HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

Obesity (Silver Spring). 2023 August ; 31(8): 2057–2064. doi:10.1002/oby.23789.

No strong evidence of the protein leverage hypothesis in pregnant women with obesity and their infants

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Abstract

Objective: Investigate the role of dietary protein on macronutrient and energy intake, maternal adiposity during pregnancy, and infant adiposity at birth.

Methods: In 41 women with obesity, early-pregnancy (13–16 weeks) protein intake was assessed with food photography and expressed as a ratio of Estimated Average Requirements in pregnancy for protein (0.88g/kg/d), herein 'protein balance'. Energy intake was measured by the intake-balance method, gestational weight gain as grams per week, and fat mass by a 3-compartment model. Spearman correlations and linear models were computed using R 4.1.1 ($p<0.05$ considered significant).

Results: Women were 27.8 ± 4.8 years of age, had a pregravid BMI of 34.4 ± 2.9 kg/m^2, and were non-Caucasian in majority (n=23, 56.1%). Protein balance in early-pregnancy was not significantly associated with energy intake across mid- and mid/late-pregnancy (β=328.7, p=0.30 and β=286.2, p=0.26, respectively) nor gestational weight gain (β=117.0, p=0.41). Protein balance was inversely associated with fat mass in early-, mid-, and late-pregnancy (β=−10.6, p=0.01, β=−10.4, p=0.03, β=−10.3, p=0.03, respectively). Protein balance did not predict infant adiposity at birth (p>0.05).

Conclusions: Low protein intake may have been present pre-pregnancy, explaining early relationships with adiposity in our cohort. The PLH is likely not implicated in the intergenerational transmission of obesity.

Keywords

Pregnancy; Adiposity; Obesity; Dietary Proteins

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Authors' contributions: JM, ADA, and LMR conceptualized the parent study. MK and LMR conceptualized the current analysis. MK prepared and analyzed the data and wrote the first draft of the manuscript. All authors critically reviewed and approved the final version of the manuscript.

Disclosure: The authors declare no conflict of interest.

Introduction

The increasing prevalence of obesity worldwide has been met by economic efficiencies in food supply. Increased development of shelf-stable and processed foods provides an increased supply of accessible and convenient foods to meet the demands of modern technological-driven, fast-paced societies. Cost-conscious foods often dilute protein in favor of the less expensive and more palatable carbohydrate (simple sugars) and fat (saturated fat). Abundance of food favoring this macronutrient distribution has led to population intakes of calorically-dense, ultra-processed foods, and an overconsumption of calories (1).

The Protein Leverage Hypothesis (PLH) posits that proportional deficits in protein intake relative to carbohydrate and fat in modern diets have resulted in weight gain due to excess energy intake required to reach daily protein or amino acid needs (2). Elegant experimental studies and surveys of experimental and population-level data have indeed provided evidence that humans overeat non-protein energy on protein-diluted diets, and undereat non-protein energy on protein-concentrated diets (3, 4). Randomized controlled trials in adults have demonstrated that these proportional reductions in the ratio of protein relative to fat and carbohydrate intake then increase energy intake (3, 5). Recent, complex mathematical and conceptual frameworks provide further compelling evidence in support of the PLH, particularly that protein intake is implicated in body weight regulation and obesity within human populations (6–8).

Previous reports using National Health and Nutrition Examination Survey data showed that only 12–13% of pregnant women consumed optimal levels of protein relative to recommendations established by the Institute of Medicine during the second and the third trimesters (9). Insufficient protein intake during pregnancy is a concern since a deficiency in specific amino acids that are important for cell metabolism and function can lead to embryonic losses, intra-uterine growth restriction, reduced postnatal growth, and neurological underdevelopment (10).

Current knowledge of protein leverage during pregnancy in humans and whether it contributes to maternal energy intake, gestational weight gain, and downstream effects on infant size at birth or childhood obesity is lacking (11, 12). As total maternal protein needs are increased throughout pregnancy in the form of turnover and deposition to the growing fetus and maternal tissues, it is recommended and essential that dietary protein be increased to support such demands. If maternal protein intake in pregnancy is not sufficient to support the increased protein demand, the PLH would suggest that pregnant women would have greater weight gain and greater downstream effects on infant adiposity. Indeed, protein leverage is driven by a regulatory, appetite-linked protein target, wherein intake of dietary protein has been shown to be effective for body weight management via mechanisms promoting satiety as well as increased energy expenditure and fat-free mass (13).

The aims of this observational study were to investigate the role of maternal dietary protein in early-pregnancy on (i) macronutrient intake (combined carbohydrate and fat) in early-pregnancy and energy intake across mid- and mid/late-pregnancy and (ii) gestational weight gain and body composition in early-, mid-, and late-pregnancy, and (iii) infant anthropometrics and body composition at birth. In women, we hypothesized that maternal diets low in protein relative to protein needs would be characterized by higher (i) intakes of carbohydrate, fat, and energy as well as (ii) gestational weight gain, fat mass, and fat mass index (FMI) in pregnancy. In infants, we hypothesized that maternal low protein intake relative to needs would be (iii) inversely associated with weight, head circumference, fat mass, and FMI as well as positively associated with fat-free mass.

Methods

Study design.

This was a secondary data analysis of the Mom Energy Expenditure (MomEE) study which has been extensively described elsewhere (14). In brief, MomEE was a state-of-the-art prospective, observational cohort study in 72 pregnant women with obesity (NCT01954342) at Pennington Biomedical Research Center (PBRC; Baton Rouge, Louisiana). This study aimed to understand the role of energy intake and energy expenditure on gestational weight gain in pregnant women with obesity and doubly labeled water, room calorimetry, and 3-compartment body composition measurements were completed. MomEE was approved and monitored by the PBRC Institutional Review Board and all participants provided verbal and written consent prior to study initiation.

Participants and recruitment.

Pregnant women who were English-speaking, aged 18 – 40 years, with obesity (body mass index [BMI]>30.0 kg/m^2), and with a single, viable, first-trimester pregnancy (<16 weeks gestation) were enrolled in the study. This secondary analysis was restricted to women with class I and II obesity (BMI 30.0 – 40.0 kg/m^2). There is an inverse association between maternal BMI and weight gain in pregnancy and we have shown that in women with class III obesity, weight gain is limited or weight loss is observed (15). Further, these pregnancies require more medical management for adverse maternal and neonatal outcomes such as gestational diabetes, hypertensive disorders, preterm birth and non-elective Caesarean delivery, which would impact the analysis and interpretations (16, 17). Women were recruited from obstetrical office referrals or through targeted print and social media advertisements. Infants of eligible women were included if they had a body composition measurement, and were excluded if they were born prematurely or small for gestational age.

Study visits.

Participants attended study visits at screening (confirmation of pregnancy-15 weeks), 1st trimester (13–16 weeks, ‘early-pregnancy’), 2nd trimester (24–27 weeks, ‘mid-pregnancy’), 3rd trimester (35–37 weeks, ‘late-pregnancy’), delivery, and <10 days after delivery for infant measurements. Relevant measurements to this analysis are highlighted below, and were conducted in-person at the PBRC.

Maternal sociodemographics.

Sociodemographic data, including enrollment age, race, marital status, household income, level of education, and living situation were obtained from self-reported questionnaires.

Gestational age was calculated using the ultrasound or last self-reported menstrual period date and confirmed from prenatal records.

Maternal anthropometrics.

Metabolic weight was recorded twice, with the participant fasting and wearing a hospital gown and undergarments only; hospital gown weight was subtracted from averaged weights. Height was measured twice using a wall-mounted stadiometer with the head in the Frankfort position. BMI was calculated as weight (kg)/height (m^2). Fat mass and fat-free mass were assessed using a 3-compartment model by isotope dilution and air displacement plethysmography (BOD POD, COSMED, Concord, CA). FMI was calculated as fat mass (kg)/height (m^2). Further details are available elsewhere (14, 15).

Infant anthropometrics.

Weight was measured with the infant nude to the nearest 5 grams on a calibrated scale (SCALE-TRONIX, White Plans, NY). Head circumference was measured using a measuring tape around the most prominent part of the infant's head immediately above the supraorbital ridges. Fat mass and fat-free mass were assessed using air displacement plethysmography (PEA POD, COSMED, Concord, CA) with a head cap covering the infant's hair. Infant percent fat mass was considered valid at or above 6%. For infants with a percent fat mass less than 6% (n=3), an adjusted fat mass was calculated (18).

Energy and dietary assessments.

Energy intake was assessed using the intake-balance method as the mean total daily energy expenditure (TDEE) measured by doubly labelled water and changes in energy stores; that is, energy deposition through changes in fat mass and fat-free mass from the 1st trimester to the 2nd trimester ('across mid-pregnancy') and to the 3rd trimester ('across mid/late-pregnancy'). Maternal dietary intakes of protein, carbohydrate, and fat were assessed over 7 days with the Remote Food Photography Method (RFPM). The RFPM is a novel, validated method for measuring food intake that capitalizes on digital photography of food provision (pre-meal photographs) and plate waste (post-meal photographs) to estimate energy, macronutrient, and micronutrient intake (19). As previously reported, daily dietary data first underwent a quality control check for gross under-reporting. Days in which energy intake was less than 60% of TDEE were excluded from the analysis.

Daily protein intake in early-pregnancy was expressed as a ratio based on the Estimated Average Requirements (EARs) in pregnancy for total protein (0.88g/kg/d) for each participant. Expressing protein intake relative to protein needs allows for an accurate characterization of protein status in the first trimester according to internationally-established dietary reference intakes (the EARs). This ratio is hereby referred to as 'protein balance'):

$$\text{Protein balance} = \frac{\text{Protein Intake}}{\text{Protein Requirements}}$$

$$= \frac{\text{Early} - \text{Pregnancy Protein Intake } (g)}{\text{Early} - \text{Pregnancy Metabolic Weight } (kg) \times 0.88\ g/kg}$$

Data analysis.

For all variables, normality assumptions were tested using Q-Q plots and the Shapiro-Wilk tests. Spearman correlations and linear models were used to examine relationships between protein balance and: (i) early-pregnancy macronutrient intake (combined carbohydrate and fat intake, g) as well as energy intake (kcal) across mid-pregnancy and mid/late-pregnancy (Aim 1), (ii) gestational weight gain (g/week), fat mass (kg and %), fat-free mass (kg and %), and FMI in the mother in early-, mid-, and late-pregnancy (Aim 2), and (iii) weight (g), head circumference (cm), fat mass (kg and %), fat-free mass (kg and %), and FMI in the infant at birth (Aim 3). Specifically, a linear regression model was performed for every outcome (independent variable) separately; protein was included in the models as the exposure variable. In the infant models, adjustments were made for fetal age, characterized as gestational age at delivery and infant age at measurement (anthropometric and body composition assessment). Log transformations were applied for non-parametric variables. Interactions by race (mother) and sex (infant) were explored. R 4.1.1.was used to perform analyses; $p<0.05$ was considered significant.

Results

Subject characteristics

Of 72 participants in the parent study, 41 women and 22 infants satisfied inclusion criteria for this ancillary study and had the required clinical data available at desired timepoints for analysis. Women were 27.5 ± 4.8 years of age, had a pregravid BMI of 34.4 ± 2.9 kg/m^2, and majority were non-Caucasian (n=23, 56.1%). Maternal protein intake in early-pregnancy was 0.88 ± 0.22 g/kg/d (range: 0.52 – 1.41 g/kg/d), and protein balance was 1.0 ± 0.2. Table 1 summarizes demographic and anthropometrics characteristics of women at enrollment and infants at birth.

Aim 1: Early-pregnancy protein, macronutrient, and energy intake

In early-pregnancy, absolute protein intake (g) did not correlate with intake of combined carbohydrate and fat (g) (R=0.025, p=0.88; Figure 1A). Protein balance was not significantly associated with maternal energy intake as measured by the intake-balance methods across mid-pregnancy (β=328.7, p=0.30; Figure 1B) or mid/late-pregnancy (β=286.2, p=0.26; Figure 1C). No significant interactions were observed between protein balance and race or obesity class in relation to energy intake at either timepoints (data not shown).

Aim 2: Maternal early-pregnancy protein ratio and maternal anthropometrics

Protein balance was not associated with gestational weight gain across pregnancy (β=117.0, p=0.41, Fig 2A). However, protein balance had a significant inverse association with fat mass (β=−10.6, p=0.01, Fig 2B) and FMI (β=−2.7, p=0.04) in early-pregnancy. Similarly, protein balance had a significant inverse association with fat mass (Fig 2 C and D: β=−10.4, p=0.03; β=−10.3, p=0.03) in mid- and late-pregnancy, but not with FMI (β=−2.65, p=0.11; β=−2.64, p=0.11), respectively.

Protein balance was not associated with percent fat-free mass (β=3.70, p=0.12; β=3.88, p=0.20; β=4.64, p=0.08) or absolute fat-free mass (β=−5.78, p=0.19; β=−5.46, p=0.24; β=−3.8, p=0.42) in early-, mid-, and late-pregnancy, respectively. No significant interactions were observed between protein balance in early-pregnancy and race or obesity class in relation to gestational weight gain, fat mass, fat-free mass, or FMI at all timepoints (data not shown).

Aim 3: Maternal early-pregnancy protein ratio and infant anthropometrics

Protein balance in early-pregnancy was not associated with weight, head circumference, fat mass (Figure 3A), percent fat mass, fat-free mass, percent fat-free mass (Figure 3B), or FMI at delivery (p>0.05). No interactions with race, sex, or maternal obesity class were observed (data not shown).

Discussion

The overarching aim of this study was to investigate whether there is evidence for the PLH in the intergenerational transmission of obesity; that is, excess maternal weight and adiposity gain in pregnancy and infant size at birth. Collectively, our state-of-the-art nutrition data for diet quality (i.e., remote food photography) and energy intake (i.e., intake-balance method) do not support a strong role for the PLH in maternal adiposity or size at birth for infants born to pregnant women with obesity. Yet, our data highlight that there are other physiological drivers of fat mass gain during pregnancy that may not be encompassed by measured dietary composition.

While we did not find strong evidence for the PLH in pregnancies that are affected by obesity, larger population-based studies should be conducted to arrive to conclusive recommendations, including studies targeting broader diversity in maternal weight status and source of protein. The effects of maternal protein intake on dietary and energy intake as well as body composition should be investigated based on source of protein (eg animal- vs plant-based) since these differ in their amino acid composition, digestibility, and absorption, as well as differences in quality (eg, ultraprocessed vs amimal- vs plant-based foods). Indeed, pregnant women are not exempt from the modern diet and may not be consuming enough or high-quality protein.

In this study, protein intake did not correlate with consumption of carbohydrates and fats. These results contrast findings from experimental studies in the general adult population, where lower proportion of energy from protein under ad libitum feeding conditions over the short-term increased consumption of calories from carbohydrates and fats, driving excess energy intake (5). Protein leverage was found to persist long-term in other experimental trials, whereby increased percent dietary protein resulted in a prolonged reduction in total energy intake. (20) It is possible that the observation period in pregnancy may not have been long enough or measurements not sensitive enough to observe changes in dietary and energy intake. In addition, the concept of the PLH may be confounded by the novelty and sensitive nature of pregnancy, whereby women may be more mindful of caloric intake during pregnancy, and thereby not alter their eating habits in a similar way to a non-pregnancy state. Indeed, despite no relationship with energy intake, women with obesity in our study with lower protein balance did have increased fat mass across all pregnancy time points, including early in pregnancy. It is plausible that those women who consumed lower protein in early-pregnancy also did so prior to pregnancy. Therefore, the relationship between low protein intake and increased fat mass early in pregnancy suggests that women's energy intake may have been increased pre-pregnancy in alignment with the PLH.

Low protein intake in women did not translate to an altered body composition in infants. To our knowledge, this is the first study to test the PLH in pregnancy, and as such there are no other studies to compare or contrast. The existing studies examining protein intake in pregnancy and infant anthropometrics including birth weight and growth are conflicting (11, 12, 21, 22). For example, while one study found no relationship between maternal protein intake in mid-pregnancy and infant birth weight (21), another found that an increase of 10 g of absolute protein intake/day was associated with a reduction in birth weight of 17.8 g (95% CI: −32.7, −3.0; P=0.02) (22). In contrast, another found that high protein intake in early-pregnancy was positively associated with weight at birth, followed by slower growth rates into childhood (11). In the context of weight, the effects of protein restriction seem to be more prominent in reducing the risk of infants born small for gestational age (23, 24). More dominant physiological drivers of changes in infant energy deposition, such as pre-gravid obesity and gestational weight gain, may therefore exist.

One potential driver of increased energy intake in response to protein restriction is fibroblast growth factor (FGF) 21. FGF21 is a predominantly liver-derived hormone that has garnered interest for its potential use as an anti-obesity therapy (25). Circulating FGF21 levels have been positively correlated with body mass index and insulin resistance (26). FGF21 levels are elevated in states of metabolic stress in both rodents and humans. For example, FGF21 represents an endocrine signal of protein restriction, and is activated during periods of reduced protein intake. In addition, FGF21 levels have been shown to increase from the first to the third trimester in pregnancy (27). Within normal physiological adaptations in pregnancy, especially as the pregnancy advances to the third trimester, insulin sensitivity decreases by 50–60% due to an increase in beta cell mass through proliferation and hypertrophy of the beta cells, consistent with increases in FGF21 levels (28, 29). However, FGF21 did not previously appear to sense changes in maternal energy stores, yet was positively correlated with maternal body mass index and fat mass throughout pregnancy in women with overweight and obesity (27). In our study, it is plausible that increased FGF21 in the presence of lower protein relative to requirements contributed to changes in body composition irrespective of energy conservation mechanisms/energy intake.

Our study is strengthened by several factors. First, it represents a diverse cohort of pregnant women with obesity, who are prone to overeating and excess weight gain. Second, we used validated objective measures to assess dietary intake (RFPM), energy intake (DLW), and body composition (BOD POD and PEA POD) (30). It is important to note that validation studies of the RFPM during pregnancy showed lower reporting of energy intake in pregnant women with obesity, potentially due to underreporting of snacks (31). While 34% of women with obesity in our study met (or exceeded) average protein requirements, emerging nitrogen balance studies suggest that protein intake requirements are rather approximately 1.2 g/kg of body weight/day during early gestation and 1.52 g/kg of body weight/day during late gestation (32). Less than 20% of women in our study consumed 1.2 g/kg of protein during early gestation, which precludes sensitivity analyses by these cut-offs. Finally, previous studies (5) have examined protein as a percentage of total energy intake as the exposure. However, these studies were experimental in nature, with a constant energy intake provided at a given point of time through researcher-provided foods. Given that energy and protein requirements change (i.e., mostly increase) across pregnancy, we have characterized protein as actual protein intake relative to the expected protein needs. This approach ensures the analysis is relevant in the context of pregnancy, which offers a robust methodological approach to our central question.

Population-wide increases in maternal obesity, excess gestational weight gain, macrosomia, and infants born large for gestational age are established factors contributing to the intergenerational transmission of obesity. Novel, emerging risk factors for obesity conditioning, such as the role of maternal diet, are increasingly being identified. Until strong scientific recommendations can be made from rigorous studies, a balanced diet fulfilling nutrition requirements for macro- and micro-nutrients during pregnancy is ideal to foster health gestational weight gain and to support healthy growth.

Acknowledgements:

The authors would like to acknowledge all participants in the MomEE study for their time and invaluable contributions. Deidentified data can be made available upon request.

Funding:

This research was funded by the National Institutes of Health (R01DK099175), the Louisiana/Pennington Nutrition Obesity Research Center (P30DK072476), and the Clinical Research Cores at Pennington Biomedical Research Center (U54G M104940). MK was funded by the Canadian Institutes of Health Research Postdoctoral Fellowship (RN439810; Application No. 459061).

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What is known already known about this subject?

- The Protein Leverage Hypothesis (PLH) posits that deficits in dietary protein drive excess energy intake to meet protein demands, promoting the development of obesity.
- In pregnancy, protein demand increases. If protein intake does not increase, the PLH would suggest that women and their infants would have comparatively greater adiposity.

What are the new findings in your manuscript?

- Pregnant women with obesity were meeting Estimated Average Requirements for protein (0.88 ± 0.22 g/kg/d), however intake was variable across women (range: 0.52 – 1.41 g/kg/d).
- Pregnant women with obesity whose protein intake was less than recommendations in early pregnancy did not consume more energy, but had an increased fat mass throughout pregnancy.

How might your results change the direction of research or the focus of clinical practice?

- Our findings emphasize the importance of conducting larger population-based studies to arrive to conclusive recommendations, including studies targeting broader diversity in maternal weight status and source of protein.

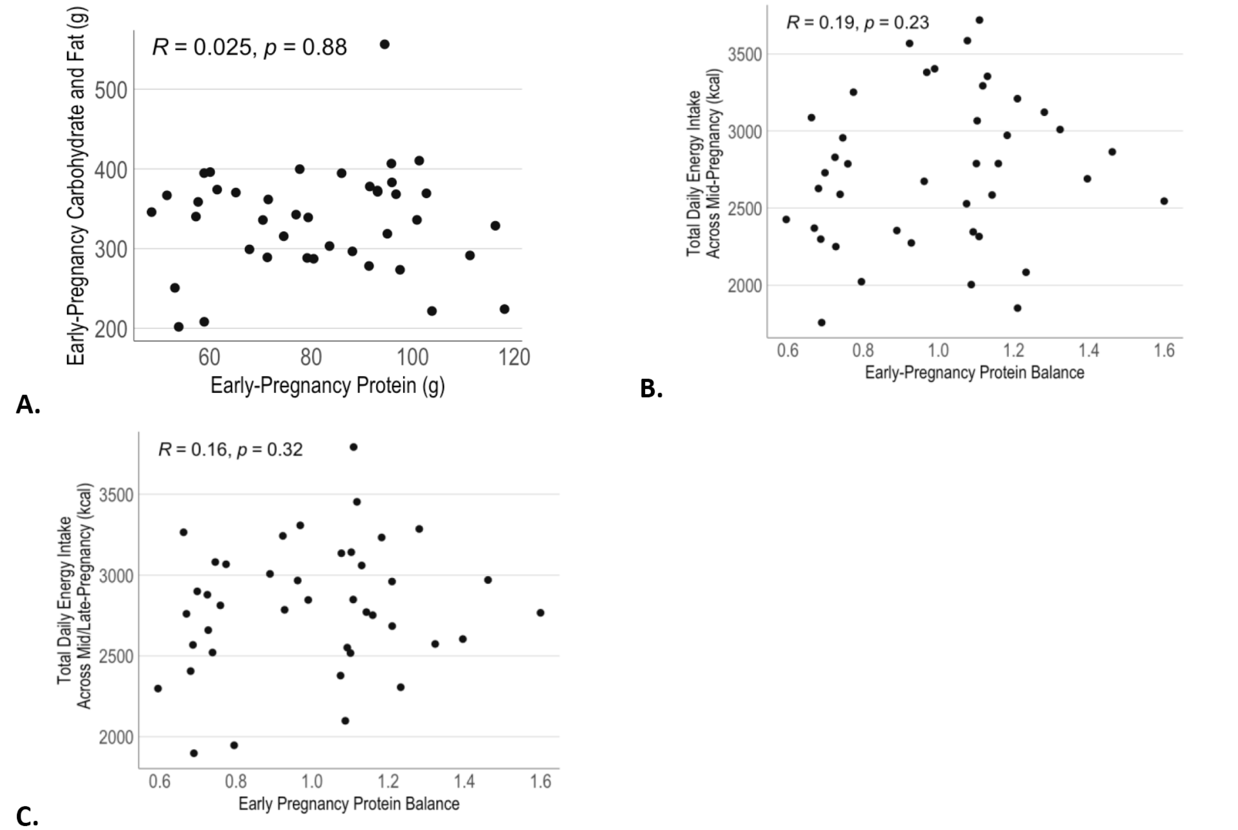

Figure 1.

Maternal dietary intake was measured by remote food photography over 7 days and energy intake by the intake-balance method. **A.** Low maternal protein intake (g) in early-pregnancy did not correlate with carbohydrate and fat (g) overcompensation in early-pregnancy, **B.** Protein balance in early-pregnancy did not correlate with energy intake (kcal) across mid-pregnancy, **C.** Protein balance in early-pregnancy did not correlate with energy intake (kcal) across mid/late-pregnancy.

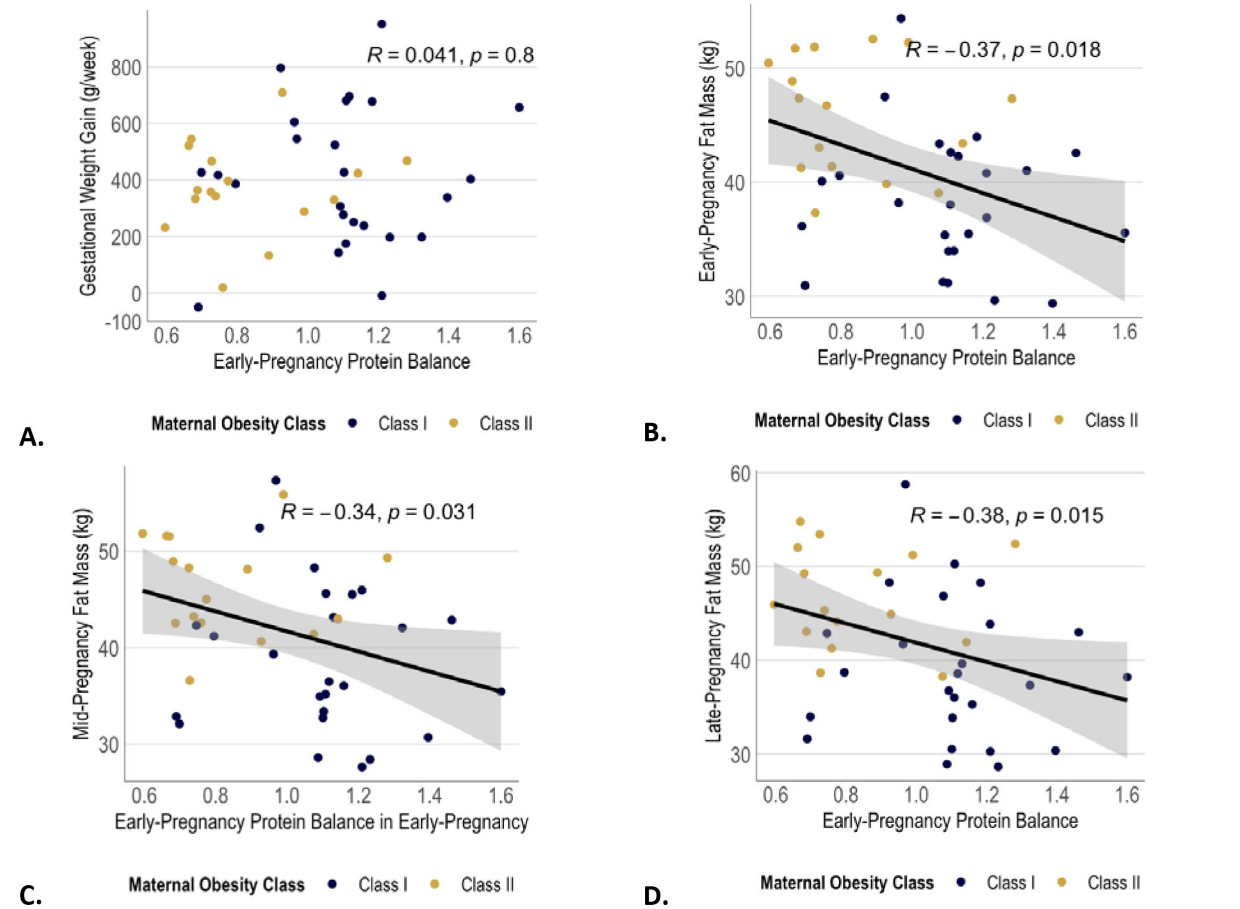

Figure 2.

A. Protein balance in early-pregnancy was not associated with gestational weight gain (g/week), **B.** Protein balance in early-pregnancy was inversely associated with fat mass (kg) in early-pregnancy (13–16 weeks), **C.** Protein balance in early-pregnancy was inversely associated with fat mass (kg) in mid-pregnancy (24–27 weeks), **D.** Protein balance in early-pregnancy was inversely associated with fat mass (kg) in late-pregnancy (35–37 weeks).

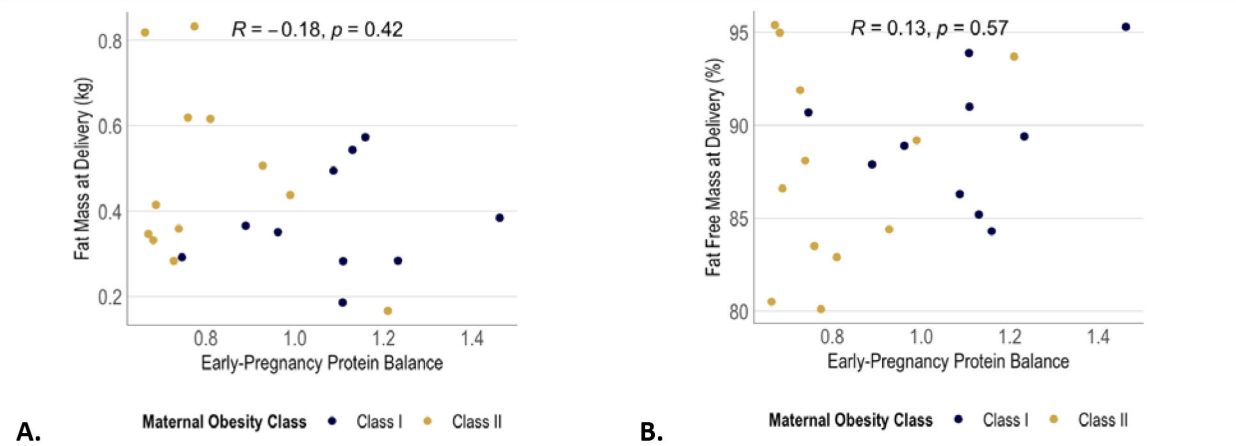

Figure 3.

A. Protein balance in early-pregnancy was not significantly associated with infant fat mass at delivery, **B.** Protein balance in early-pregnancy was not significantly associated with infant percent fat-free mass at delivery.

Table 1.

Demographic and anthropometrics characteristics of participants

	Women (n=41)
Age, years	27.5 ± 4.8
Race, n (%)	
Caucasian	18 (43.9)
Non-Caucasian	23 (56.1)
Black or African American	19 (46.3)
White	18 (43.9)
Other	4 (9.8)
Body Mass Index (BMI; kg/m^2)	34.4 ± 2.9
Protein balance $^{\phi}$	1.0 ± 0.2
Protein intake, n (%)	
Below EAR of 0.88 g/kg/d	27 (65.9)
Above or equal to EAR of 0.88 g/kg/d	14 (34.1)
Gestational Age at Delivery, days	39.3 ± 0.99
	Infants (n=22)
Age, days	7.1 ± 1.2
Sex (n, %)	
Female	7 (31.8)
Male	5 (68.2)
Weight, kg	3.4 ± 0.4
Head circumference, cm	35.1 ± 1.5

MeanSD unless otherwise noted.

$^{\phi}$Daily protein intake in early-pregnancy was expressed as a ratio based on the Estimated Average Requirements in pregnancy for total protein (0.88g/kg/d) for each participant. This ratio is hereby referred to as ‘protein balance’.