# The Association of Maternal Protein Intake during Pregnancy in Humans with Maternal and Offspring Insulin Sensitivity Measures

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## ABSTRACT

The purpose of this review is to critically evaluate the studies assessing the relations between protein intake during human pregnancy and insulin sensitivity measures in the mother and offspring, and to get a better understanding of the knowledge gaps that still exist. Overall, there is insufficient evidence to conclude about implications of higher amounts of protein intake during pregnancy on maternal or offspring insulin sensitivity. However, studies show a relation between protein quality and insulin sensitivity, such that animal protein may be associated with negative outcomes and plant protein may be associated with positive insulin sensitivity outcomes. There is an urgent need for standardized studies using comparable terminology to evaluate any potential relations between insulin sensitivity in mothers and offspring and truly low and high maternal protein intake while maintaining eucaloric balance to better inform about optimal protein dosage and quality during this period. *Curr Dev Nutr* 2019;3:nzz055.

## Introduction

The Developmental Origins of Health and Disease theory states that the intrauterine environment conditions the growing offspring for a spectrum of metabolic outcomes ranging from optimal metabolic health to the development of metabolic diseases (1). Maternal diet affects the intrauterine environment and can affect several metabolic variables such as glucose homeostasis and insulin sensitivity (2, 3). Independent of maternal diet, pregnancy is characterized by a state of accelerated development of insulin resistance (4), which may promote the development of gestational diabetes mellitus (GDM) (5) and lead to the development of subsequent disease states such as diabetes and obesity of the offspring later in life (2, 3). Thus, insulin sensitivity, glucose metabolism, and insulin regulation during pregnancy must be closely monitored to optimize the health of both the mother and offspring.

Dietary protein has been suggested to be a modulator of glucose metabolism and insulin regulation in males and nonpregnant females, and the amount and type of protein consumed may influence these metabolic outcomes. However, there is no strong consensus of these findings in the general population (males and nonpregnant females). Specifically, 1 recent review aggregating several studies assessing the effects of short- and long-term higher-protein diets determined that the effects of acute (1 wk–6 mo) and chronic (>6 mo) protein diets consisting of >20% of total energy intake (TEI) from protein (considered the higher end of current recommendations of 10–35%, and >1.5 $g \cdot kg^{-1} \cdot d^{-1}$ for an average 68-kg individual) on insulinemic action in healthy, nonobese, nonpregnant female and male populations are equivocal (6). In pregnancy, findings are also limited and ambiguous (7–9).

The current recommendation for protein intake during adulthood (nonpregnancy) is based on the Estimated Average Requirement (EAR) of 0.66 $g \cdot kg^{-1} \cdot d^{-1}$. To satisfy additional protein needs for newly deposited protein during growth in pregnancy, these recommendations increase to 0.88 $g \cdot kg^{-1} \cdot d^{-1}$, which remains consistent throughout the duration of pregnancy (10) (~11% of TEI considering a diet of 2000 kcal/d, and current recommendations of 10–35% TEI

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Abbreviations used: AGE, advanced glycation end-product; BCAA, branched-chain amino acid; EAR, estimated average requirement; FFM, fat-free mass; GDM, gestational diabetes mellitus; GLUT-4, glucose transporter type 4; OGTT, oral-glucose-tolerance test; TEI, total energy intake; T2DM, type 2 diabetes mellitus.

from protein). However, recent research shows that protein requirements increase from early to late pregnancy due to an exponential increase in growth of maternal and fetal tissues (11, 12). If the increased demand for adequate protein intake throughout the duration of pregnancy is not met, then impaired substrate metabolism (e.g., decreased amino acid flux) results in the inability to maintain an optimal metabolism during pregnancy (11, 12). Thus, the main concern in determining ideal, personalized recommendations for protein intake during pregnancy is to establish an appropriate amount to satisfy the balance between consuming adequate protein for fetal growth and maternal health while maintaining metabolic homeostasis. Even so, it is critical to define safe limits and types of protein intake during pregnancy in various populations (e.g., those with GDM or obesity).

Therefore, the purpose of this review is twofold: *1*) to assess the current knowledge regarding the effects of the amount and type of protein intake during pregnancy in humans on maternal and offspring insulin sensitivity measures {e.g., fasting glucose and insulin, HOMA-IR, insulin increment [determined by an oral-glucose-tolerance test (OGTT)]} and the RR of GDM as an indirect measure of insulin resistance; and *2*) to identify existing knowledge gaps regarding this topic so that further research can build a more substantial body of evidence for dietary protein recommendations during pregnancy, considering maternal and offspring insulin sensitivity outcomes.

## Current Status of Knowledge

### Protein intake

Current protein intake recommendations during pregnancy are based on factorial estimates of recommendations for healthy populations because the traditional nitrogen balance method of determining protein requirement is particularly involving. Therefore, current protein recommendations during all stages of pregnancy are set to 0.88 $g \cdot kg^{-1} \cdot d^{-1}$ adapted from the EAR, and 1.1 $g \cdot kg^{-1} \cdot d^{-1}$ adapted from the RDA for healthy nonpregnant adults (10). However, these recommendations do not consider the increased need for protein as pregnancy progresses, which has been determined using the minimally invasive indicator amino acid oxidation method (early pregnancy: 11–20 weeks of gestation, 1.2 $g \cdot kg^{-1} \cdot d^{-1}$; late pregnancy: 30–38 weeks of gestation, 1.52 $g \cdot kg^{-1} \cdot d^{-1}$) (13). Although these protein requirements are higher than current recommendations, they supply ∼14–18% of TEI, which is both within normal limits and comparable with recent NHANES data (2011–2014) that indicate women of child-bearing age (20–44 y old) consume 15.3% of total daily energy from protein (14). Further, it seems that the increased requirement for protein intake is feasible because this group of women consumed the same amount of protein before beginning the study (early pregnancy group: 1.44 ± 0.30 $g \cdot kg^{-1} \cdot d^{-1}$; late pregnancy group: 1.47 ± 0.53 $g \cdot kg^{-1} \cdot d^{-1}$) (13), supporting other findings of little overall change in dietary patterns from before pregnancy to early and late pregnancy (15). In addition, a recent study in healthy pregnant women from British Columbia found that women were consuming greater amounts of protein at 16 and 36 weeks of gestation (1.5 and 1.3 $g \cdot kg^{-1} \cdot d^{-1}$, respectively) than the current recommended amounts (16). Therefore, it seems that current consumption of protein in developed countries by pregnant women is in line with the higher protein intake recommendations during pregnancy (13).

Protein intake may elicit glycemic and insulinemic responses acutely and chronically; however, not all studies agree. Acutely, protein promotes insulin secretion which reduces glycemia (17, 18), indicating its role in anabolism in an acute setting. However, data on the short-term (1 wk to 2 mo) consequences of higher protein intake on insulinemic outcomes in healthy nonobese participants are limited and show only minor effects (19–21). For example, there was no difference in insulin sensitivity measures using an intravenous-glucose-tolerance test between 10 d of 3.0 $g \cdot kg$ fat-free mass $(FFM)^{-1} \cdot d^{-1}$ compared with 1.5 $g \cdot kg\ FFM^{-1} \cdot d^{-1}$ in healthy young (higher: 9.5 ± 1.8; lower: 7.5 ± 1.4 $10^{-5}$ $mL \cdot min^{-1} \cdot pmol^{-1} \cdot 1^{-1}$) or older (higher: 7.6 ± 1.4; lower: 6.9 ± 1.3 $10^{-5}$ $mL \cdot min^{-1} \cdot pmol^{-1} \cdot 1^{-1}$) individuals. However, these values were expressed in grams per kilogram FFM per day, and not grams per kilogram per day, arguably making the measurements more applicable because FFM is more metabolically active; however, discrepancies in units make accurate comparisons with other studies difficult. Insulin regulation and glucose metabolism may be negatively affected by chronic protein intake ranges of 0.97–1.87 $g \cdot kg^{-1} \cdot d^{-1}$ compared with 0.57–0.74 $g \cdot kg^{-1} \cdot d^{-1}$ (22–24) in nonpregnant female and male humans; however, not all studies agree (25). Thus, even in nonpregnant female and male humans, findings are inconsistent. However, with the knowledge of the potential ramifications of varying amounts and types of protein intake on insulin sensitivity measures in nonpregnant female and male populations, it is even more important to define these effects in pregnancy because of the accelerated development of insulin resistance throughout the duration of pregnancy in healthy individuals, with an even more exaggerated response in overweight and obese pregnant women.

### The effects of protein intake during human pregnancy on offspring and maternal insulin sensitivity measures

This state-of-the-art review aims to address the current state of the literature and identify priorities for future research. PubMed, Ovid, and Web of Science search engines were used to search for articles. The research focus in this field before the year 2000 was primarily protein restriction, whereas the focus of our article is on higher protein intake. As a result, the articles that are discussed here are all from after 2000. We considered all published intervention and observational studies assessing the effects of amount and type of protein intake during pregnancy on maternal and offspring insulin sensitivity measures in humans under eucaloric conditions. All classifications of maternal BMI status and GDM were accepted. All interventions that aimed to provide dietary protein during pregnancy were considered. Primary outcome measures included maternal and/or offspring insulin sensitivity measures, maternal and/or offspring plasma glucose and/or insulin concentrations, and GDM risk and/or prevalence. The keywords used to search were "protein intake," "pregnancy," "insulin sensitivity," "insulin resistance," "insulin," and "glucose." The 3 studies that are presented in **Table 1** and the 7 studies in **Table 2** are the only studies, to our knowledge, that assess these relations.

Much of the early work regarding the effect of human protein intake during pregnancy on insulin sensitivity was based on studies of famine (combined caloric and protein restriction), particularly the Dutch Hunger Winter of 1944–1945, which has been exhaustively studied (26–28). Thus, the focus of this critical review will be on studies

**TABLE 1** Summary of human studies assessing the effects of amount of protein intake during pregnancy on maternal and offspring insulin and glucose regulation outcomes[1]

| Study | Population | Dietary logs | Offspring age | "Low PRO" group | "High PRO" group | Measurements | Outcomes |
|---|---|---|---|---|---|---|---|
| Maslova et al. (9) | Offspring of Danish women | FFQ in gestational week 30 | 19–21 y | Total: 65 g/d<br>1.1 $g \cdot kg^{-1} \cdot d^{-1}$<br>16.2% TEI | Total: 104 g/d<br>1.7 $g \cdot kg^{-1} \cdot d^{-1}$<br>15.5% TEI | Divided PRO according to source (animal, vegetable). Offspring fasting insulin and glucose concentrations | No associations |
| Maslova et al. (8) | Offspring of Danish women; 608 pregnant women with GDM and 626 pregnant controls | FFQ in gestational week 25 | 9–16 y | <73.1 g/d<br><1.1 $g \cdot kg^{-1} \cdot d^{-1}$*<br><12.5% TEI | >73.1 g/d<br>>1.1 $g \cdot kg^{-1} \cdot d^{-1}$*<br>>12.5% TEI | Fasting insulin, glucose, and HOMA-IR | No associations in GDM or controls. GDM women in the lowest PRO intake group had the lowest insulin resistance |
| Shiell et al. (7) | 168 men and women born in the Aberdeen Maternity Hospital from 1948 to 1954 | 7-d food log in gestational weeks 28–30 | 40 y | <60 g/d<br><1.0 $g \cdot kg^{-1} \cdot d^{-1}$<br><10.0% TEI | >80 g/d<br>>1.3 $g \cdot kg^{-1} \cdot d^{-1}$<br>>13.4% TEI | Plasma glucose and insulin concentrations at fasting and after a standard OGTT, insulin increment | Offspring of women with high PRO intake had a reduced plasma insulin increment between fasting and 30 min (7.0% decrease in increment per 10-g/d increase in PRO) |

[1]*Assumes an average 150-pound (68-kg) woman because the study did not provide body mass for calculation of protein intake relative to body mass. GDM, gestational diabetes mellitus; OGTT, oral-glucose-tolerance test; PRO, protein; TEI, total energy intake.

examining the impact of higher amounts of protein intake on insulin sensitivity outcomes while maintaining eucaloric balance.

Comparisons of these studies are difficult to make for various reasons including the way that protein intake was expressed. For instance, no associations were found between differences in absolute protein intakes (65 g/d compared with 104 g/d) and offspring fasting insulin and glucose concentrations (9). Although the difference in absolute protein intake between groups was nearly 40 g/d, the protein intake relative to the percentage of TEI was quite similar (14.2% compared with 15.5% TEI). Thus, even if more absolute protein is consumed, if the percentage of TEI is maintained within a normal range, there may not be any impact on offspring glucose metabolism. In the other 2 studies where differences were noted, authors used quartiles to define protein intake. In 1 study (8), data were grouped based on percentage of TEI (<12.5% TEI: <1.1 $g \cdot kg^{-1} \cdot d^{-1}$ for an average 68-kg woman; >12.5% TEI, >1.1 $g \cdot kg^{-1} \cdot d^{-1}$ for an average 68-kg woman). No associations were noted between maternal protein intake and offspring fasting insulin and HOMA-IR when expressing protein intake in this way. Although this protein-defining approach seems reasonable, considering that national recommendations are provided in a range (e.g., 10–35% TEI should come from protein), more effective comparisons would be between intakes that are much closer to the low end of the range (~10% TEI) and those much closer to the high end of the range (~35% TEI), providing truly low and high protein intakes. Similarly, significant negative associations between protein intakes >13.4% TEI (>1.3 $g \cdot kg^{-1} \cdot d^{-1}$) and insulin sensitivity determined by a lower insulin increment (Table 1) have been noted (7), yet the protein intake values in the quartiles were extremely close (>13.4% compared with <10.0% TEI). Thus, it is difficult to compare amounts of protein intake in these studies because protein intake was expressed differently, and the groups may not have adequately represented the full spectrum of truly low to high protein intakes compared to recommended standards.

In addition, the health status of the pregnant woman may affect the outcomes that are being compared. There were no differences in fasting insulin and HOMA-IR of the offspring at 9–16 y old, with protein intake (<1.1 $g \cdot kg^{-1} \cdot d^{-1}$ compared with >1.1 $g \cdot kg^{-1} \cdot d^{-1}$) measured using an FFQ around gestational week 25, between pregnant women with GDM and non-GDM healthy controls (Table 1) (8). However, the authors noted that GDM-exposed offspring from the group that consumed <1.1 $g \cdot kg^{-1} \cdot d^{-1}$ tended to have lower fasting insulin and HOMA-IR, although this tendency was nonsignificant, thus, more studies were needed to further study this observation. It is important to note, however, that there was only a mean 6-g difference between total protein intake in these groups; therefore, the range may not have been wide enough to note a physiologically significant difference in outcome measures. In another study (9), GDM was initially used as a covariate in the analysis of the effects of maternal protein intake of <1.1 $g \cdot kg^{-1} \cdot d^{-1}$ compared with >1.7 $g \cdot kg^{-1} \cdot d^{-1}$ on offspring fasting insulin and glucose concentrations, but it was found that GDM status did not substantially alter the results. Therefore, it seems amount of protein intake does not have a differential effect on offspring insulin sensitivity

**TABLE 2** Summary of human studies assessing the effects of quality of protein during pregnancy on maternal and offspring insulin sensitivity measures and GDM risk[1]

| Study | Population | Dietary logs/intervention | Measurements | Outcomes |
|---|---|---|---|---|
| Maslova et al. (9) | Offspring (age 19–21 y) of Danish women | FFQ in gestational week 30 | Divided protein according to source (animal, vegetable). Offspring fasting insulin and glucose concentrations | No associations. |
| Maslova et al. (8) | Offspring (age 9–16 y) of Danish women; 608 pregnant women with GDM and 626 pregnant controls | FFQ in gestational week 25 | Fasting insulin, glucose, and HOMA-IR | Red and processed meat tended to increase insulin resistance. |
| Jamilian and Asemi (75) | 68 women with GDM | Intervention (36 weeks of gestation): Control: 0.8 g · $kg^{-1}$ · $d^{-1}$ of protein (70% animal and 30% plant proteins) Soy: same amount of protein with 35% animal protein, 35% soy protein, and 30% other plant proteins | Fasting insulin, glucose, HOMA-IR, and Quantitative Insulin Sensitivity Check Index | Soy decreased fasting insulin, glucose, and HOMA-IR, and decreased Quantitative Insulin Sensitivity Check Index. |
| Zhang et al. (49) | 13,110 pregnant women free from history of GDM from the Nurses' Health Study II | FFQ before pregnancy | RR of GDM | High consumption of red and processed meat significantly elevated the RR of GDM. |
| Pang et al. (51) | 980 multiethnic pregnant Asian women from Growing Up in Singapore Toward Healthy Outcomes cohort | 24-h dietary recall and 3-d food diary at gestational weeks 26–28 | RR of GDM | Higher intakes of animal and plant protein were associated with increased RR of GDM. Of animal protein sources, seafood and dairy proteins were significantly associated with high RR of GDM. |
| Bao et al. (29) | 21,457 singleton pregnancies reported among 15,294 pregnant women from the Nurses' Health Study II | FFQ before pregnancy | RR of GDM | Higher intake of animal protein (specifically red meat) was significantly associated with increased RR of GDM, whereas higher intake of plant protein (specifically nuts) was significantly associated with decreased RR of GDM. |
| Liang et al. (50) | 6299 pregnant women from the Nutrition in Pregnancy and Growth in Southwest China cohort | FFQ from 12 mo before pregnancy, 24-h dietary recall at first ultrasound and gestational weeks 20–22 and 33–35 | RR of GDM | Higher intake of animal protein in mid-pregnancy was significantly associated with increased RR of GDM. |

[1] GDM, gestational diabetes mellitus.

measures between pregnant women with GDM and non-GDM healthy pregnant women.

Obesity status may also affect findings. In several studies, it has been found that BMI and fat mass may explain much of the variance in outcome measures (29, 30). These implications may be the result of altered handling of amino acids from dietary protein intake (specifically meat) in overweight or obese individuals. Branched-chain amino acid (BCAA) content is particularly high in meat sources of protein, and it has been suggested (31) in a rodent model that BCAA catabolism is greater, but oxidation is lower in the fat cells of overweight and obese individuals, which "spills" excess BCAAs into circulation, contributing to an increased BCAA concentration (31). The increased BCAA pool may then contribute to impaired fatty acid and glucose metabolism, eventually potentially leading to impaired insulin regulation and glucose homeostasis. Although it is unknown whether dietary BCAAs directly exacerbate this metabolic burden in humans, they may contribute to the plasma BCAA pool. Further research should determine whether maternal obesity is the primary driver in increased plasma BCAA concentrations and whether there is a contribution of dietary protein sources to elevated plasma BCAA concentrations, for the purpose of determining direct relations between these alterations and insulin sensitivity measures in pregnant women and their offspring.

In addition, it is important to consider how the other macronutrients (carbohydrates and fats) are altered when assessing varying amounts of protein intake. According to the protein leverage hypothesis, when

the percentage of protein intake in the diet is lowered, there is a compensatory increase in TEI from carbohydrate and fat food sources to maintain consumption of optimal amounts of amino acids (because amino acids are present in protein, carbohydrate, and fat food sources), which ultimately dilutes total dietary protein intake in terms of percentage of total macronutrient intake (32). It is argued that low dietary protein intake at the expense of increases in TEI provides the impetus for the development of obesity and its comorbidities (33). On the other hand, higher dietary protein intake at the expense of lowering intake of the other macronutrients may also produce unfavorable outcomes. For example, a maternal dietary pattern characterized by high protein and low carbohydrate intake during pregnancy in Chinese women has been found to be associated with a greater risk of GDM (34). Thus, because metabolism may be changed by alterations in each of the macronutrients (protein, carbohydrates, fats), it is important to assess protein intake in relation to alterations in the other macronutrients when assessing metabolic responses. Reporting macronutrient intake as a ratio (carbohydrate:protein:fat) in each group, 1 study (9) found that the percentages were surprisingly similar relative to TEI (low-protein group: 56:14:30; high-protein group: 52:16:33). This may have contributed to the lack of noticeable differences in offspring fasting insulin and glucose concentrations between groups. However, both studies (8, 9) used multivariable analyses applying a 1:1 substitution of carbohydrates for protein, which relies on the modeling of an increase in protein at the expense of carbohydrate. In 1 of these studies, no associations were found between protein intake and offspring fasting insulin and glucose concentrations (9), but the authors noted that a nonsignificant indication of more favorable measures in the GDM-exposed offspring from mothers with lower protein intake may have been due to a decrease in carbohydrate intake.

Further, the methods of measurement of insulin sensitivity in the presented studies both are inconsistent and may not be ideal to measure true tissue insulin sensitivity. For instance, an OGTT with assessments of plasma glucose and insulin concentrations at 0, 30, and 120 min post-OGTT was used in male and female offspring (~40 y old) of mothers who consumed $>1.3$ g protein $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ and $<1.0$ g protein $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ during pregnancy (Table 1) (7). It was found that offspring of women who consumed $>1.3$ g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ had lower 30-min insulin increments than the offspring of women who consumed $<1.0$ g $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$, and in both groups concentrations were restored to baseline levels at 120-min post-OGTT, indicating that any changes were transient in nature, and thus may not perpetuate chronic alterations. Whereas the OGTT is a dynamic measure of insulin sensitivity (i.e., assessing insulin and glucose changes over time after a dietary glucose perturbation), the other studies relied on static measures of insulin sensitivity such as fasting glucose and insulin concentrations, and quantification of HOMA-IR using measures of fasting insulin and glucose (8, 9). In addition to a general need for more studies to assess the effects of dietary protein intake during pregnancy on insulin sensitivity measures, there is a special need for studies using the hyperinsulinemic-euglycemic clamp, which is considered the gold standard assessment of insulin sensitivity (35). The clamp technique would allow precise measurement of tissue insulin sensitivity and quantification of insulin resistance.

In contrast with the studies involving the effects of maternal protein intake on offspring insulin sensitivity measures, to date, there are no studies involving the effects of maternal protein intake on maternal insulin sensitivity measures. Therefore, it would be prudent to assess these effects, because the health status of the mother affects the outcomes of the offspring.

***Potential mechanisms.***

Although definitive conclusions cannot be made from the existing literature, decreased insulin sensitivity with higher amounts of protein intake may be partially explained by hypoglycemic protective mechanisms, explained in a theory from Layman and Baum (36). Meals higher in dietary protein elicit lower postprandial glucose and insulin responses than a high-carbohydrate meal (29). To maintain glucose homeostasis in the face of a high-carbohydrate meal, rapid insulin responses and peripheral uptake of glucose must occur. Conversely, in the face of a high-protein diet (in combination with a lower-carbohydrate diet), to prevent hypoglycemia, a modification in peripheral glucose uptake in the form of decreased insulin sensitivity must occur (28). Although this relation may be hypothetical and it is unknown whether higher-protein diets during pregnancy result in lower carbohydrate intakes, it provides a theory for a metabolic purpose of the modulation of insulin sensitivity measures with higher dietary protein meals (glycemic control), rather than defaulting findings to impaired metabolism.

Further mechanistic knowledge underlying the potential effects of dietary protein during pregnancy on maternal and offspring insulin sensitivity measures is mainly based on animal studies, and mechanisms are severely understudied in humans. Some potential mechanisms at play with variations in amount of protein intake may include epigenetics (37, 38), modifications of placental functioning and activity of the insulin-like growth factors (39), and overstimulation of mammalian target of rapamycin pathways (40, 41). Future research should address these gaps in the literature to determine if high-protein diets are indeed causative of these changes, or not.

## Quality of protein food source

Emerging data suggest that the type of protein food source and the amino acid composition of the protein sources are important to consider when assessing the effects of maternal dietary protein on maternal and offspring insulin sensitivity measures. In nonpregnant individuals, several studies have reported that consumption of animal protein increases the RR of type 2 diabetes mellitus (T2DM) (42–46), whereas consumption of plant protein is inversely associated with the RR of T2DM (47). In addition, it appears that the type of animal protein is important to consider. Red meat consumption, particularly processed red meat (e.g., breakfast meats, deli meats), has been found to be associated with an increased RR of T2DM (45). However, not all studies agree with this. Recently, a randomized clinical trial in people with T2DM assessed the effects of a high animal protein diet (30% animal protein, 30% fat, 40% carbohydrate) compared with a high plant protein diet (30% plant protein, 30% fat, 40% carbohydrate) over 6 wk and found that high animal protein improved insulin sensitivity (measured using the hyperinsulinemic-euglycemic clamp) and fasting glucose compared with high plant protein (48). A summary of the reviewed articles examining the associations between quality of protein during pregnancy and both offspring insulin sensitivity measures and RR of GDM is presented in Table 2.

***Effects of protein quality during pregnancy on offspring insulin sensitivity measures.***

Few studies have examined the effects of the type of protein intake during pregnancy on offspring insulin sensitivity measures (7, 8), and none have noted significant differences in offspring insulin sensitivity measured by fasting insulin and glucose and HOMA-IR when comparing maternal consumption of animal sources of protein with maternal consumption of nonanimal sources of protein. However, there was a trend toward an association between greater offspring insulin resistance and mothers who consumed higher total red meat/processed meat during pregnancy (8). Because of the large gap between pregnancy and the time of offspring assessment (offspring were aged 9–21 y), future research should analyze these associations in the first years of life because that may give a more direct relation, minimizing the need to control for outside factors that may influence outcomes in the years before assessment (e.g., exercise, diet). In addition, if the offspring are assessed in the first years of life, it may be possible to also examine the influence of type of maternal dietary protein on breast-milk composition and subsequent offspring insulin sensitivity outcomes.

***Effects of protein quality during pregnancy on maternal insulin sensitivity measures.***

Although no studies to our knowledge have assessed the associations between protein quality and maternal insulin sensitivity measures, various studies have noted associations with the RR of GDM (29, 49–51). Nonetheless, findings are not consistent in that most of these studies found an association with only total animal protein consumption (29, 49, 50), whereas 1 study found that both total animal and total plant protein intakes were related (51). Although this is convincing evidence, any noted effects of animal protein sources on the RR of GDM may be limited to the specific type of animal protein source (29, 49, 51). For instance, red meat (e.g., beef, pork) (29, 49), processed meat (e.g., cured meats) (49), and seafood (e.g., all types of fish, shellfish) (51) have been noted to be significantly associated with increased RR of GDM. Interestingly, 1 study did not find any associations between red meat intake and RR of GDM, but rather found associations between seafood protein intake and RR of GDM (51). It was noted, however, that because the sample population was Asian, the driving force of these differences may have been founded in the differences in animal protein composition between an Asian diet and a Western diet. The 2003–2004 NHANES data found that red meat comprised most of the meat protein consumption (58%) in the United States, with fish only comprising 10% (52). In comparison, the Asian population analyzed consumed most of their dietary meat protein from seafood sources (43%), and only 29% from red meat protein sources (51). Therefore, the driving force in this relation could be the total calories consumed in each of these types of protein. Dietary patterns were further analyzed by characterizing pregnant women from the Nurses' Health Study II into either a "Western" dietary pattern (red and processed meat, refined grains, French fries, pizza, and sweets) or a "prudent" dietary pattern (high intake of fruit, green leafy vegetables, fish, and poultry) (49). Strong associations with the RR of GDM were found in both dietary patterns, but the relations in the Western pattern were driven by red and processed meat intake, independent of other sources, including French fries, pizza, sweets, and refined grains. Future research should effectively compare the diets of various cultures to investigate the influence of the type of protein being consumed relative to the total calories that the protein source provides within the context of the specific dietary pattern.

Two studies found positive associations between dairy consumption and GDM risk (50, 51). Interestingly, these relations remained significant even after adjusting for other potential nutrients that may have driven the associations, such as saturated fat. Dairy is an insulin secretagogue, creating acute hyperinsulinemia after consumption (45), and long-term dairy-driven hyperinsulinemia may mediate insulin resistance (53). However, these findings are in contrast to literature that suggests that T2DM risk is lowered with increased dairy consumption (54–57). Currently, there is no explanation for the contrasting findings, thus, more research is required that will take into account the alterations in metabolism during pregnancy that may be influencing the differences found between pregnant and nonpregnant individuals, such as the ability to metabolize other constituents of dairy (e.g., several amino acids, glucose) driven by nutrient-shunting to the growing fetus.

The timing of consumption of different types of protein throughout pregnancy may also influence outcomes. Whereas studies have noted increased RR of GDM with higher dietary animal consumption before pregnancy (29, 49) and in the last trimester of pregnancy (26–28 weeks of gestation) (51), 1 study found no associations before pregnancy or in early pregnancy (at first ultrasound), but an association in mid-pregnancy (20–22 weeks of gestation) (50). In the latter study, absolute total animal protein coming from meat sources increased from prepregnancy and early pregnancy to mid-pregnancy (pre: 94 g/d; early: 84 g/d; mid: 129 g/d), whereas the percentage of animal protein intake relative to TEI only increased slightly (pre: 23%; early: 21%; mid: 26%). Therefore, regardless of the relative contribution to total calories, it seems that a higher intake of animal protein characteristic of the increasing progression of pregnancy may negatively affect the RR of GDM. Future research should compare the relative contributions of various types of animal proteins (e.g., red and processed compared with lean, dairy, and eggs) to determine if a healthier protein amino acid profile affects the RR of GDM to a similar extent.

Although not clear at the present, observed adverse changes in insulin sensitivity and/or the RR of GDM with higher red meat protein intakes during pregnancy may be mediated mostly by other nutrients within the protein source. However, it has been noted that the RR of GDM still exists even after controlling for several other nutrients in red meat animal protein sources that could potentially increase the RR of GDM, such as saturated fat, fatty acids, and cholesterol (29, 49). Therefore, even other components may be the driving force of the increase in the RR of GDM with higher red meat animal protein intake, for example, nitrates, nitrites, and/or iron (58). High concentrations of nitrates and nitrites have been implicated in the development of type 1 diabetes in offspring (59). Physiologically, nitrates and nitrites can react to form N-nitroso compounds (60), which may have toxic effects on pancreatic $\beta$-cells due to peroxynitrite, reactive nitrogen intermediates, and nitrosamine generation (60), and also $\beta$-cell autoimmunity (61). Further, iron is found in high concentrations in red meat, and an association has been shown between tissue iron stores and diabetes risk, as reviewed previously (62). Iron can be toxic for pancreatic $\beta$-cells and iron overload has been associated with $\beta$-cell failure and decrements in insulin sensitivity (62). Another component of red meat that may be responsible for this association is advanced glycation

end-products (AGEs), formed through heating and processing of meats and high-fat products. AGEs have been found to promote inflammatory markers such as C-reactive protein and TNF-$\alpha$ (63, 64), which have been positively linked with the RR of GDM and hyperglycemia (65).

Amino acid pattern of the animal protein may also be implicated in this relation owing to the effects of certain amino acids on insulin secretion, skeletal muscle glucose and glycogen metabolism, and liver glucose production (66). After ingestion, animal protein sources produce a significant increase in the plasma concentration of BCAAs (67) compared with plant-based protein sources (68). Furthermore, BCAAs constitute the majority of the rise in plasma amino acids after a meal high in a red meat animal protein (67). High dietary BCAA consumption and the metabolic signature of BCAAs have been associated with insulin resistance in healthy nonpregnant, nonobese, and obese populations (31, 69, 70) and high plasma BCAA concentrations have been associated with an increased risk of developing T2DM (71) and a decrease in insulin sensitivity measures (31, 67, 70). However, the majority of these changes have been noted in combination with other poor dietary habits (e.g., consumption of high-fat foods) (31). Further, other lifestyle habits that may negate these negative implications (e.g., lack of exercise) have not been adequately addressed or controlled for in these studies. Therefore, it is unclear whether elevated plasma BCAA concentrations are a cause of, a result of, or simply correlated with impaired insulin and glucose regulation. Furthermore, no studies have examined the relation between insulin sensitivity changes and animal protein consumption as it relates to changes in plasma BCAA concentrations in human pregnancy. However, it has recently been found that maternal diabetes may have a direct impact on embryonic BCAA concentrations and metabolism in diabetic rabbits (72), suggesting a potential programming impact of maternal diabetes on offspring BCAA regulation. Because of the increased need for protein (and thus likely high animal protein consumption) combined with a natural increase in insulin resistance throughout pregnancy, future research should thoroughly define the impact of animal protein sources on plasma BCAA concentrations as it relates to changes in insulin sensitivity throughout the entire span of pregnancy, to determine whether 1 particular stage of pregnancy is particularly sensitive to the effects of higher animal protein intake.

In contrast to many animal protein sources, plant protein sources have been reported to decrease the RR of GDM (29) and improve insulin sensitivity (73), although not all findings are consistent (51). Nut consumption, in particular, has been found to improve these measures. Nuts have a nutrient composition that includes a high content of fiber and MUFAs and PUFAs, combined with a low glycemic index (73, 74), all of which have been associated with decreased risk of diabetes and with improved insulin sensitivity (73). Plant protein intake from soy protein sources may also improve insulin sensitivity measures during pregnancy in women with GDM (75). Sixty-eight women with GDM were randomly assigned to consume either a control diet containing 0.8 g protein $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ from mostly animal sources (70% animal, 30% plant) or a soy diet containing the same amount of daily protein, but with higher amounts of plant protein (35% animal, 35% soy, 30% other plant) for 6 wk at any point during pregnancy. In pregnant women consuming soy, fasting insulin, glucose, HOMA-IR, and the Quantitative Insulin Sensitivity Check Index were significantly lower than in the control group after the intervention. Mechanistically, soy protein food may exert beneficial effects on glucose homeostasis owing to its ability to improve glucose transporter type 4 (GLUT-4) translocation as well as the oxidative and nonoxidative pathways of glucose metabolism (76). Thus, based on this study, the addition of soy protein to a maternal diet may improve glucose and insulin regulation in women with GDM; however, these findings need to be replicated in larger studies. Although there are various studies indicating the benefits of plant protein consumption, 1 study noted a significant positive association between vegetable protein intake and the RR of GDM (51). The authors mentioned that exploration of various types of vegetable proteins did not produce any significant results, indicating that total vegetable protein intake was the driving force in the noted relation, and it may be more related to the different dietary patterns of the Asian population compared with Western populations (as discussed previously). Therefore, although it may be of benefit to examine individual protein sources and/or nutrients within the protein sources, it may be more beneficial for future research to focus on the specific dietary patterns associated with eating elevated amounts of specific protein sources.

Modeling of the substitution of various protein sources has produced quite interesting results that may be much more practical for professionals when it comes to discussing nutrition in practice. By substituting different types of proteins, the negative effects of some proteins on the development of GDM could be mitigated (29). For example, substituting 1 serving/d of total red meat with poultry, fish, legumes, or nuts has been associated with a significant decrease in the RR of GDM by 29%, 33%, 33%, and 51%, respectively. Further, substituting only 5% TEI from animal protein with plant protein was enough to significantly decrease the association between RR of GDM and animal protein, by 51%. Specifically, substituting processed red meat with unprocessed red meat was also found to be associated with a decreased RR of GDM. Therefore, it will benefit future investigations to directly test the effects of substituting certain protein sources with others, specifically when it comes to maternal and offspring insulin and glucose regulation outcomes, for the purpose of relaying more practical information to professionals to use in practice.

## Limiting factors of current studies and future directions

Independent of the paucity of data, it is difficult to make definitive conclusions based on the discussed studies, owing to various limitations. There is a lack of longitudinal data sets in the offspring, with no study assessing the impact of maternal protein intake in infants, 1 study assessing adolescents (9–16 y old) (8), 1 study assessing young adults (19–20 y old) (9), and 1 study assessing older offspring (40–58 y old) (7). Longitudinal information could provide insight into the length of time to which dietary habits during pregnancy affect glucose metabolism of the offspring. Further, data on the effect of maternal protein intake on insulin sensitivity in infancy would address one of the most important time points considering the various associations between infant health (e.g., growth rate) and consequent adult outcomes (e.g., adiposity, BMI) (77); thus, future research should assess this gap.

One critical limitation in the literature is the lack of control for lifestyle habits, such as physical activity of the offspring, throughout the years of follow-up. For example, although 1 study (8) studied offspring at 9–16 y, it did not adequately control for physical activity of the offspring

within or preceding those years. However, this study controlled for maternal physical activity, which is also important to consider when examining the programming of metabolic traits. Physical activity is known to improve insulin sensitivity (78), mediated by improvements in glucose transport and metabolism (i.e., muscle GLUT-4 protein content and translocation). Further, a critical emphasis for future studies regarding this topic is that physical activity and protein intake may have interactive effects on insulin sensitivity measures (79). In this study, resistance exercise was combined with a moderately high protein diet (33% protein, ~1.2 $g \cdot kg^{-1} \cdot d^{-1}$) and energy restriction (~1400 kcal/d—women, ~1700 kcal/d—men) for 16 wk in overweight/obese, sedentary individuals with T2DM. This intervention elicited greater improvements in basal insulin concentrations than did moderately high protein diet alone, a control diet providing adequate protein intake (19% protein, ~0.7 $g \cdot kg^{-1} \cdot d^{-1}$), and adequate protein intake combined with resistance exercise (79). Because the relation between moderately high dietary protein intakes and exercise has not been reported during pregnancy, yet a synergistic effect of the combination of a moderately high protein diet and exercise on decreased T2DM instance has been reported, future research should address these relations in pregnancy where protein requirements are increased at the same time that insulin resistance develops.

Lastly, there is a lack of data on effects of maternal dietary protein intake during early and mid-gestational periods on offspring insulin sensitivity. Instead, studies used food logs and FFQ records from the latter portion of pregnancy (weeks 25–30 of gestation) (7–9). Protein requirements increase with pregnancy progression; however, no studies have compared the effects of dietary protein intake of each trimester of pregnancy on offspring insulin sensitivity outcomes. With that, we are unable to determine if one of the phases of pregnancy is more sensitive than the others, or if overall diet is what matters.

## Conclusions

Given the increase in the prevalence of obesity in women of childbearing age and the ongoing scientific discussion about associations between higher-protein diets and insulin resistance, it is crucial to fully understand the depth of the relation between the amount of maternal dietary protein intake and insulin sensitivity measures in pregnant women and their offspring. However, based on the limited-quality data available at this time, definitive conclusions regarding this topic cannot be drawn. Despite these limitations, studies have found that the source of dietary protein during pregnancy may affect maternal insulin sensitivity measures and the RR of GDM. Overall, the main status of the field examining the impact of amount and type of maternal dietary protein consumption on maternal and offspring insulin sensitivity measures is varied and scattered. If future studies clearly define low and high protein, and provide a significant spread of protein intakes, preferably including amounts that are outside of current recommendations (protein intake of 10–35% TEI), there may be more impactful findings to advance the field. Future studies must also address the limitations of present studies including, but not restricted to, longitudinal offspring cohort data including infancy time points, control for lifestyle habits of mothers and offspring including physical activity, and recording of dietary intake throughout each stage of pregnancy. Furthermore, potential impacts of protein amounts or types must be viewed in the context of dietary patterns. By addressing these gaps in the literature, improved protein recommendations during pregnancy can be established.

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