SUPPLEMENT

Protein and Amino Acid Requirements during Pregnancy[1–3]

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ABSTRACT

Protein forms an essential component of a healthy diet in humans to support both growth and maintenance. During pregnancy, an exceptional stage of life defined by rapid growth and development, adequate dietary protein is crucial to ensure a healthy outcome. Protein deposition in maternal and fetal tissues increases throughout pregnancy, with most occurring during the third trimester. Dietary protein intake recommendations are based on factorial estimates because the traditional method of determining protein requirements, nitrogen balance, is invasive and undesirable during pregnancy. The current Estimated Average Requirement and RDA recommendations of 0.88 and 1.1 $g \cdot kg^{-1} \cdot d^{-1}$, respectively, are for all stages of pregnancy. The single recommendation does not take into account the changing needs during different stages of pregnancy. Recently, with the use of the minimally invasive indicator amino acid oxidation method, we defined the requirements to be, on average, 1.2 and 1.52 $g \cdot kg^{-1} \cdot d^{-1}$ during early (~16 wk) and late (~36 wk) stages of pregnancy, respectively. Although the requirements are substantially higher than current recommendations, our values are ~14–18% of total energy and fit within the Acceptable Macronutrient Distribution Range. Using swine as an animal model we showed that the requirements for several indispensable amino acids increase dramatically during late gestation compared with early gestation. Additional studies should be conducted during pregnancy to confirm the newly determined protein requirements and to determine the indispensable amino acid requirements during pregnancy in humans. *Adv Nutr* 2016;7(Suppl):839S–44S.

Keywords: amino acids, dietary guidelines, maternal nutrition, pregnancy, protein metabolism

Introduction

Protein forms an essential component of a healthy diet in humans to support both growth and maintenance. Protein in the body plays structural (keratin, collagen) and functional (enzymes, transport proteins, hormones) roles (1, 2). Ultimately, most mammalian protein is composed of 20 different amino acids, and thus there is a need in our body for both an adequate supply of amino acids and total protein (nitrogen). The current definition of protein requirement is as follows: "the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or secretion of milk at rates consistent with good health" (2). Thus, during pregnancy, an exceptional stage of life defined by rapid growth and development and enormous maternal physiologic changes from the time

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of conception to birth, adequate dietary protein is crucial to ensure a healthy outcome.

Within several weeks of conception, adjustments in protein metabolism occur to support fetal growth and development while maintaining maternal homeostasis and preparing for lactation (3). Protein utilization from foods and deposition as new tissues are energy dependent at stages of absorption, amino acid transport, protein synthesis, and proteolysis. Thus, dietary intake during pregnancy must have sufficient energy and protein to ensure the full-term delivery of a healthy infant. The additional energy required during the full term of pregnancy has been estimated to be ~77,000 kcal (4), although the energy cost of pregnancy is not distributed equally throughout the gestational period. This is because the amount of protein deposited in maternal and fetal tissues varies during pregnancy, with nonsignificant deposition during the first trimester, gradually increasing during the second trimester, and with most occurring in the third trimester (5). Thus, protein and amino acid intake recommendations during pregnancy should be gestational stage–specific, with adequate energy to ensure all needs are met.

Protein Metabolism during Pregnancy

The total amount of additional nitrogen accumulated during pregnancy has been calculated, with 40% of maternal protein gain represented by the fetus, placenta, and amniotic fluid (6, 7). Maternal tissues represent the other 60%, which includes uterine tissue, breast tissue, adipose tissue, blood volume increases, and extracellular fluids. On the basis of calculations by Hytten (6), for a typical 12.5-kg gain in body weight during pregnancy (which includes a 3.3-kg term infant), the accretion is 148 g nitrogen, which is equivalent to 925 g protein (when using a conversion factor of 6.25) (1, 5). This additional protein accretion by the mother suggests that there must be substantial adaptations in protein metabolism that take place to accommodate this increase. Whole-body protein turnover studies conducted with the use of stable isotope tracers reported increased turnover by early pregnancy in pregnant women compared with non-pregnant women (8–13), and there is an absolute increase in protein synthesis by 15% in the second trimester and 25% during the third trimester (14). Concentrations of several amino acids in maternal plasma (including both dispensable and indispensable amino acids) decrease in early pregnancy and persist at low concentrations throughout. The decline is greatest for the glucogenic amino acids (alanine, serine, threonine, glutamine, and glutamate) and amino acids in the urea cycle (arginine, ornithine, citrulline) (15). A concomitant decrease in maternal urea synthesis and urinary urea excretion takes place early in gestation and rates remain low throughout pregnancy (7). Thus, it is hypothesized that increases in protein synthesis, combined with decreases in amino acid catabolism and thus urea synthesis, and circulating plasma amino acids are a conservation mechanism for overall retention of protein during a period of extreme demand (i.e., pregnancy) (14).

The metabolic adaptations in protein metabolism, described above, suggest that during pregnancy in well-nourished individuals, general physiologic changes act to conserve protein and nitrogen, promote protein accretion, and thereby ensure adequate nutrient supply to the fetus. Only a few studies have provided any information under conditions of altered dietary supply/availability of the primary substrates of protein synthesis (balance of the 20 amino acids). Thus, as correctly pointed out by King (3), if dietary protein intake is below some threshold required for metabolic adaptations, then women have an increased risk of poor pregnancy outcomes and appropriate nutritional intervention with adequate protein and amino acids may be necessary. Indeed, current work from Thame and Kurpad and colleagues has shown that amino acid kinetics are altered during adolescent pregnancy and in adult pregnancy with low BMIs, such that the metabolic adaptations are not adequate to sustain a healthy pregnancy outcome (16–19). Thus, identifying optimal protein and amino acid requirements during different stages of pregnancy is critical for the creation of adequate dietary intake recommendations during this crucial life stage.

Protein and Amino Acid Intake Recommendations during Pregnancy

Protein intake recommendations in humans are provided as the Estimated Average Requirement (EAR) and RDA (1). The EAR is the average daily nutrient intake estimated to meet the requirement of half of healthy individuals in a particular life stage and sex group. The RDA is an estimate of the minimum daily average dietary intake that meets the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage (1).

The EAR in pregnancy for total protein is based on the adult protein maintenance needs of 0.66 $g \cdot kg^{-1} \cdot d^{-1}$, plus the additional needs to support the newly deposited protein. Protein deposition during pregnancy was calculated from total body potassium accretion studies in healthy pregnant women who gained 13.8 kg body weight by the end of the third trimester to be 12.6 g/d (1, 2, 5). The increased requirement on a body weight basis was then estimated to be 12.6 g protein/d ÷ 57 kg (reference woman) = +0.22 g

TABLE 1 Comparison of protein recommendations during pregnancy with requirements determined by using the IAAO method[1]

	DRI (1), $g \cdot kg^{-1} \cdot d^{-1}$	IAAO $g \cdot kg^{-1} \cdot d^{-1}$	IAAO % of energy
Adults (*n* = 8)			
EAR	0.66	0.93	~10
RDA	0.80	1.2	~13
Pregnant women			
~16 wk gestation (*n* = 17)			
EAR	0.88	1.22	~13
RDA	1.1	1.66	~18
~36 wk gestation (*n* = 19)			
EAR	0.88	1.52	~17
RDA	1.1	1.77	~20

[1] Data are from references 20 and 21. EAR, Estimated Average Requirement; IAAO, indicator amino acid oxidation.

protein · kg^{-1} · d^{-1}. This value was added to the EAR for nonpregnant adults of 0.66 g protein · kg^{-1} · d^{-1} and resulted in an EAR of 0.88 g · kg^{-1} · d^{-1} (1) during pregnancy. The RDA was set at 1.1 g · kg^{-1} · d^{-1} on the basis of an additional intake of 25 g/d (**Table 1**). The FAO report (2), based on the same set of data on protein deposition during pregnancy, recommended that additional protein intakes of 0.7, 9.6, and 31.2 g/d be consumed during the first, second, and third trimesters, respectively.

To our knowledge, there have been no published data on amino acid requirements during human pregnancy. It is generally assumed that amino acid needs increase in proportion to the increased protein needs during pregnancy. Because the pregnancy EAR for total protein is 0.88 g · kg^{-1} · d^{-1}, which is 1.33 times the EAR for adults of 0.66 g · kg^{-1} · d^{-1}, each individual amino acid requirement value for nonpregnant adults is multiplied by 1.33 and rounded to the nearest whole number (1) (**Table 2**). However, our studies in swine (22) and modeling by others (23) indicate that requirements for some amino acids increase more than for others. Therefore, this assumption that amino acid requirements in humans increase in proportion to protein requirements during pregnancy is probably incorrect. The primary reason that no direct studies of amino acid requirements during pregnancy have been conducted in humans is due to the tedious, cumbersome nature of the traditional methods (e.g., nitrogen balance) previously available to determine protein and amino acid requirements.

Methods to Determine Protein and Amino Acid Requirements

Nitrogen balance method. The nitrogen balance technique, which involves the measurement of nitrogen intake and excretion, has been regarded as the gold standard for determining nitrogen (protein) requirements (1). Although conceptually this is an elegant concept, the method tends to underestimate protein/amino acid needs because it overestimates nitrogen intake and underestimates nitrogen excretion (1). In addition, the body urea pool is quite large and equilibration to a new test protein intake requires 5–7 d of adaptation (24). The length of adaptation makes the method cumbersome and time-consuming, and it is also not suitable for studying vulnerable populations, including pregnancy. In addition, when a range of protein intakes is tested, the efficiency of protein utilization has been shown to decrease to near-zero nitrogen balance (25). As nitrogen intake increases, the nitrogen response curve is nonlinear and analyzing the balance data with linear regression can be erroneous. Most of the earlier balance studies had protein intakes near-zero balance, and thus the intercept, usually determined by linear interpolation, leads to an underestimation of the true balance (26), and therefore of the requirement.

The current recommendations for protein requirements in adult humans are set at an EAR and RDA of 0.66 and 0.80 g · kg^{-1} · d^{-1}, respectively, by both the DRI (1) and FAO (1) (Table 1). These recommendations were established by fitting a linear regression analysis model to the earlier nitrogen balance data in men, where zero nitrogen balance was used as the criterion of nutritional adequacy (26). Rand et al. (26), although acknowledging that there may be some limitations to this analysis, selected studies for the final analysis, which had test intakes at around zero balance. Humayun et al. (20) included additional nitrogen balance studies, which tested higher test protein intakes, and performed a reanalysis using a 2-phase linear regression analysis model. The reanalysis resulted in the estimation of a breakpoint (EAR) of 0.91 g · kg^{-1} · d^{-1} and an upper 95% CI (population-safe, equivalent to the RDA) of 0.99 g · kg^{-1} · d^{-1}. These reanalyzed values are significantly higher than the current EAR and RDA of 0.66 and 0.80 g · kg^{-1} · d^{-1}, respectively (1). The known limitations of the nitrogen balance method, and the fact that it is not practical for determining requirements in populations such as pregnancy, made it necessary to develop newer state-of-the-art techniques.

Indicator amino acid oxidation method. The indicator amino acid oxidation (IAAO) is a minimally invasive technique that uses a stable isotope–labeled indispensable amino acid to determine amino acid requirements (27, 28). The method is based on the fundamental physiologic principle that excess amino acids cannot be stored in the body and must be partitioned between incorporation into protein and oxidation. Thus, when one indispensable amino acid is deficient for protein synthesis, then all other amino acids including an indicator amino acid (usually another indispensable amino acid such as L-1-^{13}C-phenylalanine) are in excess and therefore will be oxidized (28). With increasing intake of the limiting amino acid, oxidation of the indicator amino acid will decrease, reflecting increasing incorporation into protein. Once the requirement is met for the limiting amino acid/protein, there will be no further change in the oxidation of the indicator amino acid with increasing intake of the test amino acid. The inflection point at which the oxidation of the indicator amino acid stops decreasing and reaches a plateau is referred to as the "breakpoint." The breakpoint, which is identified with the use of 2-phase linear regression analysis, indicates the EAR of the limiting (test) amino acid/protein (29). The IAAO method requires oral isotope intake (30), collection of breath and urine samples (31), and a

TABLE 2 Amino acid intake recommendations during pregnancy[1]

Amino acid	EAR,[2] mg · kg^{-1} · d^{-1}	RDA,[3] mg · kg^{-1} · d^{-1}
Histidine	15	18
Isoleucine	20	25
Leucine	45	56
Lysine	41	51
Methionine + cysteine	20	25
Phenylalanine + tyrosine	36	44
Threonine	21	26
Tryptophan	5	7
Valine	25	31

[1] Data are from reference 1. EAR, Estimated Average Requirement.
[2] Factorial estimates based on adult amino acid requirements × 1.33 to take into account increased protein demands of pregnancy.
[3] Factorial estimate based on 24% variability from the EAR.

single study day adaptation to the test intake (32). Thus, the method is well suited for studying protein and amino acid requirements in different physiologic conditions across the life cycle. In particular, the rapid adaptation and noninvasive methods eliminate the risk due to long-term deficient intake in vulnerable populations.

The IAAO method, which was initially developed to determine indispensable amino acid requirements, has been widely applied in adult humans, in school-aged children, and in children with disease (28, 29). The first application of IAAO studies in humans to determine protein requirements in young men was by Humayun et al. (20). The study design was similar to the indispensable amino acid requirement studies, except that all amino acids in the pattern of egg protein (except for phenylalanine, as the indicator amino acid, and tyrosine) were provided from deficiency to excess. The results suggested that the protein requirement in young men was 0.93 and 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ (Table 1) and significantly higher than the current recommendations of 0.66 and 0.80 $g \cdot kg^{-1} \cdot d^{-1}$ for the EAR and RDA, respectively. Since then, the IAAO method to determine protein requirements has been applied in several populations (school-aged children, elderly women >65 y, and female octogenarians) (33–35). In all of the studies, it was shown that current recommendations are underestimates and that the mean protein requirements determined by IAAO method exceed the requirements determined by traditional nitrogen balance studies by ~30–40%. The application of the IAAO method to determine protein requirements has been criticized by some (36, 37), because the indicator amino acid, phenylalanine intake, is held constant with increasing test protein intakes and the IAAO might be limited and deficient at higher test protein intakes. However, in all of the above-mentioned studies, phenylalanine flux was unchanged with increasing intakes of protein, suggesting that phenylalanine is not limiting with any test intakes. When the flux is constant, the IAAO method reflects the partitioning between incorporation into protein synthesis and oxidation in response to the test protein intakes, and indeed identifies the requirement for protein. We have addressed this and other concerns in the responses to Letters to Editors and encourage readers to be aware of the debate in reassessing the current protein intake recommendations (36, 37). One other key discussion point is whether the higher requirement estimates derived by using the short-term IAAO method are relevant to long-term health benefits (38). Future long-term supplementation studies with the newly defined protein requirements will need to be conducted to confirm the IAAO findings.

Current Data on Protein Requirements during Pregnancy

We recently determined the protein requirements during early (~16 wk) and late (~36 wk) gestation in healthy singleton pregnant women using the IAAO method (21). The requirements were determined to be 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ (upper 95% CI: 1.66 $g \cdot kg^{-1} \cdot d^{-1}$) and 1.52 $g \cdot kg^{-1} \cdot d^{-1}$ (upper 95% CI: 1.77 $g \cdot kg^{-1} \cdot d^{-1}$) during early and late gestation, respectively. There are 2 striking observations from this result. The first is that the requirements are significantly higher than the current EAR and RDA of 0.88 and 1.1 $g \cdot kg^{-1} \cdot d^{-1}$, respectively. Second, protein needs are increased as early as 16 wk of gestation, although it was previously thought that the demand for protein would be low initially and increase substantially only by late pregnancy (3). Although these requirements appear to be considerably higher than what might be consumed during pregnancy, our recent data suggest that this is not the case. We recently completed a prospective analysis in 270 pregnant women from Vancouver, British Columbia, Canada (39), who were recruited for a separate study. FFQs were administered during early (~16 wk) and late (~36 wk) gestation, and the results showed that the range of median maternal protein consumption was 1.3–1.5 $g \cdot kg^{-1} \cdot d^{-1}$. These data were collected from women living in Vancouver, Canada, where the historic incidence of low-birth-weight infants born to women aged 20–34 y is low (~5.45%) (40). Our protein requirements determined with the use of the IAAO method are comparable to protein intake patterns of healthy pregnant women from the same population (39). When we consider protein requirements as a percentage of total energy, our IAAO study provided energy at 1.7 × resting energy expenditure for each participant and was specific to the individual. On average, the pregnant women in our study received 2305 and 2483 kcal energy/d during early and late gestation, respectively, which is similar to current energy recommendations according to the DRI and FAO (1, 4). The current protein DRI recommendation of an RDA of 1.1 $g \cdot kg^{-1} \cdot d^{-1}$ during pregnancy converted to a percentage of energy represents ~9% of energy from protein in both early and late gestation. Our estimated protein requirements during early and late gestation represent 14% and 17.5% of energy from protein, respectively. The IAAO-based protein requirements correspond to the recommended Acceptable Macronutrient Distribution Range of 10–35% for energy from protein.

Amino Acid Requirements during Pregnancy

To our knowledge, there are no published studies of amino acid requirements in humans; however, by using the pig as a model we have thus far determined the threonine (41), lysine (42), isoleucine (43), and tryptophan (44) requirements during the first and third trimesters of pregnancy. The results have revealed increases in requirements during later stages of pregnancy for all of the amino acids, although the increases varied among the amino acids. Requirements for threonine increased by 55%, lysine by 45%, isoleucine by 63%, and tryptophan by 35% during late stages of pregnancy when compared with the early stages. These studies showed that applying the same factor to all amino acids to obtain a mathematically derived requirement estimate, as done by the DRI, is erroneous. Unfortunately, similar published studies are not available on human pregnancy. We recently completed the first, to our knowledge, amino acid requirement study in human pregnancy. Lysine requirements during late gestation increased by 27% when compared with early gestation (R Elango et al., unpublished data, 2015). This is more than the increase in lysine requirements factorially calculated by DRI from the increase in protein requirement of 41 mg · kg^{-1} · d^{-1} (Table 1). The degree to which other indispensable amino acid requirements increase with progressive stages of pregnancy, and how these compare with total protein intake, needs to be determined in future studies. There have also been current swine studies that suggest that, in addition to the indispensable amino acids, there might be an increased demand for the dispensable amino acids such as arginine and glutamine for uterine and fetal development (45–47). Whether a similar demand for the indispensable amino acids exists during human pregnancy needs to be explored further.

Protein Supplementation Studies

Higher protein intakes during pregnancy have been an active area of debate for several decades (48, 49). Protein supplementation during pregnancy has also been debated because there might be competition among amino acids, which might negatively affect fetal growth, as shown in animal models (50). In a randomized controlled trial in low-socioeconomic-status pregnant women living in New York, negative pregnancy outcomes (increased risk of infants born small-for-gestational age) were reported with high-protein supplements (providing >34% of energy) (51). But several other studies in which the protein supplements were provided as food and conducted in the presence of adequate energy, showed a significant reduction in the risk of small-for-gestational age infants, suggesting prevention of intrauterine growth restriction (52–54). Thus, as discussed extensively by Imdad and Bhutta (55, 56), protein supplementation during pregnancy must be a balanced protein supplement (<25% of the total energy content) to ensure reduction in risk of small-for-gestational age infants. Furthermore, earlier studies from the Montreal Diet Dispensary found that pregnant women from lower socioeconomic groups who received an intervention with individualized nutritional rehabilitation and consumed ~100 g protein/d had the best pregnancy outcome as measured by reduced incidence of low birth weight (52). The protein requirement results obtained in our study (Table 1), when expressed as an average daily intake for the women who participated in our study, would be 79 g/d during early gestation and 108 g/d during late gestation (21) and would provide protein energy at ~15–17% of calories, which is consistent with the Montreal Diet Dispensary birth outcome data. Furthermore, Blumfield et al. (57) showed in an elegant analysis that, at protein intakes between 18% and 20% of calories from food, key micronutrient requirements, including folate, vitamin E, iron, and zinc, were met in healthy, well-nourished, pregnant women. Thus, protein supplementation during pregnancy should be in the form of food supplements, balanced (within 25% of total energy), and, as stated earlier by Prentice et al. (58), with a view to prevent intrauterine growth restriction and not as a goal to increase birth weight.

Conclusions

In conclusion, we believe that the current EAR and RDA for protein intakes during pregnancy of 0.88 and 1.1 g · kg^{-1} · d^{-1} are an underestimate. With the use of the IAAO method, we recently determined the mean protein requirements to be 1.2 and 1.52 g · kg^{-1} · d^{-1} during early and late gestation, respectively. The average daily protein intakes would be ~79 g/d (~14% of calories) during early gestation and 108 g/d (~17% of calories) during late gestation for normally nourished women gaining gestational body weight within recommendations. To our knowledge, there are currently no direct published estimates of amino acid requirements during pregnancy, but recent animal studies suggest that indispensable amino acid needs for threonine, lysine, isoleucine, and tryptophan are increased toward later stages of pregnancy, but not in the same proportion to protein requirement as during early gestation (59). Future studies are urgently needed to establish human indispensable amino acid requirements during pregnancy. More studies are also necessary to confirm the positive effect of food-based protein supplements during pregnancy, with a focus on preventing intrauterine growth restriction, to ensure that protein supplements are always as a balanced protein-energy supplement (<25% of total energy content). These studies are necessary for the development of more accurate dietary recommendations for the many populations of women throughout the world who have compromised nutrition during pregnancy due to low protein and/or energy intake or imbalanced amino acid intake.

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