⁻¹), and L-ring[²H₅]phenylalanine (prime: 2 μmol/kg; infusion rate: 2.0 μmol · kg⁻¹ · h⁻¹). A priming dose of [¹³C]sodium bicarbonate (5 mg) was given to achieve early steady state enrichment in the bicarbonate/carbon dioxide pool. After a basal study (180 min), the response to a mixed-nutrient load was evaluated by giving oral Boost with added protein (37 mL; Beneprotein, Nestlé USA, Glendale, CA) every 30 min for the next 180 min. Each 37 mL mixture corresponded to 3.96 g protein and 62.8 kcal. Four subjects in their third trimester were studied only during fasting.

Arterialized blood samples (6 mL each) were obtained at 30-min intervals throughout the study. Blood samples were centrifuged immediately in cold (4°C), and the separated plasma was frozen at -70°C until analysis. Breath samples for the measurement of ¹³C enrichment of the expired carbon dioxide were obtained at 30-min intervals throughout the study, as described previously (11). Rates of production of carbon dioxide ($\dot{V}CO_2$) and consumption of oxygen ($\dot{V}O_2$) were measured by using a canopy system (DeltaTrac II; Sensor Medics, Yorba Linda, CA). The rate of infusion of the tracer was confirmed gravimetrically by using the same equipment and tubing at the end of the study. All subjects tolerated the procedures with no serious effects.

Analytic procedures

Blood glucose was measured by the glucose oxidase method by using a glucose/lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). The concentration of total homocysteine, total cysteine, and amino acids in the plasma and in the infusates was measured by HPLC (12, 13).

Gas chromatography–mass spectrometry analysis

The methodology used to measure the enrichment of amino acid tracers in the plasma has been described (14, 15). The mass-to-charge ratios (*m/z*) of 250 (*m*₀) and 255 (*m*₅) were monitored

to measure the enrichment of L-ring[²H₅]phenylalanine. The *m/z* ratios of 234 (*m*₀), 235 (*m*₁), 236 (*m*₂), 237 (*m*₃), and 238 (*m*₄) were monitored to quantify unlabeled and labeled methionine. The *m/z* 235 (*m*₁) represented the enrichment of L-[1-¹³C]methionine, and the mass 237 (*m*₃) represented the enrichment of L-[C²H₃]methylmethionine. Multiple linear regression analyses were performed to calculate the relative enrichments and correction for natural abundance of *m*₁ ([1-¹³C]tracer) and *m*₃ ([C²H₃]methyl) methionine by using an in-house-developed software (Isomet, developed by J Kim). Enrichment of ¹³C in the carbon dioxide was quantified by isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH). The ¹³C enrichment of homocysteine in plasma was measured as described by Davis et al (16).

The whole-body rate of appearance (Ra) of phenylalanine and methionine was calculated by tracer dilution during isotopic steady state (17). The various components of methionine metabolism were calculated as described by Storch et al (18) and MacCoss et al (19). The Ra of methionine, estimated from the dilution of carboxyl [¹³C]-labeled tracer (*Q_C*), represents methionine entering the circulation from proteolysis and from an exogenous (enteral) source. The carboxyl label is retained during the conversion of methionine to homocysteine and back to methionine (Figure 1). In contrast, the methyl-labeled tracer is lost during transmethylation and replaced by an unlabeled methyl group during remethylation. Therefore, the Ra of methionine estimated from the dilution of L-[C²H₃]methionine (*Q_M*) is the sum of methionine released from protein breakdown and the methionine that is exogenously administered plus the amount that is synthesized by methylation of homocysteine.

$$\text{Ra} (1\text{-}^{13}\text{C tracer}) \text{ or } Q_C = B + I \quad (1)$$

where *B* is the appearance of methionine from protein breakdown and *I* is the methionine administered exogenously.

$$\text{Ra} (\text{C}^2\text{H}_3 \text{ tracer}) \text{ or } Q_M = B + I + RM \quad (2)$$

where *RM* is the rate of remethylation of methionine from homocysteine.

The difference between *Q_M* and *Q_C* represents the rate of remethylation:

$$Q_M - Q_C = RM \quad (3)$$

The rate of transsulfuration was assumed to be equal to the rate of oxidation of methionine and was estimated from the Ra of ^{13}C of $[1-^{13}\text{C}]$ methionine in the expired carbon dioxide (18, 20). It is assumed that during the formation of cysteine, an equimolar quantity of α -ketobutyrate is formed and is oxidized in the tricarboxylic acid cycle (Figure 1).

We did not correct the kinetic data for the intracellular enrichments of methionine; therefore, our estimates of transmethylation and transsulfuration are lower than actual results. We did measure the plasma homocysteine enrichments as an index of intracellular $[1-^{13}\text{C}]$ methionine enrichment in a small group of pregnant and nonpregnant subjects. As shown in the results, there was an effect of feeding on m_1 homocysteine/ m_1 methionine ratio. However, the ratio was not different between pregnant and nonpregnant subjects.

We did not adjust for possible carbon dioxide retention, because our previous data and those of others from healthy adults showed no significant retention of tracer during parenteral glucose infusion (21). The use of a 20% tracer retention factor would only increase our estimates of transsulfuration.

Statistical analysis

All data are reported as means \pm SDs. Statistical analyses were performed by using a commercial software program (Statistix 9.0; Analytical Software, Tallahassee, FL) The data were initially analyzed for skewness and kurtosis by using descriptive statistics. Only 2 subjects were studied longitudinally throughout the pregnancy. Therefore, the data were analyzed cross-sectionally as independent groups. Because the sample size was small and the data were not normally distributed, the differences between the 4 study groups were analyzed by nonparametric Kruskal-Wallis one-factor analysis of variance (ANOVA). When the difference between groups was significant, the data of 2 subject groups were compared by using the Mann-Whitney U test. We also repeated the same analysis by using one-factor ANOVA and Tukey corrections for post hoc comparisons, wherever there were significant group effects. However, the results of these analyses were similar to those with nonparametric tests reported here. A value of $P < 0.05$ was considered significant. Pearson's correlations were performed for linear regression analysis.

RESULTS

Subjects' clinical data are shown in Table 1. All subjects except one delivered healthy, appropriate-for-gestational-age infants at 38.7 ± 1.8 wk gestation. One subject delivered at 31 wk gestation by spontaneous labor for unknown reasons. She was studied once in the second trimester, and her data were within the range of the other subjects. The average birth weight of the newborns ($n = 17$) was 3145 g. *MTHFR* zygosity is also shown in Table 1.

Plasma concentrations of amino acids during fasting are shown in Table 2. As shown, there was a significant difference ($P < 0.05$) for total α amino nitrogen and for several individual amino acids between the 4 groups. The lower plasma concentrations of nonessential amino acids (serine, glycine, glutamine, tyrosine, asparagine, citrulline, and ornithine) were apparent during the

first trimester of pregnancy. Plasma concentrations of methionine were lower in pregnant subjects in the first and third trimester of pregnancy compared with concentrations in nonpregnant subjects. In response to protein-enriched food, there was an increase in plasma amino acid concentrations in all groups, but the increase was not significantly different between groups (data not shown).

Phenylalanine kinetics

The Ra of phenylalanine was quantified as a measure of protein turnover (Table 3). A steady state plasma tracer enrichment was established in all subjects between 60–90 min. There was a significant difference between groups for Ra of phenylalanine during fasting ($P = 0.02$). Phenylalanine Ra was significantly lower in the first trimester of pregnancy as compared with that in the nonpregnant state. The Ra of phenylalanine was significantly higher during the second and third trimesters than that in the first trimester. There was a significant positive correlation between gestational age and the Ra of phenylalanine ($r^2 = 0.26$, $P = 0.01$; Figure 2). In response to an enteral protein load, the Ra of phenylalanine increased in all groups. However, there was no significant difference between the 4 groups in response to feeding. The increase in phenylalanine kinetics during feeding is the sum of enteral absorption of dietary phenylalanine, first-pass hepatic uptake, and the suppression of protein breakdown. The contribution of these components to the measured phenylalanine Ra could not be determined by the present experimental design.

Plasma cysteine, homocysteine, and glutathione

There was a statistically significant difference between the 4 groups for total cysteine, homocysteine and cysteinylglycine concentration in the plasma during fasting (Table 4). Plasma cysteine concentrations in pregnant women were significantly lower than those in nonpregnant women and were significantly lower in the third trimester than in the first trimester. Homocysteine concentrations in pregnant women in their third trimester were significantly different from those in nonpregnant women. There was no difference in total plasma glutathione concentration between the 4 groups. In contrast, cysteinylglycine concentrations were lower during the first and second trimesters of pregnancy. Enteral protein administration did not have any significant effect on these parameters (data not shown).

Respiratory calorimetry

The rate of consumption of oxygen (Table 5), normalized for body weight, although higher in pregnancy, was not significantly different from that in nonpregnant subjects. The rate of production of carbon dioxide was significantly higher during pregnancy. As reported previously (11, 14), respiratory exchange ratio was higher in pregnant subjects than that in nonpregnant subjects. Enteral feeding resulted in an increase in $\dot{V}\text{CO}_2$, $\dot{V}\text{O}_2$, and respiratory exchange ratio in all groups.

Methionine kinetics

A steady state isotopic enrichment was achieved in all subjects, both during fasting (120–180 min) and during feeding (360–420 min). Isotopic steady state in methionine and homocysteine was

TABLE 2

Plasma amino acid concentrations (in $\mu\text{mol/L}$) during fasting in pregnant and nonpregnant women¹

	Nonpregnant	First trimester	Second trimester	Third trimester	<i>P</i> ²
Aspartate	10.13 ± 2.94	7.2 ± 1.1	6.10 ± 0.74 ^{3,4}	8.20 ± 2.15 ^{4,5}	0.004
Glutamate	41.69 ± 9.04	34.45 ± 7.1	35.00 ± 12.22	41.50 ± 9.96	NS
Asparagine	45.25 ± 10.61	33.15 ± 3.24 ³	37.10 ± 7.30	37.65 ± 6.86	0.051
Serine	133.44 ± 37.36	77.75 ± 14.7 ³	79.10 ± 20.73 ³	70.05 ± 18.67 ³	<0.001
Glutamine	611.75 ± 99.50	403.5 ± 50.8 ³	452.60 ± 43.68 ³	443.90 ± 81.01 ³	<0.001
Glycine	134.69 ± 96.88	72.15 ± 20.9 ³	82.90 ± 20.39	77.00 ± 16.25	0.026
Histidine	214.94 ± 110.17	105.9 ± 47.9	120.30 ± 51.42	121.60 ± 40.54	NS
Threonine	152.19 ± 58.17	113.75 ± 24.8	174.70 ± 60.24 ³	163.80 ± 45.55 ⁴	0.032
Citrulline	27.38 ± 11.05	14 ± 3.45 ³	14.60 ± 3.36 ³	16.65 ± 4.04 ³	0.001
Alanine	255.31 ± 65.77	186.4 ± 64.7	234.50 ± 66.86	219.50 ± 104.75	NS
Arginine	98.88 ± 43.18	44.85 ± 11.2	44.50 ± 9.43	48.70 ± 13.48	NS
Taurine	27.38 ± 20.59	15.95 ± 3.64 ³	13.60 ± 3.07 ³	10.95 ± 3.33 ^{3,4}	<0.001
Tyrosine	54.56 ± 16.88	33.25 ± 5.4 ³	33.60 ± 4.99 ³	33.80 ± 5.42 ³	0.003
Aminobutyric acid	22.88 ± 7.26	16.9 ± 3.03 ³	15.50 ± 3.32 ³	13.05 ± 3.40 ^{3,4}	0.001
Methionine	31.00 ± 1.95	24.25 ± 4.05 ³	25.20 ± 3.37 ³	23.10 ± 3.92 ³	0.001
Valine	199.50 ± 29.02	173.95 ± 28.63	158.70 ± 34.75	138.95 ± 20.70 ^{3,4}	0.001
Tryptophan	38.19 ± 4.91	36.7 ± 3.80	32.50 ± 6.32	30.40 ± 4.86 ^{3,4}	0.008
Phenylalanine	51.00 ± 13.35	37.35 ± 8.04	42.20 ± 15.85	42.00 ± 8.64	NS
Isoleucine	46.75 ± 13.60	32.7 ± 7.05	35.80 ± 8.20	39.50 ± 10.12	NS
Leucine	92.38 ± 22.63	65.1 ± 8.64	68.50 ± 16.13	70.35 ± 14.67	NS
Ornithine	41.50 ± 15.10	17.2 ± 5.14 ³	16.10 ± 2.30 ³	18.45 ± 4.88 ³	0.003
Lysine	151.44 ± 44.40	106.35 ± 20.77 ³	123.80 ± 19.73 ⁴	125.00 ± 23.73 ⁴	<0.001
Total	2482.2 ± 438.7	1652.75 ± 119.5 ³	1572.4 ± 688.0 ³	1794.1 ± 282.0 ³	0.002

¹ All values are means ± SDs.² Obtained by using Kruskal-Wallis one-factor ANOVA.³ *P* < 0.05 compared with nonpregnant subjects (Mann-Whitney *U* test).⁴ *P* < 0.05 compared with first trimester subjects (Mann-Whitney *U* test).⁵ *P* < 0.05 compared with second trimester subjects (Mann-Whitney *U* test).

also evaluated by extending the fasting period in 5 subjects (third trimester) from 180 to 300 min and was found not to change with extended fasting.

The Ra of methionine, measured by using a [¹⁻¹³C]methionine (*m*₁) tracer, was $\approx 20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during fasting (Table 6). The Ra of methionine was not significantly different between the 4 groups. A significant positive correlation was seen between the Ra of methionine and gestational age ($r^2 = 0.15$, *P* = 0.02). The fraction of methionine oxidized (or the fractional rate of transsulfuration of methionine) measured by the rate of appearance of ¹³C of methionine in expired carbon dioxide is shown in Figure 3. There was a significant difference between groups for the fractional rate (*P* < 0.001) and the total rate (*P* = 0.004) of transsulfuration of methionine (Table 6). The fractional and total rate of transsulfuration during fasting was significantly higher during the first trimester of pregnancy compared with the third trimester and the nonpregnant state (Figure 3).

The Ra of methionine, quantified by using methyl-labeled (*m*₃) tracer, is also shown in Table 6. As shown, there was a significant difference between groups (*P* < 0.001), with the Ra in pregnant women in the first trimester being lower than that in nonpregnant subjects and higher than in all groups during the third trimester. The rate of remethylation of methionine, calculated from the difference between Ra measured by [¹⁻¹³C] and Ra measured by [²H₃]tracer, was highest in the third trimester and significantly different from that in the first trimester and in the nonpregnant state. Transmethylation—ie, the sum of remethylation and transsulfuration—was higher in subjects in the third trimester than that in subjects in the first trimester and in nonpregnant subjects.

Enteral protein load resulted in an increase in methionine kinetics, transsulfuration, remethylation, and transmethylation in all groups. However, the magnitude of change (Δ) in any of the variables was not significantly different between groups.

TABLE 3

Rate of appearance of phenylalanine (in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) in pregnancy¹

	Nonpregnant	First trimester	Second trimester	Third trimester	<i>P</i> ²
Fasted	42.1 ± 10.2 (8) ³	31.9 ± 4.2 (10)	40.9 ± 6.1 (5) ³	43.5 ± 12.1 (10) ³	0.019
Fed	56.5 ± 11.5 (8)	45.0 ± 7.8 (10)	56.6 ± 17.0 (5)	51.1 ± 9.1 (5)	NS
Δ^4	14.41 ± 4.58 (8)	13.15 ± 5.75 (10)	15.65 ± 11.26 (5)	12.59 ± 2.89 (5)	NS

¹ All values are means ± SDs; *n* in parentheses.² Obtained by using Kruskal-Wallis one-factor ANOVA.³ *P* < 0.05 compared with first trimester subjects (Mann-Whitney *U* test).⁴ Fed minus fasted states.

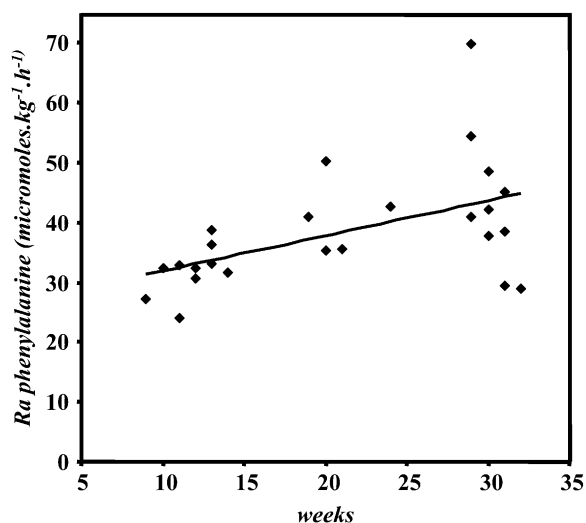


FIGURE 2. Relation between gestational age and the rate of appearance (Ra) of phenylalanine ($y = 0.59x + 26.0$; $r^2 = 0.26$; $P = 0.01$). The Ra of phenylalanine was measured during pregnancy by tracer dilution using [$^2\text{H}_5$]phenylalanine.

^{13}C Enrichment (m_1) of homocysteine, a measure of intracellular methionine enrichment, was examined in 3 subjects in the first trimester, 2 subjects in the third trimester, and 3 nonpregnant subjects. During fasting, the ratio of m_1 enrichment of plasma homocysteine and plasma methionine was 0.41 ± 0.05 ($n = 5$) in pregnant subjects and 0.35 ± 0.02 ($n = 3$) in nonpregnant subjects. During feeding, m_1 homocysteine/ m_1 methionine ratio was 0.71 ± 0.05 during pregnancy and 0.75 ± 0.04 in nonpregnant subjects. With the assumption that homocysteine is a surrogate for intracellular methionine enrichment, the “true” kinetics of methionine would be 25% higher during fasting and 13% during feeding. However, there will be no relative change between the groups. The increase in the ratio of m_1 homocysteine/ m_1 methionine during feeding, in the presence of lower m_1 homocysteine/ m_1 methionine ratio in the plasma, suggests a suppression of intracellular (mostly hepatic) proteolysis.

MTHFR zygosity and methionine metabolism

We analyzed methionine Ra, transsulfuration, transmethylation, and plasma concentrations of cysteine, homocysteine, and glutathione in relation to *MTHFR* zygosity. No significant effect of *MTHFR* zygosity was observed.

DISCUSSION

The key findings of the present study are that the fractional and total rate of transsulfuration of methionine was higher in the first trimester than that in the third trimester and the nonpregnant state, and transmethylation of methionine was higher in the third trimester than that in the first trimester and in the nonpregnant state (Figure 3, Table 6). The whole-body, weight-specific rate of protein breakdown (Ra phenylalanine) was lower in the first trimester and increased to levels comparable to those in nonpregnant women and in pregnant women in the third trimester of pregnancy.

As anticipated, total plasma amino acids during fasting were significantly lower in pregnant subjects than those in nonpregnant subjects, and the differences for most of the amino acids persisted in the fed state. These data are similar to those reported by us and by others previously (11, 14, 22). The mechanism of pregnancy-induced hypoaminoacidemia is unclear and may be related to the lower rate of whole-body proteolysis observed early in pregnancy and the increase in volume of distribution later in pregnancy. The lower concentrations of serine, glycine, and methionine suggest changes in methionine and one-carbon metabolism during pregnancy.

We measured the Ra of phenylalanine to quantify the whole-body rate of protein breakdown. Pregnancy resulted in a significantly lower rate of proteolysis during the first trimester followed by an increase with advancing gestation. The lower rate of protein breakdown during the first trimester may be the consequence of the development of pregnancy-induced accelerated starvation (23) and an increase in plasma fatty acids and insulin resistance (24, 25). Studies in nonpregnant subjects suggest that elevated fatty acids directly cause a decrease in the rate of protein breakdown in addition to causing resistance to insulin action (24).

Phenylalanine Ra was significantly higher in the second and third trimesters than in the first trimester (Table 3, Figure 2). Although not statistically significant, methionine Ra was also higher in the second and third trimesters than in the first trimester. These data are consistent with those of Thompson and Halliday (26), who showed an increase in the rate of protein turnover with advancing gestation, and are in contrast with others showing no change in protein turnover with advancing gestation (14, 27, 28). The differences between various studies may be related to the amino acid tracer used, phenylalanine compared with leucine, or whether the studies were cross-sectional or longitudinal. The discordance between rate of efflux of leucine and phenylalanine across the leg was also observed by

TABLE 4

Plasma concentrations (in $\mu\text{mol/L}$) of total cysteine, homocysteine, glutathione, and cysteinylglycine during fasting¹

	Nonpregnant	First trimester	Second trimester	Third trimester	P^2
Total cysteine	318.34 ± 15.42	285.16 ± 44.62^3	$243.93 \pm 27.49^{3,4}$	$248.96 \pm 34.67^{3,4}$	<0.001
Homocysteine	6.00 ± 1.29	5.10 ± 1.56	4.52 ± 1.66	4.23 ± 1.17^3	0.040
Total glutathione	3.98 ± 1.24	2.96 ± 0.85	3.34 ± 0.67	3.67 ± 1.09	NS
Cysteinylglycine	1020.6 ± 120.38	840.63 ± 63.77^3	800.92 ± 145.14^3	1022 ± 466.95	0.016

¹ All values are means \pm SDs.

² Obtained by using Kruskal-Wallis one-factor ANOVA.

³ $P < 0.05$ compared with nonpregnant subjects (Mann-Whitney *U* test).

⁴ $P < 0.05$ compared with first trimester subjects (Mann-Whitney *U* test).

TABLE 5
Respiratory calorimetry measurements in pregnant subjects¹

	Nonpregnant	First trimester	Second trimester	Third trimester	P ²
$\dot{V}O_2$ (mmol · kg ⁻¹ · h ⁻¹)					
Fasted	9.51 ± 1.02 (8)	8.88 ± 0.78 (10)	8.45 ± 0.33 (5)	8.99 ± 1.19 (10)	NS
Fed	9.56 ± 1.22 (8)	10.25 ± 1.00 (10)	9.80 ± 0.73 (5)	9.40 ± 1.18 (5)	NS
$\dot{V}CO_2$ (mmol · kg ⁻¹ · h ⁻¹)					
Fasted	6.56 ± 0.60 (8)	7.63 ± 0.94 (10) ³	6.72 ± 0.65 (5) ³	7.71 ± 1.22 (10)	0.01
Fed	8.04 ± 1.01 (8)	9.07 ± 1.03 (10) ^{3,4}	7.75 ± 0.56 (5) ³	8.26 ± 1.22 (5)	0.05
RER					
Fasted	0.78 ± 0.08	0.86 ± 0.05	0.79 ± 0.06	0.83 ± 0.08	NS
Fed	0.84 ± 0.07	0.88 ± 0.04	0.79 ± 0.08	0.88 ± 0.08	NS

¹ All values are means ± SDs; *n* in parentheses. $\dot{V}O_2$, rate of oxygen consumption; $\dot{V}CO_2$, rate of production of carbon dioxide; RER, respiratory exchange ratio.

² Obtained by using Kruskal-Wallis one-factor ANOVA.

³ *P* < 0.05 compared with nonpregnant subjects (Mann-Whitney *U* test).

⁴ *P* < 0.05 compared with second trimester subjects (Mann-Whitney *U* test).

Morrison et al (29) in their study of human subjects with and without cirrhosis. Their data show that phenylalanine efflux from the leg (skeletal muscle) was higher in patients with cirrhosis than in controls, whereas the efflux of leucine was lower and the whole-body kinetics of leucine were unchanged, suggesting a local (skeletal muscle) metabolism of leucine. These studies also underscore the problem of comparing data from different studies in which different amino acid tracers have been used to quantify protein turnover. The gestation-related change/increase in protein turnover (Figure 2) may be the consequence of greater demands for amino acids imposed on the mother by the fetus (22). In response to feeding, there was a similar magnitude of increase in phenylalanine Ra in all subjects, which suggested no effect of pregnancy on splanchnic uptake of phenylalanine.

We used the Storch et al model (18) of tracer infusion to quantify methionine metabolism, with the difference that we did

not use doubly [¹³C,²H₃] labeled methionine but used 2 different methionine tracers. This required a more-stringent gas chromatography–mass spectrometry analysis of m₁ and m₃ enrichments. We did observe a greater variability in m₃ enrichment as compared with m₁ enrichment, resulting in a greater variability in our estimation of Ra of methionine using m₃ tracer and consequently in the rate of remethylation. Whether the observed variance is analytical or biological cannot be certain. However, on the basis of the achievement of tracer steady state and replicate analysis of biological samples, we feel that it may be biological, although its significance is unclear.

Our data show that transsulfuration of methionine, both as a fraction of methionine Ra and actual, was higher during the first trimester. In addition, plasma homocysteine concentrations were higher in the first trimester than those during the second and third trimesters. A high rate of transsulfuration during the first trimester would be expected to result in higher plasma

TABLE 6
Methionine kinetics (in μmol · kg⁻¹ · h⁻¹) during pregnancy¹

	Nonpregnant	First trimester	Second trimester	Third trimester	P ²
Ra					
m ₁ Tracer					
Fasted	21.12 ± 4.90 (8)	18.25 ± 2.73 (10)	20.53 ± 4.36 (5)	22.66 ± 4.96 (10)	NS
Fed	35.15 ± 5.47 (8)	28.99 ± 3.34 (10)	32.85 ± 9.39 (5)	34.11 ± 4.74 (5)	NS
m ₃ Tracer					
Fasted	24.68 ± 6.38 (8)	21.34 ± 7.53 (10)	24.68 ± 8.28 (5) ³	39.70 ± 6.75 (10) ⁴	<0.001
Fed	47.69 ± 17.52 (8)	36.88 ± 15.41 (10)	43.88 ± 20.60 (5)	61.61 ± 15.26 (5)	NS
Transsulfuration					
Fasted	2.16 ± 0.61 (8)	3.45 ± 0.76 (10) ^{3,4}	2.92 ± 0.49 (4)	2.56 ± 0.91 (9)	0.004
Fed	8.38 ± 1.93 (8)	11.52 ± 2.12 (10) ⁴	9.45 ± 3.05 (4)	9.13 ± 1.89 (4)	0.036
Remethylation					
Fasted	6.16 ± 6.82 (7)	5.68 ± 6.09 (8) ³	8.06 ± 9.92 (4)	17.04 ± 8.65 (10) ⁴	0.021
Fed	17.04 ± 16.96 (7)	12.10 ± 12.21 (8)	17.47 ± 21.46 (4)	27.51 ± 15.69 (5)	NS
Transmethylation					
Fasted	8.18 ± 6.84 (7)	9.04 ± 5.70 (8) ³	10.98 ± 9.44 (4)	19.21 ± 9.58 (9) ⁴	0.067
Fed	25.18 ± 17.33 (7)	23.80 ± 11.76 (8)	26.92 ± 19.96 (4)	35.48 ± 19.31 (4)	NS

¹ All values are means ± SDs; *n* in parentheses. Ra, rate of appearance; m₁ tracer, [¹³C]methionine; m₃ tracer, [C²H₃]methionine.

² Obtained by using Kruskal-Wallis one-factor ANOVA.

³ *P* < 0.05 compared with third trimester subjects (Mann-Whitney *U* test).

⁴ *P* < 0.05 compared with nonpregnant subjects (Mann-Whitney *U* test).

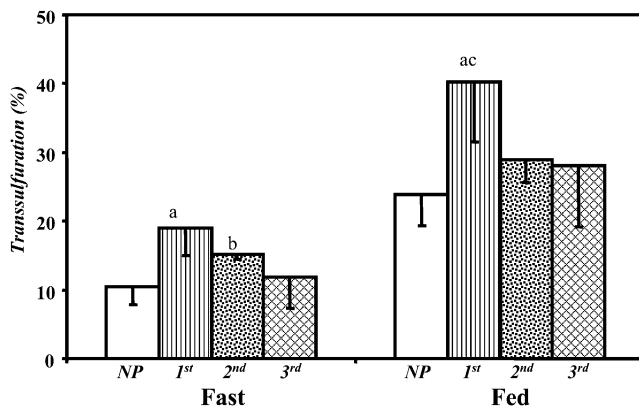


FIGURE 3. Fractional (%) rate of transsulfuration of methionine in pregnant subjects during fasting and feeding. Transsulfuration was quantified by the rate of appearance of ^{13}C of $[1-^{13}\text{C}]$ methionine in expired carbon dioxide (details in text). Values are means \pm SDs. ^aFirst trimester compared with third trimester and nonpregnant subjects, $P < 0.05$ (Mann-Whitney U test); ^bsecond trimester compared with nonpregnant subjects, $P < 0.05$ (Mann-Whitney U test); ^cfirst trimester compared with second trimester subjects, $P < 0.05$ (Mann-Whitney U test). □, nonpregnant (NP); ▨, first trimester (1st); ▩, second trimester (2nd); ▤, third trimester (3rd).

homocysteine concentrations, and a decrease in transsulfuration during the third trimester would result in lower plasma homocysteine concentrations in the third trimester as reported here and by others (30). The biological significance of the high rate of transsulfuration during the first trimester remains speculative. The activity of cystathionine β synthase is low, and that of cystathionine γ lyase is absent in the fetal liver in humans (reviewed in reference 3). The high rate of transsulfuration in the first trimester, therefore, could be aimed at providing cysteine and glutathione to the fetus. The need for glutathione by the developing embryo has been suggested on the basis of data relating oxidant injury to the development of fetal malformations (31). A high rate of transsulfuration would also result in an obligatory requirement for methionine and is consistent with the high rate of NTDs in women with lower dietary intake of methionine (32, 33) and with fetal growth retardation in animal models of restricted methionine intake during pregnancy (34, 35).

The rate of transmethylation was high during the third trimester as compared with early pregnancy and the nonpregnant state. The high rate may be related to high methylation demands by the growing fetus and the placenta. In this context, Gaull et al (36) observed that the activity of methionine synthase, the key enzyme catalyzing remethylation of methionine, is high in the fetal liver, which suggests a high rate of remethylation of homocysteine in the fetus. Our measurements of transmethylation in the third trimester are the sum of rates of transmethylation both in the maternal compartment as well as those in the conceptus, and may represent a high contribution of the fetus. Additionally, maternal and fetal polymorphism of methionine synthase MTR 2756G has been shown to be an important risk factor for uteroplacental insufficiency (37).

We did not observe any effect of *MTHFR* polymorphism on methionine metabolism in pregnancy. Whether the lack of any effect is due to the routine folate supplementation will not be known, because folic acid is prescribed to all pregnant women, and flour and bread are fortified with folate (1). Additionally, our study subjects, except for one, were all heterozygotes.

In summary, data from the present study show that the whole-body rate of protein turnover is lower in the first trimester of pregnancy than that in the second and third trimesters and in the nonpregnant state. The rate of transsulfuration of methionine is higher in the first trimester, and the rate of transmethylation is higher in the third trimester than at other times during pregnancy and in nonpregnant women. The high rate of transsulfuration during early gestation suggests a higher requirement for methionine, whereas high transmethylation late in gestation suggests a greater need for the methyl donors betaine and folate. The clinical implications of these data remain to be determined and would require such measurements in abnormal states.

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