Maternal protein intake in pregnancy and offspring metabolic health at age 9–16 y: results from a Danish cohort of gestational diabetes mellitus pregnancies and controls

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ABSTRACT

Background: Recent years have seen strong tendencies toward high-protein diets. However, the implications of higher protein intake, especially during developmentally sensitive periods, are poorly understood. Conversely, evidence on the long-term developmental consequences of low protein intake in free-living populations remains limited.

Objective: We examined the association of protein intake in pregnancy with offspring metabolic health at age 9–16 y in a longitudinal cohort that oversampled pregnancies with gestational diabetes mellitus (GDM).

Design: Six hundred eight women with an index pregnancy affected by gestational diabetes mellitus and 626 controls enrolled in the Danish National Birth Cohort were used for the analysis. Protein (total, animal, vegetable) intake was assessed by using a food-frequency questionnaire in gestational week 25. The offspring underwent a clinical examination including fasting blood samples and a dual-energy X-ray absorptiometry scan (subset of 650) from which metabolic outcomes were derived. Multivariable analyses were conducted applying a 1:1 substitution of carbohydrates for protein.

Results: The mean ± SD protein intake in pregnancy was 93 ± 15 g/d (16% ± 3% of energy) in GDM-exposed women and 90 ± 14 g/d (16% ± 2% of energy) in control women. There were overall no associations between maternal protein intake and offspring fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). We found that maternal total protein intake was associated with a tendency for a higher abdominal fat mass percentage (quartile 4 compared with quartile 1: 0.40 SD; 95% CI: −0.03, 0.83 SD; $P = 0.07$) in GDM-exposed offspring and a tendency for a higher total fat mass percentage among male offspring (quartile 4 compared with quartile 1: 0.33 SD; 95% CI: −0.01, 0.66 SD; $P = 0.06$), but a small sample size may have compromised the precision of the effect estimates. GDM-exposed offspring of mothers with a protein intake in the lowest decile (≤12.5% of energy compared with >12.5% of energy) had lower fasting insulin (ratio of geometric means: 0.82; 95% CI: 0.68, 0.99; $P = 0.04$) and a tendency toward lower HOMA-IR (ratio of geometric means: 0.82; 95% CI: 0.66, 1.02; $P = 0.07$), but there was no evidence of associations with body composition. Male offspring seemed to derive a similar benefit from a maternal low protein intake as did GDM-exposed offspring.

Conclusions: Overall, our results provide little support for an association of maternal protein intake in pregnancy with measures of offspring metabolic health. Further studies in larger cohorts are needed to determine whether low maternal protein intake in pregnancy may improve glucose homeostasis in GDM-exposed and male offspring. *Am J Clin Nutr* 2017;106:623–36.

Keywords: pregnancy, gestational diabetes mellitus, protein, insulin, body composition

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Supplemental Tables 1–5 and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: AF%, abdominal fat mass percentage; DNBC, Danish National Birth Cohort; FFQ, food-frequency questionnaire; GDM, gestational diabetes mellitus; GW, gestational week; LM%, lean mass percentage; MetS, metabolic syndrome; PA, physical activity; TF%, total fat mass percentage; T2D, type 2 diabetes mellitus.

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INTRODUCTION

There is now irrefutable evidence that perturbations acting on the developing fetus in pregnancy can have important implications for later chronic disease development, including type 2 diabetes mellitus (T2D) and related metabolic and cardiovascular outcomes (1, 2). Dietary risk factors for these outcomes are well-described in adult populations (3–7), but less is understood about which dietary exposures in pregnancy influence metabolic outcomes in the offspring. Studies in animal models have demonstrated the relevance of maternal protein intake during gestation and lactation. Much of the original work was done examining the effect of maternal low-protein (8% of energy), isocaloric diets on offspring glucose homeostasis and body composition; dietary calories were typically maintained by increasing carbohydrate content. These studies showed that, despite a lower birth weight, low-protein–exposed animals become more obese and glucose intolerant as they aged compared with offspring of control dams fed a standard chow diet (8–11). In the few animal studies studying high-protein (30–53% of energy) diets, prenatal protein exposure has been found to increase fat mass (12–15) and levels of signaling molecules, such as resistin, that are associated with reduced insulin sensitivity (16). These studies were completed in a variety of species (rats, cats), across different dietary protein content, and in different time periods (gestation, lactation, weaning) and may not be completely applicable to the human setting because of differences in early development and physiologic adaptation to a certain macronutrient composition. Results from observational studies in humans have been more conflicting and have reported direct (17), inverse (18), and null (19–21) associations of maternal protein intake with offspring weight and adiposity. Furthermore, studies with long-term follow-up are sparse. We were aware of only one study linking maternal diet to glucose metabolism in 40-y-old offspring and found an inverse association for maternal protein intake in pregnancy with plasma insulin increment between fasting and 30 min (22). However, this was a small study based on a subsample of 168 participants from the original cohort (n = 549), and the study lacked detailed information on maternal and offspring lifestyle confounders. With the rising interest in high-protein diets, examining a high protein intake alongside low protein exposure is becoming increasingly relevant.

We recently showed that maternal protein intake, especially from animal sources, was associated with higher risk of overweight in 19- to 21-y-old offspring in a population-based Danish cohort (17). However, in those data we were not able to determine whether this association was driven by lean or fat mass because the study lacked body-composition data. In the present study, we examined both maternal high- and low-protein intake in midpregnancy in relation to clinical markers of glucose homeostasis and body composition in the offspring at 9–16 y using a nested cohort of the DNBC (Danish National Birth Cohort) that oversampled gestational diabetes mellitus (GDM) pregnancies. We hypothesized a relation between higher maternal protein intake, in particular from animal sources, and increased offspring insulin resistance and adiposity. Poorer metabolic outcomes were expected among the GDM-exposed compared with control offspring, possibly acting via infant macrosomia (23). We furthermore hypothesized that higher levels of insulin resistance and adiposity would be present in the control offspring of mothers consuming low-protein diets. Given the lack of dietary studies focused on offspring metabolic health in women with GDM, we could not a priori infer the influence of low protein in these offspring.

METHODS

Study population

The study population was nested within the DNBC, which enrolled 101,045 pregnancies between 1996 and 2002 and has been described in detail elsewhere (24). In brief, DNBC participants completed 4 telephone interviews in gestational weeks (GWs) 15 and 30, and then completed 6- and 18-mo postpartum interviews. The offspring of the cohort have been followed up with questionnaires several times in childhood (at 7 and 11 y; the 14-y follow-up is ongoing). The participant flow for the study population is shown in **Figure 1**. We identified 1350 DNBC pregnancies with a suspected or confirmed GDM diagnosis and 2629 randomly selected DNBC control pregnancies. Information about potential GDM pregnancies was extracted from the GW 30 and 6-mo interviews, and the Danish National Patient Register (International Classification of Diseases–10 codes O244 and O249) (25). We retrieved medical records for these women, which were examined by 3 clinicians. Clinician-verified GDM diagnoses were based on either on WHO published cutoffs for a 2- or 3-h oral-glucose-tolerance test (**Supplemental Table 1**) (26, 27). Recruiting offspring born from GDM and non-GDM was part of a feasibility study of offspring born from women enrolled in the Diabetes and Women's Health study (25). Because our cohort included an overrepresentation of GDM offspring, who are generally at a higher risk of overweight and T2D, this cohort best served the purpose of reproducing prior findings and extending them to include physiologically relevant outcomes.

In 2012–2014, the identified GDM-exposed and control mothers and offspring were invited by mail, e-mail, or phone to participate in a clinical follow-up examination. The study was set up to examine developmental programing of dysmetabolic traits and risk of metabolic disease among offspring of GDM versus control mothers, and the first paper describing the initial clinical and metabolic comparisons between these 2 groups is currently under review for publication in another journal (LG Grunnet, S Hansen, L Hjort, CM Madsen, FB Kampmann, AC Baun Thuesen, C Granstrom, M Strøm, E Maslova, R Frikke-Schmidt, P Damm, J Chavarro, FB Hu, SF Olsen, A Vaag, unpublished results, 2017). Participation rates were 47% (n = 608) for GDM mother-offspring dyads and 43% (n = 626) for controls. The main known reason for nonparticipation was lack of time. Two GDM cases that were not verified by the clinicians were recoded as controls, yielding 606 GDM mother-offspring dyads and 628 control dyads. Of the 1234 participants who underwent a clinical examination, we excluded 33 twins (31 GDM) and 3 triplets (all GDM) to avoid strongly correlated measures and potentially different risk profiles of multiple gestations; 80 siblings were kept in the analysis to preserve power. Of the remaining 1198 participants, 923 also had information on diet. The final sample size varied depending on missing outcome data with 770–783 mother-offspring pairs available for the HOMA-IR and insulin analysis and 506 mother-offspring pairs for the body-composition analysis.

Mothers in the DNBC provided written, informed consent for themselves and on behalf of their children. The Regional Scientific Ethics Committee for the municipalities of Copenhagen and Frederiksberg approved all study protocols, and all procedures were in accordance with the Declaration of Helsinki. The clinical follow-up

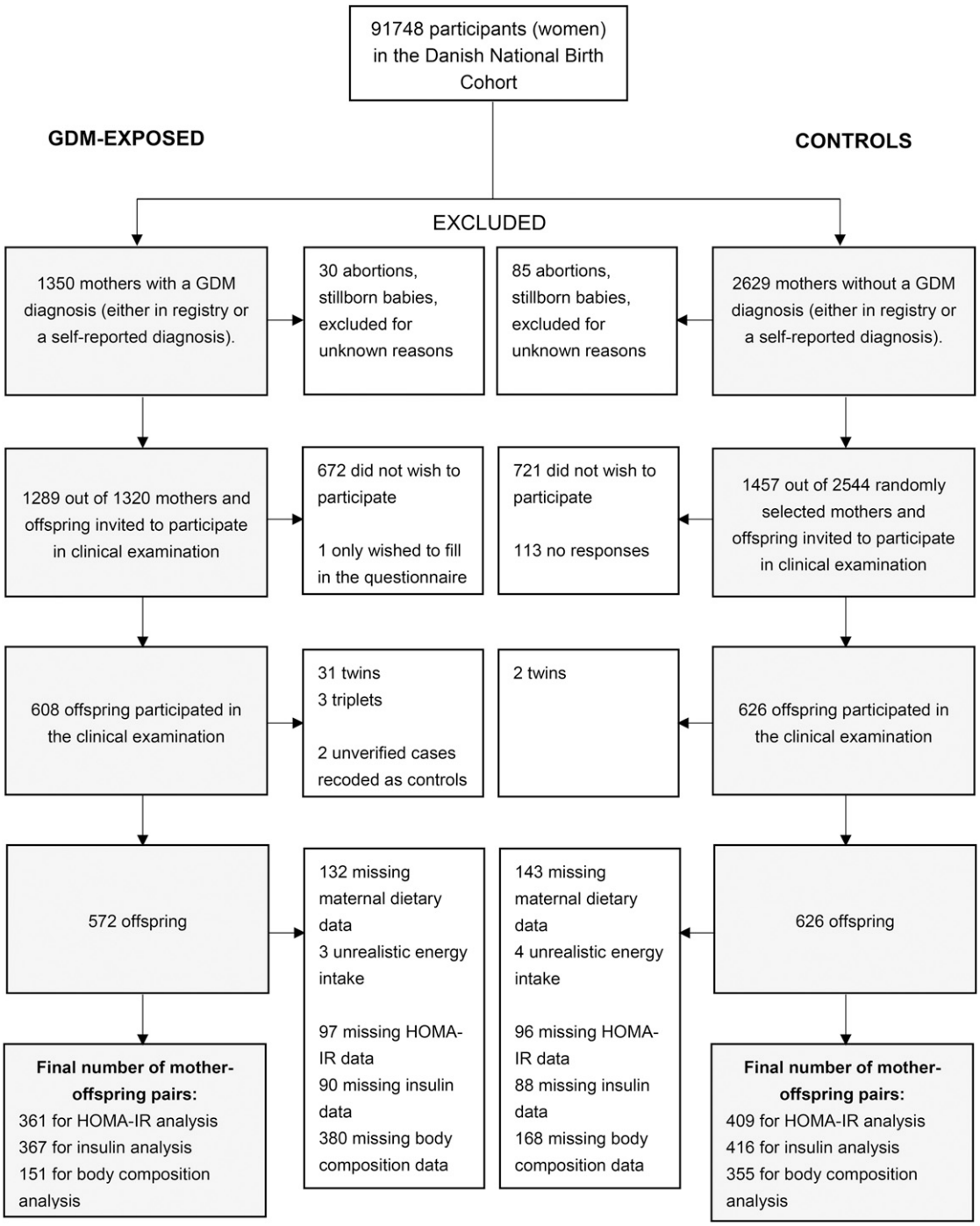

FIGURE 1 Flowchart of enrollment and examination of women and offspring in the Diabetes and Women's Health Study. Each woman was defined as either a case or a control in all her pregnancies, thus redefining some control pregnancies to case pregnancies. A woman who participated with >1 pregnancy was defined as a case if she had ≥1 case pregnancy, regardless if the case pregnancy was the first one or not. There were ∼50 women with pregnancies initially defined as both cases and controls that were redefined as cases. Women who only had pregnancies that ended with abortions or stillborn babies were excluded. Some women with live-born children were also excluded for an unknown reason. Later in this stage, 470 extra control women were included. GDM, gestational diabetes mellitus.

study followed the same procedures with regard to consent and approval of study protocols (H-4–2011-045 and H-4-2013-129).

Assessment of protein intake

A 360-item semiquantitative food-frequency questionnaire (FFQ) covering the women's diet in the previous 4 wk was mailed to the DNBC participants in GW 25 (28). By using assumptions of standard portion sizes, intakes of different food items in grams per day were calculated, and nutrient intake was quantified by using the National Food Institute's Food Composition Databank version 6.02 (www.foodcomp.dk). All nutrients were energy-adjusted by using the residual method (29). Pregnant women who reported intakes resulting in unrealistic energy

intake estimates (arbitrarily set to <2500 or >25,000 kJ/d) were removed. The FFQ has been validated against 7-d weighed food diaries and biomarkers of selected nutrients (n = 88) with a moderate correlation (Spearman ρ = 0.44) found between protein intake from food diaries and the FFQ (30).

To allow for differences in "packaging" of protein alongside other nutrients, additives, and environmental chemicals, we furthermore divided protein intake by source of intake (animal, vegetable). Animal protein came from dairy, eggs, meat, and fish products, and vegetable protein from cereal, vegetable, and fruit products and vegetable oils.

Outcome assessment

The clinical examination included offspring metabolic, anthropometric, and body-composition measurements. A fasting blood sample was obtained during the examination and plasma, serum, and buffy coat stored. We used sandwich electrochemiluminescence immunoassay to determine plasma insulin levels (Roche Diagnostics). HOMA-IR was calculated as ([(fasting plasma insulin × fasting plasma glucose)/22.5] × 0.144) (31). The weight and height of the offspring were measured 2 or 3 times if the difference between measurements differed by >0.5 kg or >0.5 cm. BMI was calculated as kg/m^2 by using the average of repeated measures. Overweight was defined by using the International Obesity Task Force age- and sex-specific cutoff points (32). Body-composition measurements were evaluated in a subset of the offspring with a dual-energy X-ray absorptiometry scan (Lunar Prodigy). For the purpose of this analysis, we used lean mass percentage (LM%), total fat mass percentage (TF%), and abdominal fat mass percentage (AF%), which were standardized separately for the GDM-exposed and control offspring to z scores (mean ± SD: 0 ± 1) to allow for more direct comparisons with other studies. A composite metabolic syndrome (MetS) score was also assessed and is described in the **Supplemental Methods**.

Covariate assessment

We chose our model covariates a priori based on described biological and social pathways linking the covariates as predictors of offspring metabolic health or as correlates of maternal diet (33–37). Parental covariates included maternal age, sociodemographic position (based on parental occupation and education: high-level position, medium-level position, skilled, unskilled, or unemployed, or student), parity (nulliparous, 1 prior child, or ≥2 prior children), maternal prepregnancy BMI (≤18.5, 18.6–24.9, 25.0–29.9, 30.0–34.9, or ≥35), maternal smoking (nonsmoker, occasional smoker, or smoker), partner smoking in pregnancy (nonsmoker or smoker), and maternal physical activity (PA; none, 0–120 min/wk, or >120 min/wk). Maternal PA has been described in detail elsewhere (38). In brief, we defined PA as minutes per week of any physical activity, regardless of intensity. We also adjusted for offspring sex, age, and puberty status (Tanner stage ≥2 for breast development for female offspring and testes size ≥4 mL for male offspring in a subsample of the children, n = 896). Breastfeeding and offspring macronutrient intake, dietary quality (Healthy Eating Index based on Danish nutritional recommendations), and physical activity at the 14-y follow-up were considered in a secondary analysis. This analysis examined the possibility of pregnancy protein intake acting as a marker of postnatal diet and lifestyle but were excluded from the initial analyses because they could be construed as intermediate variables on the causal pathway.

Missing data for the main model covariates varied between 0.5% and 4.59%. A missing indicator was used to account for missing data with missingness >1%.

Statistical analysis

Analyses were performed separately for the GDM-exposed and control groups because of potential confounding by GDM exposure and to assess potential effect modification by maternal hyperglycemia. Offspring metabolic differences between the 2 groups have been demonstrated in a prior paper (LG Grunnet, et al., personal communication, 2017), and stratified results may have both biological and clinical relevance. P-interaction values are reported by using the cross product of the group and protein exposure.

We calculated means ± SDs and frequencies (as percentages) of maternal macronutrient intakes and sociodemographic covariates for suspected and verified GDM-exposed women and for controls. Statistically significant differences between GDM-exposed and controls were determined by using F test or chi-square test for continuous and categorical variables, respectively. All P values accounted for correlations among siblings by using linear mixed regression models with a compound symmetry covariance structure specified in the REPEATED statement in PROC MIXED.

We used substitution models to examine the relation between maternal protein consumption and offspring metabolic health. This was accomplished by using an isocaloric model that included all energy-contributing nutrients. Carbohydrate intake was allowed to vary by excluding it from the model. This made it possible to interpret the effect estimates as a 1:1 substitution of carbohydrates for protein. Fat substitution was not examined because of potential overlap between fat and protein in many of the same foods, e.g., meat and dairy, making the interpretation of effect estimates difficult. A substitution model was chosen because free-living, weight-stable individuals tend to substitute calories from different food sources rather than increase their caloric intake, and these results may therefore be more pertinent when providing recommendations. Although pregnant women are generally advised to increase their caloric intake starting with the second trimester (39), studies have not found differences in caloric intake across trimesters and postpartum (40, 41). We examined total protein intake and 2 major types of protein by food sources (i.e., animal and vegetable protein) as the main exposures to evaluate both protein quantity and quality. Total protein intake is also more translatable to nutritional recommendations. To capture any potential nonlinear associations, maternal protein intake was additionally evaluated in quartiles. Low protein intake was arbitrarily defined as the lowest 10th percentile of the percentage of energy intake (≤12.5% of energy) because this allowed us to examine more extreme intake without compromising too much power. "Low" protein was used as a relative term and was not analogously to similar terminology used in animal studies. Markers of glucose homeostasis and body composition were considered the primary outcomes. In secondary analyses, we also evaluated the associations of major

protein-contributing food sources: maternal meat (red meat and processed meat, white meat) and dairy intake with offspring outcomes.

We used multivariable linear mixed regression models with a compound symmetry covariance structure to estimate β coefficients and 95% CIs for continuous untransformed outcomes. Ratios of geometric means (95% CIs) were calculated for log-transformed outcomes. For binary outcomes, we used generalized linear mixed regression models with a log Poisson distribution to calculate RRs and 95% CIs. Because of the limited sample size, we first adjusted for parental sociodemographic position, maternal age, parity, maternal BMI, maternal smoking, and offspring age and sex (model A and main model). In a second model, we also adjusted for maternal physical activity, partner smoking, and offspring puberty status as additional markers of parental lifestyle and offspring maturity (model B). To avoid overadjustment and potential collider-stratification bias (42), suspected mediators (gestational weight gain, birth weight) were left out of the models; Spearman correlations with the exposure and outcomes were examined for potential insight into mediation pathways. Finally, we also examined potential effect modification by offspring sex as an additional variable of metabolic susceptibility to intrauterine protein exposure because there are demonstrable sex differences in, e.g., growth pathways and vulnerability to diet that may be mediated by placental responses (11, 43, 44). *P*-interaction values from the offspring sex and exposure cross product are reported.

All tests were 2-sided, and we used a threshold of $P < 0.05$ to denote statistical significance. The analyses were performed by using the Statistical Analyses System software (release 9.4; SAS Institute).

RESULTS

Study characteristics

The total protein intake in pregnancy was 93 ± 15 g/d (16% ± 3% of energy) in GDM-exposed women and 90 ±14 g/d (16% ± 2% of energy) in control women. This was comparable to the national average for women 18–75 y old (15% of energy) (45). **Table 1** shows the differences between GDM-exposed and control mother-offspring dyads in macronutrient intake and sociodemographic characteristics. Protein, primarily from animal sources, and fat intakes were significantly higher in women with GDM, although this translated into modest differences of 3 g/d. Carbohydrate intake was, on average, 6 g/d lower in GDM-exposed women compared with controls. In general, women with GDM were multiparous, were of lower sociodemographic position, were overweight or obese, gained less gestational weight, exercised less, and smoked more in pregnancy compared with control women. There was no difference in offspring birth weight or proportion of male offspring in the GDM-exposed compared with the control group. Women with a clinician-verified GDM diagnosis (n = 342) had covariate distribution similar to the GDM-exposed group with the exception of a higher proportion of obese women (33% compared with 26%) in the former group.

The medians (IQRs) for the outcomes among the GDM-exposed offspring were 2.2 (1.5–3.1), 69.9 pmol/L (47.9–94.6 pmol/L), 29.8% (24.2–35.5%), and 66.3% (61.0–71.9%) for HOMA-IR, fasting insulin, TF%, and LM%, respectively. The corresponding levels for the control offspring were 1.9 (1.4–2.5), 61.1 pmol/L (46.5–80.3 pmol/L), 25.4% (21.1–30.7%), and 70.5% (65.6–74.6%). The metabolic differences between the 2 groups are evaluated in detail in another paper (LG Grunnet et al., personal communication, 2017).

Compared with GDM-exposed women who did not have dietary data (n = 132), GDM-exposed women who provided dietary data (n = 440) were more likely to be nulliparous (38% compared with 31%), to be of high-medium sociodemographic position (51% compared with 41%), to exercise (>0 min/wk: 31% compared with 20%), and to have a BMI in the range 18.6–24.9 (40% compared with 30%). There were no differences for maternal age; parental smoking; gestational weight gain; and offspring birth weight, fasting insulin, HOMA-IR, and body composition. We did not find any differences for control women with (n = 483) compared with those without dietary data (n = 143).

Maternal protein intake and offspring glucose homeostasis

None of the interaction terms reached statistical significance (**Tables 2** and **3**). We found no evidence of an association of maternal total (Tables 2 and 3), animal, or vegetable protein intake with offspring fasting plasma insulin or HOMA-IR in either the GDM-exposed or control group (data not shown). Using a clinically verified GDM diagnosis in a subsample of women with medical records available (n = 219–224) did not change the results and neither did adjustment for offspring TF%. Results were not different for male compared with female offspring (**Figure 2**A).

Protein intake was not correlated with either gestational weight gain or birth weight in either group (Spearman ρ: −0.08 to 0.05); gestational weight gain and birth weight were also not associated with markers of insulin resistance (Spearman ρ: −0.16 to −0.04).

Maternal protein intake and offspring body composition

None of the interaction terms reached statistical significance (Tables 2 and 3). There was no statistically significant association of maternal total protein intake with offspring LM%, although results were stronger among the controls (quartile 4 compared with quartile 1: −0.23 SD; 95% CI: −0.51, 0.04 SD), and the unadjusted results in this group reached statistical significance (Tables 2 and 3). Maternal prepregnancy BMI accounted for majority of the attenuation in the effect estimate, reducing it by 19% compared with 0–13% for the other covariates. The results for animal protein mirrored those of total protein in the control group only, and there were no associations for vegetable protein in either group (data not shown). In male offspring, there was a trend toward significant inverse association of maternal protein intake with LM% (quartile 4 compared with quartile 1: −0.32 SD; 95% CI: −0.65, 0.02 SD) that was not seen for female offspring (Figure 2B).

We could not confidently infer an association between maternal total protein intake and either TF% or AF% in the GDM-exposed or control mother-offspring dyads (Tables 2 and 3). The results were strongest, although the CIs overlapped unity, for AF% among GDM-exposed pairs (quartile 4 compared with quartile 1: 0.40 SD; 95% CI: −0.03, 0.83 SD). We found no evidence for an

TABLE 1
Parental characteristics across GDM-exposed and control mother-offspring dyads[1]

	GDM-exposed mother-offspring dyads (n = 572)	Control mother-offspring dyads (n = 626)	P	Verified GDM cases[2] (n = 342)
Maternal midpregnancy dietary intake				
Energy, kcal/d	2340 ± 637[3]	2338 ± 549	0.99	2337 ± 646
Protein, g/d	93 ± 15	90 ± 14	0.002	93 ± 15
Animal protein	61 ± 16	59 ± 15	0.02	61 ± 15
Vegetable protein	29 ± 6	29 ± 6	0.09	30 ± 6
Carbohydrate, g/d	315 ± 37	321 ± 34	0.004	316 ± 36
Fat, g/d	81 ± 17	79 ± 15	0.02	81 ± 17
Red and processed meat, g/d	89 ± 39	77 ± 36	<0.0001	89 ± 37
White meat, g/d	25 ± 18	26 ± 19	0.19	25 ± 18
Dairy, g/d	654 ± 469	660 ± 434	0.90	630 ± 453
Parental characteristics				
Maternal age, y	32.1 ± 4.4	31.1 ± 4.2	0.17	32.6 ± 4.5
Nulliparous	36[4]	50	<0.0001	35
Sociodemographic position			<0.0001	
High- or medium-level position	48	62		50
Skilled	27	23		27
Unskilled	15	8		13
Student/unemployed	4	5		4
Maternal prepregnancy BMI, kg/m^2			<0.0001	
≤18.5	1	7		1
18.6–24.9	38	72		31
25–29.9	28	13		27
30–34.9	16	5		21
≥35	10	1		12
Gestational weight gain, kg/wk	0.38 ± 0.35	0.47 ± 0.20	<0.0001	0.32 ± 0.39
Maternal physical activity, min/wk			<0.0001	
0	66	59		66
>0 to <120	18	21		18
≥120	11	18		10
Maternal smoking in pregnancy			0.02	
Nonsmoker	71	77		69
Partner smoking			0.04	
Nonsmoker	67	69		66
Offspring characteristics				
Birth weight	3757 ± 629	3580 ± 517	0.12	3696 ± 653
Male offspring	52	51	0.53	51

[1] Statistically significant differences between the GDM-exposed and control groups were determined by using F test or chi-square test for continuous and categorical variables, respectively. All P values were corrected for correlations among siblings. GDM, gestational diabetes mellitus.
[2] Based on hospital journal assessments by the medical specialists (midwife or medical doctor).
[3] Mean ± SD (all such values).
[4] Percentage (all such values).

association with either adiposity outcome when we split protein into animal and vegetable protein (data not shown). There was a potential direct relation with TF% (quartile 4 compared with quartile 1: 0.33 SD; 95% CI: −0.01, 0.66 SD) among male offspring (Figure 2C).

Using clinically verified GDM cases (n = 98) did not change any of the associations but widened the CIs. Our findings did not support an association with overweight offspring (**Supplemental Table 2**). Gestational weight gain and birth weight were only weakly associated with measures of adiposity in the GDM-exposed (Spearman ρ: −0.10 to −0.23) and control (Spearman ρ: −0.04 to −0.17) groups.

Maternal protein intake and offspring MetS score

The individual MetS components tracked well with an increasing MetS score (**Supplemental Table 3**). GDM-exposed or control offspring profiles were similar within the lowest 2 quartiles of the MetS score, but the GDM-exposed offspring displayed a worse metabolic profile starting with the third quartile. In the highest quartiles, fasting glucose and insulin, BMI, and waist circumference were higher in the GDM-exposed, whereas mean values for systolic blood pressure, HDL, and triglycerides were the same for both groups. We found no significant association of maternal total protein intake with the MetS score stratified on GDM status (**Supplemental Table 4**) or sex (data not shown).

Low protein intake (≤12.5% of energy compared with >12.5% of energy)

The protein percentage of energy was 11% ± 1% in the low-protein group (n = 41) and 17% ± 2% in the remaining sample (n =

TABLE 2
The association of maternal total protein intake in midpregnancy with offspring fasting insulin, HOMA-IR, and body composition at age 9–16 y in the GDM-exposed mother-offspring dyads[1]

	Unadjusted effect estimates	Adjusted effect estimates (model A)[2]	Adjusted effect estimates (model B)[3]
Glucose homeostasis (n = 361–367)			
Fasting plasma insulin,[4] pmol/L			
Per 10 g protein/d[5]	1.02 (0.98, 1.06)	1.02 (0.98, 1.06)	1.02 (0.98, 1.07)
Total protein Q1[6]	1 (Ref)	1 (Ref)	1 (Ref)
Total protein Q2	1.08 (0.94, 1.26)	1.06 (0.91, 1.22)	1.04 (0.88, 1.23)
Total protein Q3	0.96 (0.83, 1.12)	0.94 (0.82, 1.09)	0.94 (0.79, 1.12)
Total protein Q4	1.06 (0.90, 1.23)	1.04 (0.89, 1.21)	1.06 (0.90, 1.26)
HOMA-IR[4]			
Per 10 g protein/d	1.02 (0.98, 1.06)	1.02 (0.98, 1.06)	1.02 (0.98, 1.07)
Total protein Q1	1 (Ref)	1 (Ref)	1 (Ref)
Total protein Q2	1.12 (0.96, 1.32)	1.06 (0.90, 1.25)	1.06 (0.88, 1.27)
Total protein Q3	0.96 (0.82, 1.14)	0.95 (0.81, 1.12)	0.95 (0.79, 1.16)
Total protein Q4	1.08 (0.91, 1.28)	1.04 (0.88, 1.23)	1.07 (0.89, 1.31)
Body composition (n = 151)			
Lean mass percentage z score, SD[7]			
Per 10 g protein/d	−0.01 (−0.12, 0.09)	−0.06 (−0.16, 0.04)	−0.09 (−0.20, 0.02)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	0.04 (−0.45, 0.52)	−0.30 (−0.77, 0.17)	−0.24 (−0.76, 0.28)
Total protein Q3	0.03 (−0.44, 0.51)	0.15 (−0.28, 0.59)	0.23 (−0.27, 0.72)
Total protein Q4	−0.12 (−0.57, 0.34)	−0.32 (−0.75, 0.11)	−0.35 (−0.82, 0.12)
Total fat mass percentage z score, SD[7]			
Per 10 g protein/d	0.02 (−0.09, 0.12)	0.06 (−0.04, 0.16)	0.08 (−0.03, 0.20)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	−0.02 (−0.51, 0.46)	0.31 (−0.16, 0.78)	0.25 (−0.27, 0.77)
Total protein Q3	−0.03 (−0.51, 0.45)	−0.16 (−0.59, 0.28)	−0.22 (−0.72, 0.28)
Total protein Q4	0.13 (−0.33, 0.59)	0.33 (−0.10, 0.77)	0.35 (−0.12, 0.83)
Abdominal fat mass percentage z score, SD[7]			
Per 10 g protein/d	0.03 (−0.07, 0.13)	0.08 (−0.02, 0.18)	0.10 (−0.01, 0.21)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	0.02 (−0.45, 0.50)	0.35 (−0.12, 0.82)	0.27 (−0.25, 0.79)
Total protein Q3	0.04 (−0.44, 0.51)	−0.04 (−0.48, 0.40)	−0.11 (−0.63, 0.40)
Total protein Q4	0.19 (−0.26, 0.64)	0.40 (−0.03, 0.83)	0.41 (−0.06, 0.89)

[1] P-interaction values obtained by a cross product of the group affiliation (GDM-exposed or control) and the exposure (protein intake) did not attain statistical significance for any of the outcomes or models. GDM, gestational diabetes mellitus; Q, quartile; Ref, reference.

[2] Mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, fat intake, and energy intake; and offspring age and sex.

[3] Mixed linear regression additionally adjusted for maternal physical activity, partner smoking, and offspring puberty status (subsample of n = 896).

[4] Unadjusted and adjusted ratios of geometric means (95% CIs).

[5] A 10-g daily substitution of carbohydrates for protein.

[6] The medians (IQRs) for Q1, Q2, Q3, and Q4 were 77 g/d (70–80 g/d), 89 g/d (87–91 g/d), 98 g/d (95–100 g/d), and 108 g/d (105–117 g/d), respectively.

[7] Unadjusted and adjusted mean differences (95% CIs).

399) of the GDM-exposed mothers; the respective intakes in the control mothers were 11% ± 1% (n = 48) and 16% ± 2% (n = 435).

None of the interaction terms reached statistical significance. Low maternal protein intake was associated with 16–18% lower plasma insulin in GDM-exposed and male offspring (**Table 4**). There was also a trend toward significant associations for HOMA-IR in these offspring. Adjusting for TF% strengthened the associations for male offspring. Associations were stronger among the clinically verified GDM cases compared with the GDM-exposed group (data not shown). Inverse associations with adiposity measures among GDM-exposed and male offspring were of similar magnitude to the protein quartile analysis but did not reach statistical significance (P = 0.15–0.37). We found no support for a relation with any other body-composition measurements or the MetS score stratified on GDM exposure or sex (data not shown).

Secondary analysis

We observed a direct association between maternal intake of red and processed meat and offspring fasting insulin and HOMA-IR in both control and male offspring, with the strongest results present for HOMA-IR (**Table 5**). None of the associations for body composition reached statistical significance, although the directionality was the same as for the protein analysis (data not

TABLE 3
The association of maternal total protein intake in midpregnancy with offspring fasting insulin, HOMA-IR, and body composition at age 9–16 y in the control mother-offspring dyads[1]

	Unadjusted effect estimates	Adjusted effect estimates (model A)[2]	Adjusted effect estimates (model B)[3]
Glucose homeostasis (n = 409–416)			
Fasting plasma insulin,[4] pmol/L			
Per 10 g protein/d[5]	0.99 (0.96, 1.02)	1.01 (0.98, 1.04)	1.01 (0.97, 1.05)
Total protein Q1[6]	1 (Ref)	1 (Ref)	1 (Ref)
Total protein Q2	1.05 (0.93, 1.19)	1.06 (0.95, 1.19)	1.03 (0.90, 1.19)
Total protein Q3	1.02 (0.90, 1.14)	1.01 (0.90, 1.13)	1.02 (0.89, 1.16)
Total protein Q4	0.96 (0.85, 1.09)	1.01 (0.90, 1.14)	1.00 (0.86, 1.15)
HOMA-IR[4]			
Per 10 g protein/d	0.99 (0.95, 1.02)	1.01 (0.97, 1.04)	1.01 (0.97, 1.05)
Total protein Q1	1 (Ref)	1 (Ref)	1 (Ref)
Total protein Q2	1.07 (0.94, 1.21)	1.07 (0.96, 1.21)	1.04 (0.90, 1.21)
Total protein Q3	1.02 (0.90, 1.16)	1.01 (0.90, 1.14)	1.02 (0.89, 1.17)
Total protein Q4	0.96 (0.84, 1.09)	1.01 (0.89, 1.14)	0.99 (0.84, 1.15)
Body composition (n = 355)			
Lean mass percentage z score, SD[7]			
Per 10 g protein/d	−0.10 (−0.18, −0.02)	−0.05 (−0.12, 0.02)	−0.06 (−0.14, 0.02)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	−0.10 (−0.40, 0.21)	−0.12 (−0.39, 0.15)	0.03 (−0.27, 0.34)
Total protein Q3	−0.27 (−0.57, 0.02)	−0.06 (−0.32, 0.21)	0.00 (−0.29, 0.30)
Total protein Q4	−0.34 (−0.64, −0.04)	−0.23 (−0.51, 0.04)	−0.20 (−0.51, 0.11)
Total fat mass percentage z score, SD[7]			
Per 10 g protein/d	0.10 (0.01, 0.18)	0.05 (−0.02, 0.12)	0.06 (−0.02, 0.14)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	0.10 (−0.21, 0.40)	0.12 (−0.15, 0.39)	−0.04 (−0.35, 0.27)
Total protein Q3	0.28 (−0.02, 0.58)	0.06 (−0.21, 0.32)	−0.01 (−0.31, 0.29)
Total protein Q4	0.34 (0.04, 0.64)	0.23 (−0.04, 0.50)	0.19 (−0.12, 0.50)
Abdominal fat mass percentage z score, SD[7]			
Per 10 g protein/d	0.07 (−0.01, 0.15)	0.04 (−0.04, 0.11)	0.03 (−0.05, 0.12)
Total protein Q1	0 (Ref)	0 (Ref)	0 (Ref)
Total protein Q2	0.05 (−0.25, 0.35)	0.05 (−0.23, 0.33)	−0.11 (−0.43, 0.22)
Total protein Q3	0.26 (−0.04, 0.56)	0.07 (−0.20, 0.34)	−0.03 (−0.35, 0.28)
Total protein Q4	0.26 (−0.04, 0.57)	0.18 (−0.10, 0.46)	0.12 (−0.21, 0.44)

[1] P-interaction values obtained by a cross product of the group affiliation (gestational diabetes mellitus–exposed or control) and the exposure (protein intake) did not attain statistical significance for any of the outcomes or models. Q, quartile; Ref, reference.

[2] Mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, fat intake, and energy intake; and offspring age and sex.

[3] Mixed linear regression additionally adjusted for maternal physical activity, partner smoking, and offspring puberty status (subsample of n = 896).

[4] Unadjusted and adjusted ratios of geometric means (95% CIs).

[5] A 10-g daily substitution of carbohydrates for protein.

[6] The medians (IQRs) for Q1, Q2, Q3, and Q4 were 75 g/d (70–78 g/d), 86 g/d (84–88 g/d), 95 g/d (92–97 g/d), and 105 g/d (102–111 g/d), respectively.

[7] Unadjusted and adjusted mean differences (95% CIs).

shown). Other maternal protein sources (white meat, dairy products) were not related to offspring fasting insulin and HOMA-IR (data not shown). However, maternal white-meat consumption was associated with a higher AF% (quartile 4 compared with quartile 1: 0.45 SD; 95% CI: 0.00, 0.90 SD) in the GDM-exposed group (**Table 6**). Among the controls, maternal dairy was related to a higher TF% (quartile 4 compared with quartile 1: 0.33 SD; 95% CI: 0.05, 0.61 SD). Maternal white-meat and dairy consumption was also directly associated with a higher TF% and AF% in male, but not female, offspring. Interactions terms reached statistical significance only for the sex-specific analyses (Tables 5 and 6).

Apart from offspring animal protein intake in the control group, there were no differences in breastfeeding duration and offspring macronutrient and physical activity at the 14-y follow-up across quartiles of maternal total protein intake (**Supplemental Table 5**). However, adjusting for offspring animal protein intake in the control group did not alter our results (data not shown).

DISCUSSION

Contrary to our hypotheses, we found little support for associations of maternal protein intake with offspring indicators of insulin resistance and adiposity in a high-risk population of

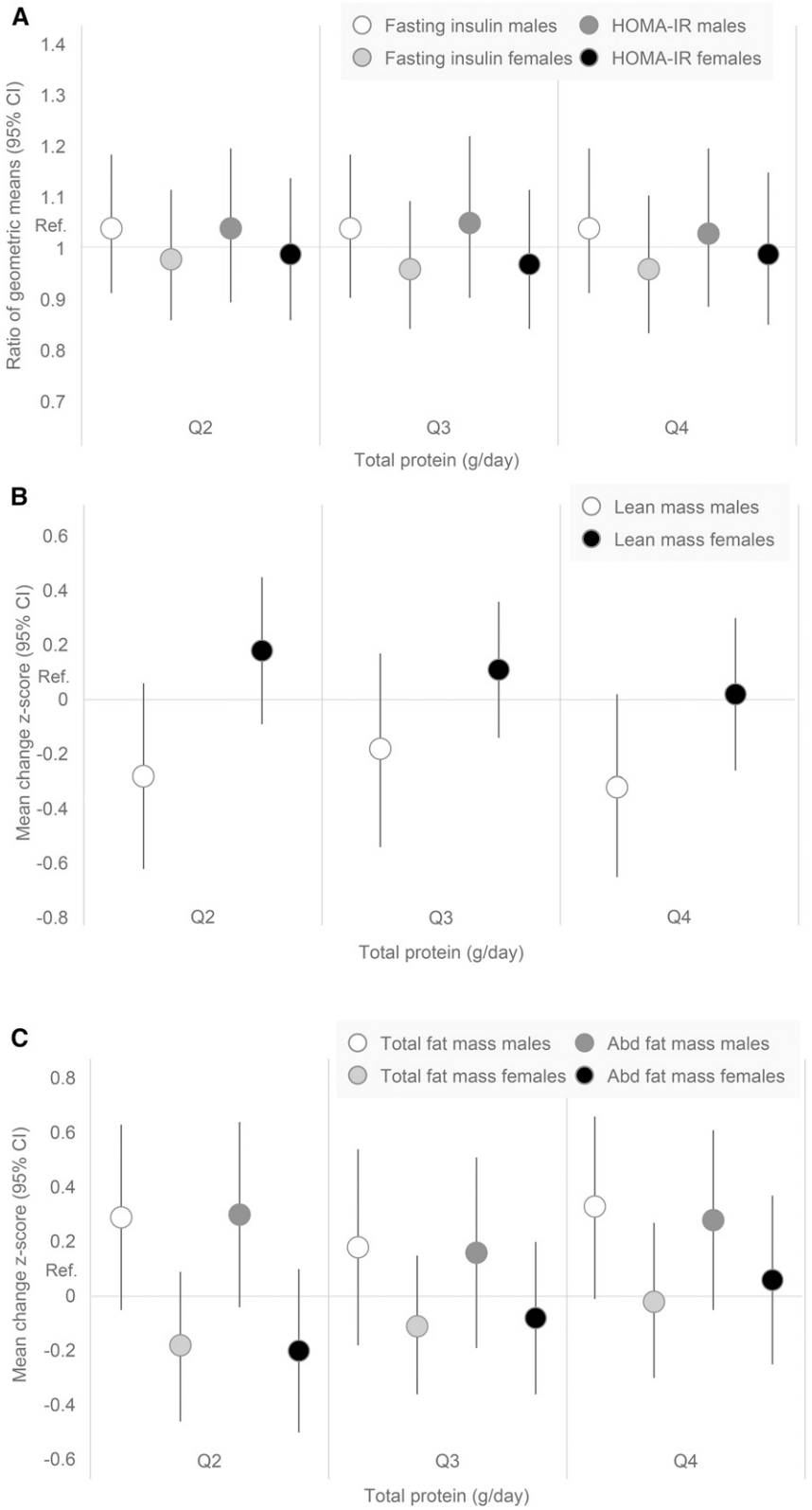

FIGURE 2 The multivariable association of maternal total protein intake in midpregnancy with offspring fasting insulin and HOMA-IR (A), lean mass percentage (B), and total and abdominal fat mass percentages (C) in male (n = 252–398) and female (n = 254–383) offspring at age 9–16 y. Effect estimates and 95% CIs were obtained by mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, and energy intake; and offspring age and sex. The ratio of the geometric means comparing Q4 to Q1 for the fasting insulin were 1.04 (95% CI: 0.91, 1.20; P-trend = 0.59) in male offspring and 0.96 (95% CI: 0.84, 1.11; P-trend = 0.53) for female offspring. The equivalent effect estimates for HOMA-IR were 1.03 (95% CI: 0.89, 1.20; P-trend = 0.68) and 0.99 (95% CI: 0.85, 1.15; P-trend = 0.84) for male and female offspring, respectively. The mean difference in z score for lean mass percentage was −0.32 (95% CI: −0.65, 0.02; P-trend = 0.11) and 0.02 (95% CI: −0.26, 0.30; P-trend = 0.82). For the total fat percentage these

(*continued on next page*)

TABLE 4
The association of maternal low protein intake (≤12.5% of energy compared with >12.5% of energy) in midpregnancy with offspring fasting plasma insulin and HOMA-IR at age 9–16 y[1]

Outcome	Unadjusted	*P*	Adjusted (model A)[2]	*P*	Adjusted (model B)[3]	*P*
Fasting plasma insulin, pmol/L						
Mother-offspring pairs						
GDM (*n* = 367)	0.84 (0.69, 1.03)	0.10	0.82 (0.68, 0.99)	0.04	0.82 (0.66, 1.03)	0.09
Control (*n* = 416)	1.00 (0.87, 1.15)	0.97	0.96 (0.84, 1.09)	0.54	0.99 (0.85, 1.16)	0.93
P-interaction	0.26		0.41		0.36	
Offspring						
Male (*n* = 400)	0.83 (0.70, 0.98)	0.03	0.84 (0.70, 0.98)	0.03	0.85 (0.68, 1.06)	0.16
Female (*n* = 383)	1.00 (0.85, 1.17)	0.99	0.95 (0.81, 1.12)	0.56	0.96 (0.82, 1.14)	0.67
P-interaction	0.16		0.29		0.30	
HOMA-IR						
Mother-offspring pairs						
GDM (*n* = 361)	0.85 (0.68, 1.07)	0.16	0.82 (0.66, 1.02)	0.07	0.82 (0.63, 1.06)	0.13
Control (*n* = 409)	1.01 (0.87, 1.17)	0.87	0.96 (0.84, 1.11)	0.58	0.99 (0.84, 1.17)	0.94
P-interaction	0.30		0.55		0.44	
Offspring						
Male (*n* = 395)	0.83 (0.68, 1.00)	0.05	0.83 (0.68, 1.00)	0.05	0.84 (0.64, 1.08)	0.17
Female (*n* = 375)	1.02 (0.86, 1.22)	0.81	0.96 (0.81, 1.14)	0.66	0.97 (0.81, 1.16)	0.72
P-interaction	0.14		0.29		0.31	

[1] Values are ratios of geometric means (95% CIs). The percentage of energy comparison was made between the lowest decile and the 9 higher deciles. GDM, gestational diabetes mellitus.

[2] Mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, and energy intake; and offspring age and sex.

[3] Mixed linear regression additionally adjusted for maternal physical activity, partner smoking, and offspring puberty status (subsample of *n* = 896).

GDM-exposed pregnancies and a control group. There was a modest, but not significant, increase in offspring abdominal adiposity with a higher maternal protein intake in GDM-exposed offspring, which did not translate into a higher risk of being overweight. Maternal red- and processed-meat consumption was associated with higher insulin resistance among control and male offspring, whereas white meat and dairy appeared to increase adiposity in GDM-exposed and control offspring, respectively. On the contrary, GDM-exposed women in the lowest decile of protein intake had offspring with lower insulin resistance; this association was also evident in male offspring.

Unlike studies on the adverse effects of undernutrition and accompanying low protein exposure in pregnancy (46, 47), the literature on protein intake at the higher end of the distribution remains scarce. Most of these studies have focused on weight and adiposity-related measurements and varied considerably in age range from prebirth to 11 y. Most studies did not find an association of maternal protein intake with offspring outcomes (19–21). Two of these studies measured intake in the first trimester (20, 21) and 1 in the third trimester (19). Assessment of the age of the offspring also varied from the first 6 mo of life (21) to midchildhood (age 5–10 y) (19, 20). None of these studies included metabolically susceptible populations. A study of fetal abdominal and thigh sites with the use of ultrasound examinations showed an inverse association between maternal protein intake across pregnancy and percentage abdominal fat, and abdominal fat in the fetus was highest when maternal protein intake was <16% of energy (18). This was contrary to results from our study of 19- to 21-y-old Danish adults, where maternal protein intake in GW 30 increased the offspring's risk of being overweight (17). Compared with the present study population, this cohort of Danish adults was older and with potentially more developed adiposity, allowing for easier detection of any present associations. Shiell et al. (22) examined maternal macronutrient intake in late pregnancy in relation to plasma insulin in the 40-y-old offspring and found that maternal protein intake was associated with lower insulin secretion after an oral-glucose-tolerance test. In fact, the highest offspring insulin secretion was found for maternal intake ≤60 g protein/d (~10% of energy). Our study did not assess insulin secretion, and we found no association of higher protein intake with measures of insulin resistance. However, low protein was associated with improved insulin resistance in the GDM-exposed group. Combined, there are currently few data in humans supporting the idea that protein intake in pregnancy affects offspring plasma insulin levels, insulin secretion, or insulin resistance, even under conditions of maternal hyperglycemia.

Quite contrary to the findings in animals, low protein intake, defined as the percentage of energy in the lowest decile of intake, was associated with lower fasting insulin levels and insulin

Figure 2. (continued) were 0.33 (95% CI: −0.01, 0.66; *P*-trend = 0.11) and −0.02 (95% CI: −0.30, 0.27; *P*-trend = 0.82), and for the abdominal fat percentage these were 0.28 (95% CI: −0.05, 0.61; *P*-trend = 0.16) and 0.06 (95% CI: −0.25, 0.27; *P*-trend = 0.77) for male and female offspring, respectively. *P*-trend values were quantified by inputting the exposure quartile medians as an ordinal variable into the regression model. Abd, abdominal; Q, quartile; Ref., reference.

TABLE 5
The multivariable association of maternal intake of red and processed meat in midpregnancy with offspring fasting insulin and HOMA-IR at age 9–16 y[1]

Outcome	Per 10 g protein/d	Q1	Q2	Q3	Q4
Fasting plasma insulin, pmol/L					
Mother-offspring pairs					
GDM (n = 365)	1.00 (0.99, 1.02)	1 (Ref)	1.23 (1.07, 1.43)	1.12 (0.96, 1.30)	1.06 (0.91, 1.25)
Control (n = 416)	1.02 (1.00, 1.03)	1 (Ref)	1.11 (0.99, 1.23)	1.09 (0.98, 1.22)	1.17 (1.04, 1.31)
P-interaction	0.87		0.19	0.19	0.75
Offspring					
Male (n = 398)	1.02 (1.00, 1.03)	1 (Ref)	1.16 (1.02, 1.32)	1.14 (1.00, 1.30)	1.19 (1.03, 1.35)
Female (n = 383)	1.00 (0.99, 1.02)	1 (Ref)	1.04 (0.91, 1.19)	1.08 (0.94, 1.25)	1.03 (0.89, 1.19)
P-interaction	0.02		0.30	0.33	0.03
HOMA-IR					
Mother-offspring pairs					
GDM (n = 359)	1.00 (0.99, 1.02)	1 (Ref)	1.27 (1.08, 1.49)	1.14 (0.97, 1.35)	1.09 (0.92, 1.31)
Control (n = 409)	1.02 (1.00, 1.03)	1 (Ref)	1.11 (0.98, 1.25)	1.09 (0.97, 1.23)	1.19 (1.04, 1.34)
P-interaction	0.98		0.26	0.19	0.70
Offspring					
Male (n = 393)	1.02 (1.01, 1.04)	1 (Ref)	1.21 (1.05, 1.39)	1.21 (1.03, 1.39)	1.27 (1.08, 1.48)
Female (n = 375)	1.00 (0.98, 1.02)	1 (Ref)	1.02 (0.89, 1.17)	1.08 (0.93, 1.25)	1.01 (0.86, 1.17)
P-interaction	0.01		0.19	0.23	0.01

[1] Values are adjusted ratios of geometric means (95% CIs). Mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, and energy intake; and offspring age and sex. GDM, gestational diabetes mellitus; Q, quartile; Ref, reference.

resistance in our data. In animal studies in which low-protein diets are fed to dams during gestation and/or lactation, offspring are usually born growth restricted but remain insulin sensitive until adulthood (48–51). With age these offspring accumulate more body fat and become glucose intolerant, suggesting physiologic maladaptation when faced with the metabolic burden of postnatal life (8, 50, 52). Although it can be argued that the GDM-exposed offspring in our study experience the early phases of increased insulin sensitivity, it is important to consider that "low" protein intake in our population was higher than what is used in most animal studies (8% of energy typically), and only 1% of women reported an intake <10% of energy. This could suggest a nonlinear association where the extreme low intake encountered in animal studies and during famines disrupts developmental processes in adipocytes and pancreatic cells in the growing fetus. These processes may be remedied with protein intake above a certain threshold, whereas increasing protein intake may adversely affect adiposity development through, for example, insulin-like growth factor I–mediated promotion of adipogenesis (53, 54). Better insulin resistance measurements were found in a protein-supplementation trial in an undernourished population, although it was not clear whether supplementation in pregnancy or childhood had the largest effect (55). The lack of association with insulin resistance in the higher categories of protein intake does not preclude that glucose intolerance may eventually develop, especially in individuals who continue to accumulate visceral body fat. We found that abdominal adiposity was higher, although not significantly so, in offspring of GDM-exposed pregnancies in which the mother consumed protein in the highest quartile. The lack of association with a combined phenotype of metabolic syndrome could be due to modest or differential associations with the individual components.

This is the first study to our knowledge to demonstrate a beneficial association of low protein intake with long-term metabolic health of GDM-exposed offspring, even after adjustment for maternal BMI. Higher protein intake, specifically from animal sources, has previously been related to GDM and T2D in adults (56). Although we saw no associations with animal protein, this raises an intriguing possibility of whether a relatively minor reduction in protein intake could mitigate some adverse programming by GDM. Why maternal red-meat intake was associated with higher insulin and HOMA-IR in control, and not GDM-exposed, offspring remains unclear but could be attributed to other components of meat, such as nitrites and nitrates or iron (57) because protein intake varied only by 6 g/d between the lowest and highest quartiles. It is also possible that GDM-exposed mothers misreported red-meat intake according to mismeasured or unmeasured factors, e.g., glycemic control. White-meat and dairy intake was only associated with adiposity and was not consistent across the different groups based on GDM exposure and sex stratification. This may be because of other nutritional components acting as active agents. For example, dairy is also an important source of saturated fat. However, in an analysis of dairy and birth weight in the DNBC, higher birth weight was attributed to the protein rather than the fat in dairy (58). We did not find any correlations between protein intake and birth weight or birth weight and the outcomes. However, this does not remove the possibility that nonlinear associations may exist within a more complex mediator network. Overall, the effect sizes in our analysis were modest, and the lack of differences between cases and controls could be attributed to power issues.

Sensitivity of male offspring to nutritional insults in pregnancy have long been known in animal models (8, 11). Differences in metabolic risk factor development have also been observed in humans (59). We found that male offspring were more protected by low protein and had a slightly elevated risk of insulin resistance with higher maternal red meat intake. This greater sensitivity to intrauterine malnutrition may stem from the faster development of the preimplantation male embryo (60).

TABLE 6
The multivariable association of maternal intake of white meat and dairy in midpregnancy with offspring adiposity at age 9–16 y[1]

Outcome	Per increment food intake	Q1	Q2	Q3	Q4
Total fat mass percentage *z* score, SD					
White meat,[2] g/d					
Mother-offspring pairs					
GDM (*n* = 151)	0.07 (−0.02, 0.15)	0 (Ref)	0.22 (−0.26, 0.70)	0.39 (−0.06, 0.84)	0.37 (−0.08, 0.82)
Control (*n* = 355)	0.02 (−0.03, 0.07)	0 (Ref)	0.04 (−0.23, 0.30)	0.09 (−0.17, 0.36)	0.13 (−0.13, 0.40)
P-interaction	0.14		0.14	0.21	0.19
Offspring					
Male (*n* = 252)	0.04 (−0.01, 0.09)	0 (Ref)	0.44 (0.09, 0.80)	0.36 (0.04, 0.68)	0.46 (0.15, 0.77)
Female (*n* = 254)	−0.02 (−0.07, 0.04)	0 (Ref)	−0.08 (−0.35, 0.20)	−0.03 (−0.32, 0.26)	−0.13 (−0.43, 0.16)
P-interaction	0.07		0.02	0.02	0.001
Dairy,[3] g/d					
Mother-offspring pairs					
GDM (*n* = 151)	0.01 (−0.02, 0.05)	0 (Ref)	0.21 (−0.27, 0.68)	−0.21 (−0.71, 0.29)	0.06 (−0.47, 0.60)
Control (*n* = 355)	0.03 (0.00, 0.05)	0 (Ref)	0.12 (−0.14, 0.38)	0.23 (−0.04, 0.49)	0.33 (0.05, 0.61)
P-interaction	0.90		0.49	0.11	0.21
Offspring					
Male (*n* = 252)	0.03 (0.01, 0.06)	0 (Ref)	0.22 (−0.11, 0.56)	0.04 (−0.29, 0.37)	0.34 (0.01, 0.67)
Female (*n* = 254)	−0.01 (−0.03, 0.02)	0 (Ref)	−0.04 (−0.31, 0.24)	0.05 (−0.23, 0.33)	−0.05 (−0.35, 0.25)
P-interaction	0.06		0.22	0.65	0.13
Abdominal fat mass percentage *z* score, SD					
White meat,[2] g/d					
Mother-offspring pairs					
GDM (*n* = 151)	0.09 (0.01, 0.17)	0 (Ref)	0.19 (−0.29, 0.66)	0.32 (−0.12, 0.76)	0.45 (0.00, 0.90)
Control (*n* = 355)	0.02 (0.03, 0.06)	0 (Ref)	0.02 (−0.25, 0.29)	0.13 (−0.14, 0.41)	0.10 (−0.17, 0.37)
P-interaction	0.05		0.13	0.29	0.06
Offspring					
Male (*n* = 252)	0.03 (−0.02, 0.09)	0 (Ref)	0.43 (0.09, 0.78)	0.34 (0.03, 0.65)	0.37 (0.06, 0.67)
Female (*n* = 254)	−0.00 (−0.07, 0.06)	0 (Ref)	−0.08 (−0.38, 0.23)	0.06 (−0.26, 0.37)	−0.07 (−0.39, 0.25)
P-interaction	0.24		0.02	0.11	0.01
Dairy,[3] g/d					
Mother-offspring pairs					
GDM (*n* = 151)	0.01 (−0.02, 0.05)	0 (Ref)	0.31 (−0.14, 0.77)	−0.11 (−0.60, 0.38)	0.13 (−0.40, 0.66)
Control (*n* = 355)	0.02 (−0.01, 0.05)	0 (Ref)	0.19 (−0.08, 0.46)	0.27 (−0.00, 0.54)	0.28 (0.00, 0.57)
P-interaction	0.91		0.56	0.21	0.42
Offspring					
Male (*n* = 252)	0.02 (−0.00, 0.05)	0 (Ref)	0.29 (−0.04, 0.63)	0.17 (−0.16, 0.50)	0.18 (−0.16, 0.53)
Female (*n* = 254)	−0.01 (−0.04, 0.02)	0 (Ref)	−0.00 (−0.30, 0.30)	0.11 (−0.19, 0.42)	−0.02 (−0.34, 0.31)
P-interaction	0.08		0.14	0.59	0.18

[1] Values are adjusted mean differences (95% CIs). Mixed linear regression adjusted for parental sociodemographic position; maternal age, parity, prepregnancy BMI, smoking, and energy intake; and offspring age and sex. GDM, gestational diabetes mellitus; Q, quartile; Ref, reference.
[2] White meat increment = 10 g/d
[3] Dairy increment = 100 g/d

Imbalances in the nutritional environment may alter early developmental events, such as cell proliferation in the inner cell mass and trophectoderm, potentially influencing gastrulation, stem cell allocation, and placental function (61). None of the reviewed studies included dietary data specific to the pre- or periconceptual period, and the effect of a high protein intake in this period is lacking data. Unlike other studies (14, 17), we did not find increased adiposity among females exposed to higher maternal protein, but this could be because of still-underdeveloped adiposity in this young population.

This study is not without limitations. The most important limitation includes potential reverse associations due to a likely overlap between GDM diagnosis and FFQ administration dates. In a small subset of women with data on GDM diagnosis date (*n* = 177), we found no difference in protein or any other macronutrient intake between those diagnosed before and after the FFQ date. This, however, does not preclude that differences existed among the remaining women. The fact that the results were in the same direction as a previous analysis in an older Danish cohort (17), however, reassures that any bias from reverse associations seems to be modest. Furthermore, such modest bias may have masked a stronger underlying association if a disproportional number of women who received their diagnosis before the FFQ underreported their protein intake. The use of an isocaloric model could be considered inappropriate in pregnancy. In our study, women were asked about intake in the last month, which is more likely to adhere to the isocaloric assumption. Furthermore, we only measured the dietary exposure over a short period of time and at 1 time point in midpregnancy, which may have led us to miss exposures outside this time range. However, studies with multiple dietary measurements in pregnancy have found strong correlations between dietary intake

across trimesters (18). The use of a substitution model relies on the change in 2 nutrients, an increase in protein at the expense of carbohydrates. Therefore, we cannot exclude that the observed result may have been due to a decrease in carbohydrates. There were few differences in sociodemographic and lifestyle covariates between participants and nonparticipants in the GDM-exposed and control groups based on available dietary data, but importantly, there were no differences for the offspring outcomes. In observational studies, we cannot exclude potential residual confounding by unmeasured or poorly measured confounders. In our analysis we adjusted for both parental and offspring covariates, including a more detailed examination of offspring dietary and lifestyle factors, but these did not substantially alter the effect estimates.

The strengths of this study include its longitudinal nature and wealth of information on both the mother and the offspring over the 9–16 y of follow-up. This allowed us to consider and evaluate many covariates as potential confounders and minimize residual confounding. This information also included detailed dietary data from midpregnancy, although we cannot preclude that nondifferential measurement error may have attenuated some of the results toward the null. Unlike the self-reported nature of the exposure, we used clinical data for the outcomes. This minimized the likelihood of differential measurement error and provided us with an outcome closer to the physiologic points of interest. The inclusion of >600 GDM-exposed pregnancies is a unique feature in itself. It allowed us to examine the associations for offspring exposed to GDM, which form a specific clinical risk group.

In conclusion, our findings did not support an association between maternal protein intake and offspring metabolic health, with the exception of maternal low protein intake in GDM-exposed offspring. Protein intake below the current consumption amounts, but above amounts reported in nutritional emergencies, seemed to be associated with a beneficial insulin response in these offspring. These associations were somewhat mirrored in those for maternal red-meat consumption and were consistent for male offspring, another group that is potentially more susceptible to nutritional insults. These findings need to be clarified in future studies, especially preconceptually and across pregnancy trimesters and in older offspring where effects may be more pronounced. The study of more vulnerable subgroups of offspring by maternal phenotype and sex is of particular interest for more targeted prevention approaches.

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