Protein Requirements of Healthy Pregnant Women during Early and Late Gestation Are Higher than Current Recommendations1–4

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Abstract

Background: Adequate maternal dietary protein intake is necessary for healthy pregnancy. However, current protein intake recommendations for healthy pregnant women are based on factorial calculations of nitrogen balance data derived from nonpregnant adults. Thus, an estimate of protein requirements based on pregnancy-specific data is needed.

Objective: The objective was to determine protein requirements of healthy pregnant women at 11–20 (early) and 31–38 (late) wk gestation through use of the indicator amino acid oxidation method.

Methods: Twenty-nine healthy women (24–37 y) each randomly received a different test protein intake (range: 0.22–2.56 g · kg−1 · d−1) during each study day in early (n = 35) and late (n = 43) gestation; 7 women participated in both early and late gestation studies. The diets were isocaloric and provided energy at 1.7 × resting energy expenditure. Protein was given as a crystalline amino acid mixture based on egg protein composition, except phenylalanine and tyrosine, which were maintained constant across intakes. Protein requirements were determined by measuring the oxidation rate of L-[1-13C]phenylalanine to 13CO2 (F13CO2). Breath and urine samples were collected at baseline and isotopic steady state. Linear regression crossover analysis identified a breakpoint (requirement) at minimal F13CO2 in response to different protein intakes.

Results: The estimated average requirement (EAR) for protein in early and late gestation was determined to be 1.22 (R2 = 0.60; 95% CI: 0.79, 1.66) and 1.52 g · kg−1 · d−1 (R2 = 0.63; 95% CI: 1.28, 1.77) gestation; 7 women participated in both early and late gestation studies. The diets were isocaloric and provided energy at 1.7 × resting energy expenditure. Protein was given as a crystalline amino acid mixture based on egg protein composition, except phenylalanine and tyrosine, which were maintained constant across intakes. Protein requirements were determined by measuring the oxidation rate of L-[1-13C]phenylalanine to 13CO2 (F13CO2). Breath and urine samples were collected at baseline and isotopic steady state. Linear regression crossover analysis identified a breakpoint (requirement) at minimal F13CO2 in response to different protein intakes.

Conclusions: These estimates are considerably higher than the EAR of 0.88 g · kg−1 · d−1 currently recommended by the Dietary Reference Intakes. To our knowledge, this study is the first to directly estimate gestational stage-specific protein requirements in healthy pregnant women and suggests that current recommendations based on factorial calculations underestimate requirements. This trial was registered at clinicaltrials.gov as NCT01784198. J Nutr doi: 10.3945/jn.114.198622.

Keywords: protein, requirements, pregnancy, IAAO, human, stable isotopes

Introduction

Adequate maternal dietary protein intake during pregnancy is essential for positive pregnancy outcomes (1). Protein is not only necessary for healthy growth and development of the fetus, but also for accretion in maternal tissues like the heart, blood, breast, and uterus, and fetal-support tissues including the placenta and extra-embryonic membranes (2). The majority of protein weight gain in the fetus occurs during the latter half of pregnancy, suggesting that the need for increased dietary protein is limited to late gestation (1, 2). However, many of the maternal adaptations involving protein and nitrogen metabolism occur early in pregnancy, before there is a substantial increase in fetal demand (3). This suggests that pregnant women may also require additional dietary protein during the early stages of gestation, although the additional dietary protein required remains unclear. However, it is clear that “optimal” or “balanced” protein intake is important to prevent intrauterine growth restriction and infant low birth weight (LBW) (4). LBW infants are at an increased risk of neonatal morbidities and mortality, and are more likely to experience...
chronic diseases such as cardiovascular disease, kidney disease, obstructive airway disease, and obesity later in life (5–7). Thus, increased understanding of maternal dietary protein requirements throughout pregnancy is necessary to promote fetal health.

Nutritional recommendations set by the Institute of Medicine are published as the DRIs (8). The DRIs currently recommend an estimated average requirement (EAR) and RDA of 0.88 and 1.1 g · kg⁻¹ · d⁻¹, respectively, of high-quality protein during pregnancy. The recommendations were derived by a factorial approach with use of data from nitrogen balance studies of non-pregnant adults, and mean protein deposition during pregnancy based on studies of total body potassium (8).

Protein requirement data specific to pregnancy are lacking because of the practical and ethical concerns of using the nitrogen balance method, which requires long-term deficient test intakes, to study a pregnant population (8). However, the development of the minimally invasive stable isotope-based technique, indicator amino acid oxidation (IAAO) (9, 10), to study protein requirements (11–14) has made it possible to examine protein needs of pregnant women directly. The IAAO technique uses a 1-¹³C-labeled amino acid tracer to measure changes in rate of tracer amino acid oxidation in response to graded intakes of protein. The objective of the current study was to use the IAAO technique to examine protein requirements of healthy pregnant women during 2 distinct gestational stages: early (11–20 wk) and late (32–38 wk) pregnancy.

### Methods

**Participants.** Twenty-nine healthy women (24–37 y) participated in this study at the Clinical Research and Evaluation Unit, British Columbia Women’s and Children’s Hospital, Vancouver, Canada (Table 1). Women who participated in this study were not experiencing nausea or vomiting, reported no pregnancy complications, including gestational diabetes mellitus, had not recently (<18 mo) given birth, reported a pre-pregnancy BMI between 18.5 kg/m² and 25 kg/m², and were in apparent good health. Two women reported using an antidepressant during their pregnancy (Venlafaxine HCL, Effexor; Pfizer), 1 woman reported using a steroid inhaler, and 1 woman reported using an over-the-counter stool softener. No medications were taken on the study day. None of the women studied reported consuming alcohol, cigarettes, or illicit substances at any time during their pregnancy. Informed written consent was obtained from each participant. Financial compensation for transportation costs incurred by participating in the study was offered to all participants. An honorarium was offered to all participants upon completion of each study day. All procedures were approved by the Research Ethics Board of the British Columbia Women’s and Children’s Hospital.

**Experimental design.** The study design was based on the minimally invasive IAAO model (9, 10) used previously to determine protein requirements in healthy adults (11) and healthy children (12). The test intake protocol more closely followed that of the school-aged children (12) and included individual graded test protein intakes that ranged from deficiency to excess. Unlike healthy adults, rapid physiologic changes in pregnant women make it more difficult to use the conventional approach to IAAO that requires few test intakes with several subjects per level (11). Participants were grouped based on the flexiblity of completing 1–4 study days during each gestational stage. Seven women participated in both early and late gestation stages. If participants chose to complete >1 study per gestational stage, study days were separated by ≥5 d.

To determine eligibility, all potential participants were invited to the Clinical Research and Evaluation Unit, Child & Family Research Institute, for a pre-study appointment for blood glucose assessment, body composition analysis, and resting energy expenditure (REE) measurements. Participants arrived for this appointment after a 12-h fast and were instructed to minimize physical exertion before the appointment (i.e., no morning exercise). A fasted blood glucose concentration ≥6.7 mmol/L is indicative of gestational diabetes mellitus and therefore precluded participation (15). Body composition was measured by 3-site (biceps, triceps, and subscapular) skinfold thickness assessment [Harpenden calipers; Baty International (16–18)]. Three-site skinfold thickness assessment with site-, gender-, and age-specific factors was used (16, 17) to assess fat-free mass. REE was measured while fasted by continuous, open-circuit indirect calorimetry (Vmax Encore; Viasys). A brief medical and pregnancy history was also collected to screen for medication use, pregnancy complications, and general health. Two days before each study day, participants consumed a maintenance diet supplying 1.5 g protein · kg⁻¹ · d⁻¹. Maintenance diet protein recommendations were based on food sources favored by each participant, as indicated by 2-d food records collected during the pre-study appointment. Participants also kept a 2-d food record during the maintenance diet to ensure consistency of dietary protein intake among participants. For the 7 women who participated in both early and late gestation stages, a separate pre-study assessment was performed at each stage to ensure provision of adequate calories for the study day.

On the study day, participants arrived at the Clinical Research and Evaluation Unit after a 12-h fast and were tested for fasting blood glucose concentrations with use of a finger prick glucometer test to verify normal glucose values (One Touch Ultra2; LifeScan Canada). Participants were randomly assigned to receive a test protein intake (range: 0.22–2.56 g · kg⁻¹ · d⁻¹). Maintenance diet protein recommendations were based on food sources favored by each participant, as indicated by 2-d food records collected during the pre-study appointment. Participants also kept a 2-d food record during the maintenance diet to ensure consistency of dietary protein intake among participants. For the 7 women who participated in both early and late gestation stages, a separate pre-study assessment was performed at each stage to ensure provision of adequate calories for the study day.

**Study diets.** The study-day diet was consumed as 8 isocaloric and isonitrogenic meals provided hourly, each meal representing one-twelfth of the daily energy requirement. The daily energy requirement was calculated as 1.7 × REE for each participant. Each meal consisted of small protein shake and protein-free cookies. The shakes consisted of protein-free liquid formula made with protein-free powder (PFD1; Mead Johnson Nutrition), flavored drink crystals (Tang and Kool-Aid; Kraft Canada), and corn oil (Mazola; ACH Food Companies), and test protein was provided as a crystalline L-αmino acid mixture (Ajinomoto) based on egg-protein composition, with the exception of phenylalanine and tyrosine, which were maintained constant at 30.5 and 61 mg · kg⁻¹ · d⁻¹, respectively. As previously described (13), presence of relative excess tyrosine is necessary to minimize retention of the ¹³C label in the tyrosine pool. This ensures a sensitive partitioning of the carboxyl carbon of phenylalanine between incorporation into protein or oxidation. The amounts of phenylalanine and tyrosine provided in the current study were comparable to earlier adult and child protein IAAO-requirement studies (11, 12) that were shown to sensibly display a breakpoint in phenylalanine oxidation in response to test diet intakes. Amino acid compositions of select test protein intakes are provided in Supplemental Table 1. To maintain the

### TABLE 1 Characteristics of pregnant women during early and late gestation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Early gestation</th>
<th>Late gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>16.5 ± 2.6</td>
<td>35.4 ± 1.8</td>
</tr>
<tr>
<td>Age, y</td>
<td>30.6 ± 3.9</td>
<td>30.3 ± 2.8</td>
</tr>
<tr>
<td>Pre-pregnancy weight, kg</td>
<td>60.2 ± 10.4</td>
<td>59.9 ± 10.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.1 ± 7.2</td>
<td>165.3 ± 7.3</td>
</tr>
<tr>
<td>Pre-pregnancy BMI, kg/m²</td>
<td>22.1 ± 2.9</td>
<td>21.8 ± 2.9</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>46.3 ± 7.1</td>
<td>53.0 ± 7.3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>22.1</td>
<td>24.8</td>
</tr>
<tr>
<td>REE, kcal/d</td>
<td>1370 ± 180</td>
<td>1480 ± 197</td>
</tr>
<tr>
<td>Previous pregnancies, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>77</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>≥2</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, unless otherwise indicated. REE, resting energy expenditure.
2 Twenty-nine healthy pregnant women completed the study (Nearly = 17 and Nlate = 19, with 7 women completing both early- and late-gestation studies).
diets as isocaloric, the carbohydrate content was adjusted with varying protein intakes. Energy from test protein intakes constituted 3–21% of the total energy provided by the diets. The remaining energy came from 37% fat and 42–60% carbohydrate energy. With the exception of water, participants were not permitted to consume anything besides the experimental diets during the study day. All participants were taking daily prenatal multivitamin supplements for the duration of enrollment with this study.

**Tracer protocol.** During each study day the participants consumed 4 hourly meals before the oral tracer protocol. An oral priming dose of 0.176 mg·kg⁻¹ of NaN₁³CO₂ [99 atom percent excess (APE); Cambridge Isotope Laboratories] and 0.66 mg·kg⁻¹ of L-[¹³C]phenylalanine (99 APE; Cambridge Isotope Laboratories) was provided for carbon dioxide retained by the body because of bicarbonate fixation, and the rate of carbon dioxide production was measured with use of open-circuit indirect calorimetry (V_{\text{MAX Encore; Viasys (19, 20). Baseline breath samples were collected 45, 30, and 15 min before the tracer protocol began, and isotopic steady state urine samples were collected every 15 min beginning 2.5 h after tracer protocol began. Breath samples were stored at room temperature in airtight bags until analysis. To assess whole-body amino acid pool L-[¹³C]phenylalanine enrichment, 2 baseline and 4 isotopic steady state urine samples were collected with use of sterile urine collection hats. Baseline urine samples and analysis were performed as described previously (11).

**Sample collection and analysis.** During each study day breath and urine samples were collected before (baseline) and after (isotopic steady state) the introduction of tracer amino acid. To measure the oxidation rate of L-[¹³C]phenylalanine to CO₂, 3 baseline and 6 isotopic steady state breath samples were collected with use of disposable exhalation tubes (Labco), and the rate of carbon dioxide production was measured with use of open-circuit indirect calorimetry (V_{\text{MAX Encore; Viasys (19, 20). Baseline breath samples were collected 45, 30, and 15 min before the tracer protocol began, and isotopic steady state urine samples were collected every 15 min beginning 2.5 h after tracer protocol began. Breath samples were stored at room temperature in airtight bags until analysis. To assess whole-body amino acid pool L-[¹³C]phenylalanine enrichment, 2 baseline and 4 isotopic steady state urine samples were collected with use of sterile urine collection hats. Baseline urine samples were collected 45 and 15 min before the tracer protocol began, and isotopic steady state urine samples were collected every 30 min beginning 2.5 h after tracer protocol began. Urine samples were preserved with 200 μL of 3.4 mol/L HCl per 10 mL sample and stored at -80°C.

Breath CO₂ enrichment was determined with use of continuous-flow isotope ratio MS (Isoprime) and expressed as APE when compared against a reference standard of compressed CO₂ (19, 20). Urinary [¹³C]phenylalanine enrichments were analyzed with an API 40,000 triple quadrupole mass spectrometer (Applied Biosystems-MDS Sciex) coupled to an Agilent 1100 high-performance LC system (Agilent Technologies Canada) (11, 12).

System operations and data acquisition were controlled with use of Analyst NT software (version 1.4.1; Applied Biosystems). Sample preparation and analysis were performed as described previously (11).

**Calculation of isotopic kinetics.** L-[¹³C]phenylalanine kinetics were calculated with use of the stochastic models described by Waterlow et al. (21) and Matthews et al. (22). These models have been applied previously to assess L-[¹³C]phenylalanine kinetics in IAAO studies (11, 23, 24). Whole-body flux was calculated as:

\[ Q = \frac{i}{E_i/\gamma - 1} \]

Q represents phenylalanine flux (μmol·kg⁻¹·h⁻¹), i is the dose of L-[¹³C]phenylalanine (μmol·kg⁻¹·h⁻¹), and E_i and E_γ are the isotopic enrichments as mole fractions (molecules percent excess) of the isotope solution and urinary phenylalanine, respectively, at plateau isotopic steady state.

The rate of phenylalanine tracer oxidation (F₁3CO₂, μmol·kg⁻¹·h⁻¹) was calculated as:

\[ F_{13CO_2} = \frac{\left(FCO_2/\left(E_{CO_2}\right)\left(44.6/60\right)/W(0.82)\right)}{100} \]

FCO₂ represents carbon dioxide production (mL/min), E_{CO₂} is the CO₂ enrichment in breath at plateau isotopic steady state (APE), W is the weight (kg) of the subject, 44.6 (μmol/L) and 60 (min/h) are constants used to convert FCO₂ to μmol/h, 0.82 is the correction factor for carbon dioxide retained by the body because of bicarbonate fixation, and 100 is used to convert APE to a fraction (25).

**Statistical analysis.** Results are reported as means ± SDs. Physiologic measurements on study day and protein intake data on the 2 days before study day were compared between the 2 periods of gestation with Student’s t test. Urinary phenylalanine flux data were analyzed with use of repeated measures ANOVA (version 19; SPSS).

Estimates of the mean protein requirement during each stage of pregnancy were derived by breakpoint analysis using a 2-phase linear regression crossover model of the F₁3CO₂ data (12). The method selects the model with minimum residual SE in a stepwise partitioning of protein intake values (x) between 2 regression lines. The lines are estimated for each candidate breakpoint with use of mixed models (Proc Mixed, Statistical Analysis Systems, SAS/STAT, version 9.0; SAS Institute) to account for repeated measures within subject, as well as missing observations, because not all women participated in multiple study days (26). Using I as an indicator variable equal to 0 for x values left of the breakpoint and 1 for x values to the right, the model is Y = β₀+β₁x+β₂+β₃Ix, where Y = F₁3CO₂, x = protein intake, β₀ = left line intercept, β₀+β₂ = right line intercept, β₁ = left line slope, and β₁+β₃ = right line slope. Therefore, Y = β₀+β₁x+β₂+β₃Ix for the right line. Equating these, β₀+β₁x = (β₀+β₂) + (β₁+β₃)x, and solving for x yields the breakpoint at x = (β₂+β₃). The final model that best fit the data with the lowest SE, lowest root mean square error, and the highest R² identified the breakpoint.

Protein intake at the breakpoint represents the estimated average protein requirement. The 95% CI was calculated with use of Fieller’s theorem (27): 95% CI = breakpoint ± t_{d,f}_{\text{df}} × SE, where SE is the SE of the combined regression lines, df is the associated with the residual mean square of the best-fit model, and α is the 95% CI level (12). Significance was set at P < 0.05 for all analyses.

**Results**

**Subject characteristics.** Twenty-nine healthy pregnant women completed the study (N_{early} = 17 and N_{late} = 19), with 7 women completing both early and late gestation studies. Participant weight gain at the time of study was within the normal range (~4.2 kg during early gestation, and ~12.4 kg during late gestation) as per the most recent 2009 gestational weight gain recommendations (28), and blood glucose measurements indicated normal glucose metabolism (Table 2). Energy intakes were adequate based on individual measured REE during each gestation, and subjects adhered well to the standardized protein intake of 1.5 g·kg⁻¹·d⁻¹ during the 2 d preceding each study day (Table 2).

**Phenylalanine flux.** Phenylalanine flux measured from urine samples did not change significantly with different test protein intakes during both early (P = 0.21) and late gestation (P = 0.26) (Supplemental Figure 1), similar to earlier IAAO studies (11, 12, 14). This provides evidence that the precursor pool for the IAAO did not change significantly with increasing test protein intakes, and suggests that the changes in oxidation were inversely proportional to whole-body protein synthesis.

**Table 2.** Study day assessments of healthy pregnant women during early and late gestation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Early gestation</th>
<th>Late gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 35)</td>
<td>(n = 43)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.4 ± 10.5</td>
<td>71.4 ± 9.1*</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.77 ± 0.39</td>
<td>4.56 ± 0.42*</td>
</tr>
<tr>
<td>VCO₂, L/min</td>
<td>0.173 ± 0.023</td>
<td>0.181 ± 0.035</td>
</tr>
<tr>
<td>Protein intake before study day, g·kg⁻¹·d⁻¹</td>
<td>1.44 ± 0.30</td>
<td>1.47 ± 0.53</td>
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</table>

*Significantly different from early gestation (P < 0.05).
L-[1-13C]phenylalanine oxidation. The F13CO2 declined with increasing protein intakes up to an intake of ~1.2 and 1.5 g · kg⁻¹ · d⁻¹ in early and late gestation pregnant women, respectively (Figures 1 and 2). Additional increases in protein intake did not produce changes in F13CO2 values, indicating a plateau in the rate of labeled phenylalanine incorporation for protein synthesis. Two-phase linear regression crossover analysis of F13CO2 data identified a breakpoint in early gestation at 1.22 g · kg⁻¹ · d⁻¹ (R² = 0.60; 95% CI: 1.66 g · kg⁻¹ · d⁻¹) and in late gestation at 1.52 g · kg⁻¹ · d⁻¹ (R² = 0.63; 95% CI: 1.28, 1.77 g · kg⁻¹ · d⁻¹). The breakpoint estimates represent the minimum protein intake required to meet the needs of 50% of the population (EAR), and the upper 95% CI values indicate the population-safe recommendation (RDA).

Discussion

The estimated average protein requirements for healthy pregnant women during early and late gestation were determined to be 1.22 g · kg⁻¹ · d⁻¹ and 1.52 g · kg⁻¹ · d⁻¹, respectively (Figures 1 and 2). These results suggest that dietary protein needs are increased compared with nonpregnant adults from early gestation (~16 wk) onwards. Compared to the DRI recommended EAR (0.88 g · kg⁻¹ · d⁻¹) for protein intake during pregnancy, our early gestation estimate is 39% higher and our late gestation estimate is 73% higher (8). Compared to the IAAO-derived EAR for protein requirement in healthy nonpregnant adults, our early and late gestation estimates are 32% and 63% higher, respectively (11). The increased need for dietary protein detected in early gestation indicates that maternal adaptations to protein metabolism are established early in pregnancy, and this agrees with previous findings that suggest changes to protein and nitrogen metabolism occur during the first trimester in anticipation of fetal needs (1, 3). The substantial difference in protein requirements between nonpregnant, early gestation, and late gestation women indicates that protein demand increases steadily as pregnancy progresses. Thus, it may be more appropriate to provide stage-specific protein intake recommendations.

In the DRI (2005) report, a single protein requirement study was considered for the determination of pregnancy intake recommendations (8). King et al. (29) conducted the only other direct estimate of protein requirement, studying pregnant adolescents during the third trimester through use of the nitrogen balance method. Given the paucity of pregnancy-specific protein requirement data available, the well-established concerns with the nitrogen balance method (30), and the absence of other stage-specific protein requirement studies, further investigation is essential for adding resolution to the nutritional demands of pregnancy. The experimental design used in this study addresses several details overlooked by the earlier study (i.e., energy intake controlled, adult subjects, inclusion of early gestation requirement study).

It is important to consider these results in the context of what healthy pregnant women are eating ad libitum and how protein requirement factors into the diet as a percentage of energy. The 2003–2004 NHANES and the Canadian Community Health Survey Cycle 2.2 reported protein intakes in the United States and Canada, respectively, but excluded pregnancy-specific data from the analysis (31, 32). We recently completed a prospective analysis of 270 pregnant women from Vancouver, British Columbia, who were recruited for a separate study (33). Dietary FFQs were administered during early (~16 wk) and late (~36 wk) gestation, and the results found that median maternal protein consumption ranged from 1.3 to 1.5 g · kg⁻¹ · d⁻¹. Thus, maternal protein intake patterns of women from British Columbia, where historically the incidence of LBW infants born to women aged 20–34 y is low (~5.45%), are comparable to our estimates of protein requirements of healthy pregnant women from the same population (34). Protein metabolism does not operate in isolation, particularly during pregnancy. Thus, it is useful to consider protein requirements as a percentage of total energy. Energy was provided at 1.7 × REE for each participant and was specific to the individual. On average, we provided 2305 kcal/d and 2483 kcal/d of energy to the early and late gestation groups, respectively. Using the DRI formula (8) to calculate estimated energy requirement for pregnant women weighing the same as our average participant in early and late gestation (Table 2), 2488 kcal/d and 2695 kcal/d is recommended by the DRI, respectively. Thus, the energy provided in this study is comparable to DRI energy recommendations. It
should be noted that DRI recommendations for energy intake during pregnancy are unlike protein recommendations in that they are based on a substantial body of evidence derived from studies of pregnant women (8). The current protein DRI recommendations of 1.1 g · kg\(^{-1} · \text{d}^{-1}\) during pregnancy converted to a percentage of energy represents ~9% of energy from protein in both early and late gestation. Our estimated protein requirement during early and late gestation represents 14% and 17.5% energy from protein, respectively. The acceptable macronutrient distribution range is 10–30% of energy from protein (8). Thus, despite our estimates of protein requirements being significantly higher than the current DRI protein intake recommendation for pregnancy on a g · kg\(^{-1} · \text{d}^{-1}\) basis, our estimates are more consistent with the current acceptable macronutrient distribution range.

In a recent meta-analysis, Imdad and Bhutta (4) reviewed the effect of balanced protein energy supplementation during pregnancy on birth outcomes. Balanced protein energy supplementation was defined as nutritional supplementation during pregnancy where proteins provided <25% of the dietary energy. The results indicated that balanced protein energy supplementation had a significant reduction (31%) in risk of small-for-gestational-age infants, especially among undernourished women. Studies from the Montréal Diet Dispensary also offer similar findings where pregnant women from lower socioeconomic groups were intervened with individualized nutritional rehabilitation (35, 36). Women who consumed ~100 g/d had the best pregnancy outcome as measured by reduced incidence of low birth weight (35). Based on body weights in the current study, the average daily protein intake recommendations would be 79 g/d during early gestation and 108 g/d during late gestation, which is consistent with the Montréal Diet Dispensary birth outcome data (36). Cucó et al. (37) longitudinally followed maternal intake of macronutrients in a cohort of well-nourished Spanish women before conception and in weeks 6, 10, 26, and 38 of pregnancy and its impact on birth weights. Protein intake had the most significant impact when multiple linear regression models were used to assess the impact of macronutrient intakes on birth weight. Similar relations of positive impacts of protein intakes during pregnancy on birth weight have been reported earlier by Godfrey et al. (38).

The IIAO method developed to determine protein requirements has been criticized by others (39, 40) because we hold phenylalanine constant across all test intakes, the response observed in F\(^{13}\)CO\(_2\) has been suggested to be reflective of its own excess or limitation, rather than the protein intake. However, we have addressed this and other these concerns in our reply to authors (39, author reply) and in a recent study determining protein requirements in octogenarian women (14). Briefly, the key point is that phenylalanine is never limiting in our test intakes because tyrosine is in excess and held constant at all intakes. Furthermore, phenylalanine flux remains unchanged across all test intakes during both early and late gestation, and the response in the F\(^{13}\)CO\(_2\) is reflective of the overall pattern of demand for protein synthesis. The results of the current study corroborate previous IIAO studies that suggest current protein intake recommendations underestimate requirements (11, 12, 14). Differences in protein requirements between early and late gestation indicate that protein recommendations should reflect the changing metabolic demands of pregnancy. Pregnancy-specific protein/amino acid requirement data are lacking in part because of the ethical and technical constraints of applying the nitrogen balance method to a pregnant population (41). The minimally invasive IIAO method represents a new avenue for exploring amino acid requirements in vulnerable populations such as pregnant women. Recently we showed that the dietary requirement for lysine (42) and threonine (43) in sows increases by 30–60% in late gestation. Further studies are needed to define gestation-stage–specific essential amino acid requirements during human pregnancy.

Acknowledgments
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References


