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Effect of a high-protein and low-glycaemic index diet during pregnancy in women with overweight or obesity on offspring metabolic health—A randomized controlled trial

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Summary

Background: Maternal obesity and excessive weight gain during pregnancy are associated with higher birth weight and increased risk of childhood obesity.

Objective: This study investigated the effect of a high-protein and low-glycaemic-index (HPLGI) diet during pregnancy on offspring body composition and metabolic health.

Methods: We conducted a dietary intervention study in pregnant women with a pre-pregnancy BMI of 28–45 kg/m^2 who were randomly assigned to an HPLGI diet or a moderate-protein moderate-glycaemic-index (MPMGI) diet. A total of 208 offspring born to these women were followed-up from birth to 5 years of age.

Results: No differences were found on BMI z-scores at different ages; however, offspring born to women on the HPLGI diet exhibited 0.43 mmol/L higher glucose levels ($p = 0.017$) at birth compared with the MPMGI diet. At 3 years of age, HPLGI offspring had 0.09 mmol/L lower levels of HDL-cholesterol ($p = 0.018$) and 16% higher levels of triglycerides ($p = 0.044$). At 5 years of age, they had 0.25 mmol/L higher total cholesterol levels ($p = 0.027$) and 0.27 mmol/L higher LDL-cholesterol levels ($p = 0.003$) compared with the MPMGI diet.

Conclusion: An HPLGI diet during pregnancy may lead to adverse metabolic outcomes in the offspring, necessitating further investigation into long-term health implications.

KEYWORDS
dietary intervention, fetal programming, offspring metabolic profile, pregnancy, prevention of childhood obesity

Abbreviations: APPROACH, an optimized programming of healthy children; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DEXA, dual-energy X-ray; FFM, fat-free mass; FM, fat mass; GDM, gestational diabetes mellitus; GWG, gestational weight gain; HDL, high-density lipoprotein; HPLGI, high-protein low-glycaemic-index; IGFBP-3, insulin-like growth factor binding protein-3; IGF-1, insulin-like growth factor-1; LDL, low-density lipoprotein; MPMGI, moderate-protein moderate-glycaemic-index; MRI, magnetic resonance imaging; RCT, randomized controlled trial; SD, standard deviation; SE, standard error; TG, triglycerides.

1 | INTRODUCTION

Globally, in 2014, approximately 38.9 million pregnant women had overweight and 14.6 million had obesity.[1] In the United States, nearly 40% of women in the childbearing age (20–39 years) have obesity,[2] while in Denmark, the same proportion (40%) of women aged 25–34 years are overweight and 17.4% have obesity.[3] The presence of maternal obesity, particularly in combination with excessive gestational weight gain (GWG), has consistently been linked to elevated offspring body mass index (BMI) z-score and increased risk of developing obesity and obesity-related metabolic complications later in life.[4–6] Thus, implementing lifestyle interventions during pregnancy with long-term follow-up of the offspring is needed to reduce the risk of obesity and improve relevant metabolic risk factors in the offspring.

Dietary interventions aimed at preventing excessive GWG confer benefits for the mother, including decreased risk of caesarean section, improved glucose metabolism and reduced postpartum weight retention.[7–10] Specifically, low-glycaemic-index diets have been associated with lower GWG, reduced maternal fasting glucose level, lower birth weight and lower risk of giving birth to an infant large-for-gestational-age.[11–14] The effect of high protein intake during pregnancy on offspring health outcomes, however, remains inconclusive. Some studies found a positive association between maternal protein intake during pregnancy and higher birth weight,[15] while others do not support such a relationship.[16] One study found an association between high protein intake and increased fetal abdominal fat percentage measured by ultrasound,[17] while another long-term study found an association between higher protein intake during pregnancy and increased fat-free mass in children at the age of 6 years.[18] These observations suggest that dietary habits during pregnancy can affect body composition in the offspring, not only during gestation and at birth, but also for many years later. However, further long-term follow-up studies are needed.

The APPROACH (An optimized programming of healthy children) study was a unique randomized controlled trial (RCT) with a dietary intervention consisting of a high-protein and low-glycaemic-index (HPLGI) diet during pregnancy in women with overweight or obesity.[7] In the APPROACH RCT, women on the HPLGI diet had significantly lower GWG compared with women randomized to the control diet, but there were no significant differences in offspring birth weight.[7] This unique follow-up study of the offspring aimed to assess the effect of the HPLGI diet intervention during pregnancy on risk factors for metabolic diseases in offspring during the first 5 years of life. Due to reduced GWG in women following the HPLGI diet during pregnancy, we hypothesized that this would result in lower BMI z-scores and a more beneficial metabolic profile in the offspring of these women.

2 | METHODS

2.1 | Study design

The APPROACH cohort study is a follow-up study of offspring born to mothers participating in the APPROACH RCT, during their first 5 years of life. The RCT was conducted at Copenhagen University Hospital Herlev-Gentofte, Denmark, from January 2014 to December 2017. Offspring assessments until age 3 were performed at Copenhagen University Hospital Herlev-Gentofte, Denmark, and the Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark, at the age of 5. All study procedures were conducted in accordance with the Helsinki II Declaration. Women and their partners participating in the study received both written and oral information about the study before signing an informed consent. The APPROACH study was approved by the Ethical Committee of the Capital Region of Denmark (H-3-2013-119) and was registered at ClinicalTrials.gov (NCT01894139).

2.2 | Participants

The pregnant women participating in the APPROACH RCT were recruited during their first-trimester scan, performed between 11 weeks + 2 days and 13 weeks + 6 days of gestation. To be eligible for the study, women had to be ≥18 years old, have a singleton pregnancy and have a pre-pregnancy BMI ranging from ≥28 to ≤45 kg/m^2. Exclusion criteria included multiple pregnancies, allergies or intolerance to dairy products or fish, weight loss exceeding 10 kg in the past year, excessive alcohol consumption (more than 14 units per week), drug abuse and underlying disorders that could interfere with the intervention (e.g., diabetes, kidney disease, cancer, liver disease or any known active metabolic disease). This paper includes data from the mothers completing the intervention with a live birth.

2.3 | Intervention

The pregnant women were allocated in groups of 4–8 and were randomly assigned as clusters to one of two diets. The diets were either an HPLGI diet (25%–28% of energy from protein and glycaemic index ≤55) or a diet according to the Nordic Nutrition Recommendations[19] with no instructions on the glycaemic index (i.e., 15%–18% of energy from protein and glycaemic index ~60), moderate-protein, moderate-glycaemic-index (MPMGI) diet. Information on maternal dietary protein intake and glycaemic index was obtained by a 29-item food frequency questionnaire during gestational weeks 15, 28 and 36, and dietary energy and macronutrient intake was obtained by a 24-hour recall during gestational weeks 21 and 32. The 29-item food frequency questionnaire was validated against a 4-day weighed food record in 31 of the included women.[20] Throughout the dietary intervention phase, there were no significant differences in total energy intake in gestational weeks 21 and 32.[7] The HPLGI group reported a mean intake of ~25% protein, whereas the MPMGI group reported a mean intake of ~18% protein. At baseline, the glycaemic index was similar between the two groups. However, during the intervention, the HPLGI diet was ~8 units lower in glycaemic index compared with the MPMGI diet.[7] Moreover, analyses of urinary urea excretion confirmed the self-reported protein intake.

Both the HPLGI diet and the MPMGI diet were consumed ad libitum, and recommended food servings were based on the targeted nutrient composition according to the randomization group. Participants attended seven group-based dietary sessions and two individual dietary consultations throughout pregnancy to facilitate dietary adherence.

2.4 | Outcomes

The outcomes of this study included markers of adiposity, such as offspring BMI z-score, fat mass (FM), fat-free mass (FFM), skinfold thicknesses and circumference measurements. Furthermore, we measured fasting glucose levels, insulin levels and lipid profiles, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides (TG).

BMI z-scores were calculated based on the World Health Organization 2009 standards for sex-specific BMI-for-age, weight-for-age and length-for-age.[21] These calculations were performed at various time-points: at birth, 6 months of age (±2 weeks), 18 months of age (±4 weeks), 3 years of age (±4 weeks) and 5 years of age (±4 weeks). Blood samples were drawn at birth (cord blood), and fasting venous blood samples were drawn at 3 years of age (after 2 hours of fasting) and at 5 years of age (after an overnight fast of ≥8 h).

At birth, midwives at the Copenhagen University Hospital Herlev-Gentofte measured body weight, length, circumferences and collected cord blood, while trained study personnel conducted measurements of anthropometry at 6 months of age, 18 months of age, 3 years of age and 5 years of age and sampling blood at 3 and 5 years of age. Length/height was measured using a non-elastic measuring tool to the nearest 0.5 cm. Offspring body weight was measured using a medical beam scale (Tanita, Illinois, USA) with a precision of 10 grams. Body weight at 3 and 5 years of age and FM at 5 years were assessed using a bioimpedance weight scale (InBody 570, USA) with a precision of 0.1 kg. Abdominal circumference was measured to the nearest 0.1 cm using a non-elastic measuring tape. Abdominal skinfold thickness, biceps skinfold thickness and triceps skinfold thickness were performed by the same person using a Harpenden skinfold calliper (Baty International, UK), to the nearest 0.1 mm at all time-points. All circumference and skinfold measurements are reported as the average of three measurements.

Blood samples at all follow-up time-points were collected in lithium heparin tubes, centrifuged and stored at least −70°C. However, whole blood glucose at ages 3 and 5 years were analysed using the Hemocue Glucose 201+ (HemoCue, Danmark) on the same day as the blood sampling. Offspring plasma cord blood glucose level at birth was measured using a colorimetric method, on a Pentra 400 analyser (Horiba ABX). Insulin in serum was measured by immunoassay on the Immulite 2000 analyser (Siemens Healthcare, UK); samples that read below the detection limit of the standard assay (14.4 mU/L) were re-analysed using an ultra-high-sensitive ELISA kit from Mercodia AB (cat no. 10-1132-01, lot no. 35009). Total cholesterol, LDL, HDL and TG from plasma were analysed on Pentra 400 (Horiba ABX SAS, France).

Gestational age was determined from the crown-rump length measurement at the first-trimester ultrasonic scan (performed between 11 weeks + 2 days and 13 weeks + 6 days) and given as the number of days to the date of delivery. The age of the offspring was calculated in months and years from birth to the visit day. Breastfeeding information and introduction to solid foods were collected by questionnaires at 6 months of age, asking 'What kind of nutrient supply did the child receive at 0–2 months, 2–4 months and 4–6 months' and 'When was the child introduced to solid foods'.

2.5 | Statistical analysis

Data from offspring who participated in the follow-up at any time until the age of 5 years were analysed. We did a sensitivity analysis of maternal characteristics of those who were included in the study ($n = 279$) compared with those who gave birth ($n = 208$). Additionally, we examined the differences between those who dropped out before the examinations at 3 years and 5 years of age, with those who were participating at 3 years ($N = 166$) and 5 years of age ($N = 141$) with no differences in maternal age and pre-pregnancy BMI. Additionally, offspring characteristics at birth were similar between those who dropped out during the follow-up period and those who remained in the study. Maternal characteristics were presented as mean with standard deviation (SD) for normal distributed values and median (Q1; Q3) for skewed values. An *F*-test was used to determine the differences between groups. Gestational weight gain was analysed by using linear mixed models including time, group and baseline maternal weight as fixed factors, and a group-by-time interaction with random effects of ID. Available case data of offspring who participated in the follow-up at any time until the age of 5 years were analysed using a linear mixed model that included time and group as fixed factors with random effects for subject ID to look at the difference in offspring outcomes between the two groups. We chose not to adjust for cluster randomization in the statistical analysis, as there was no indication that heterogeneity within clusters varied between the two experimental groups. We selected the statistical model to assess our hypothesis regarding how maternal dietary intervention during pregnancy impacts the health of offspring at different ages. We checked the linear mixed models for normal distribution using qq-plots and residual plots to check for heteroscedasticity and linearity. Outcomes that were normally distributed are presented as estimated means with standard error (SE), and the difference between the two groups is presented as an estimate with a 95% confidence interval (CI). When the model did not fulfil the criteria for normal distribution, heteroscedasticity or linearity, the outcome was log-transformed and is presented as the exponentiated effect estimate, interpreted as a ratio (%) with 95% CI. The BMI z-score distributions are given in density plots, and skewness was assessed using the Shapiro–Wilk test. A simple linear regression model was used to analyse the difference between the two groups in FM, FFM and fat percentage at 5 years of age. All statistical analyses were performed with the use of the statistical program R (version R 4.2.2; R Foundation for Statistical Computing). A two-sided *p*-value of ≤0.05 was considered statistically significant.

3 | RESULTS

The study included a total of 208 offspring, born to women with overweight or obesity participating in the APPROACH RCT, with 104 in the HPLGI diet group and 104 in the MPMGI diet group. The women were similar in age and pre-pregnancy BMI; however, women in the HPLGI group were 0.5 cm ($p = 0.046$) higher and had a weight gain of 1.71 kg ($p < 0.001$) less than those in the MPMGI group. Furthermore, while the total energy intake was similar, the macronutrient composition of the diets varied. Women in the HPLGI group consumed a higher amount of protein, a lower amount of carbohydrates and a reduced glycaemic index, along with a higher percentage of fat in gestational week 21 (Table 1). Maternal lipid and glucose metabolism did not differed between the groups, except for lower TG in gestational week 28 in the HPLGI group (Table S1). Infants born to mothers from both groups had similar sex distributions (45% female, 55% male), and ~3% and ~7% were born pre-term in the HPLGI group and MPLGI group, respectively, with no difference between groups. The dropout rate was 6.3% before the follow-up at 6 months, 5.6% between 6 and 18 months, 9.8% between 18 months and 3 years, and 15.1% between 3 and 5 years (Figure 1). The total dropout rate was 33% over the 5 years of follow-up in both groups. No differences were found in the duration of breastfeeding or the age at which offspring were introduced to solid foods (Table S2).

3.1 | Offspring markers of adiposity

BMI z-scores were not significantly different between the HPLGI and MPMGI groups at birth, 3 years and 5 years of age. At 18 months of age, the HPLGI diet group had larger skinfold thickness at the biceps ($p = 0.009$) and triceps ($p = 0.015$) compared with the MPMGI diet group. At 5 years of age, the HPLGI group had larger biceps skinfold thickness ($p = 0.029$) and abdominal skinfold thickness ($p = 0.022$)

TABLE 1 Maternal characteristics.

	N	HPLGI	*N*	MPMGI	(HPLGI-MPMGI Estimate)	*p*-value
Maternal age	104	30.8 (4.7)	104	30.6 (4.9)	0.24 (−1.07;1.55)	0.718
Pre-pregnancy weight (kg)	104	96.3 (13.1)	104	95.8 (12.4)	0.50 (−2.98;3.99)	0.776
Pre-pregnancy height (cm)	104	168.9 (6.0)	104	167.2 (6.3)	1.71 (0.03;3.38)	**0.046**
Pre-pregnancy BMI (kg/m^2)	104	32.69 (30.74;35.64)	104	32.99 (31.25;36.68)	−0.53 (−1.59;0.53)	0.324
BMI categories						**0.034**
Overweight, %	5	14.4	15	4.8		
Obese, %	99	85.6	89	95.2		
GWG	104	6.78 ± 0.42	104	8.49 ± 0.28	−1.71 (−2.57–0.85)	**<0.001**
Nulliparous, %	103	55.3	104	55.8		0.535
Energy intake, kJ						
GW 21[a]	103	7572 (1923)	101	7250 (2025)	322 (−222;867)	0.245
GW 32[a]	97	7380 (1802)	98	7273 (1925)	107 (−419;634)	0.688
Fat, E%						
GW 21[a]	103	32.7 (8.95)	101	29.8 (7.29)	2.94 (0.82;5.06)	**0.006**
GW 32[a]	97	32.0 (7.86)	98	30.3 (7.88)	1.62 (−0.60;3.85)	0.152
Carbohydrate, E%						
GW 21[a]	103	42.6 (8.49)	101	51.8 (7.45)	−9.14 (−11.35;-6.94)	**<0.001**
GW 32[a]	97	42.5 (8.27)	97	51.8 (8.05)	−9.35 (−11.65;-7.04)	**<0.001**
Protein, E%						
GW 21[a]	103	24.7 (5.8)	101	18.4 (4.6)	6.22 (4.77;7.66)	**<0.001**
GW 32[a]	97	25.5 (5.5)	98	17.8 (3.8)	7.77 (6.42:9.11)	**<0.001**
Glycaemic index						
GW 15[b]	103	53.9 (4.75)	104	53.8 (5.32)	0.06 (−1.32;1.44)	0.932
GW 28[b]	103	44.4 (4.84)	99	53.4 (4.21)	−8.96 (−10.23;-7.70)	**<0.001**
GW 36[b]	101	45.9 (4.91)	90	54.3 (4.28)	−8.39 (−9.71;-7.07)	**<0.001**

Note: Values are mean (SD) for normal distributed values and median (Q1;Q3) for skewed distributions and estimate (95% CI) for between-group differences. GWG was analysed by using linear mixed models included time, group and baseline maternal weight as fixed factors, and a group-by-time interaction with random effects of ID. GWG is presented as adjusted means ± SE. E%, percentage of total energy intake. Bold indicates significantly different from the MPMGI group.

Abbreviations: HPLGI, high-protein low-glycaemic-index; MPMGI—moderate-protein moderate-glycaemic-index.

[a]Data from 24-h recall.

[b]Data from FFQ.

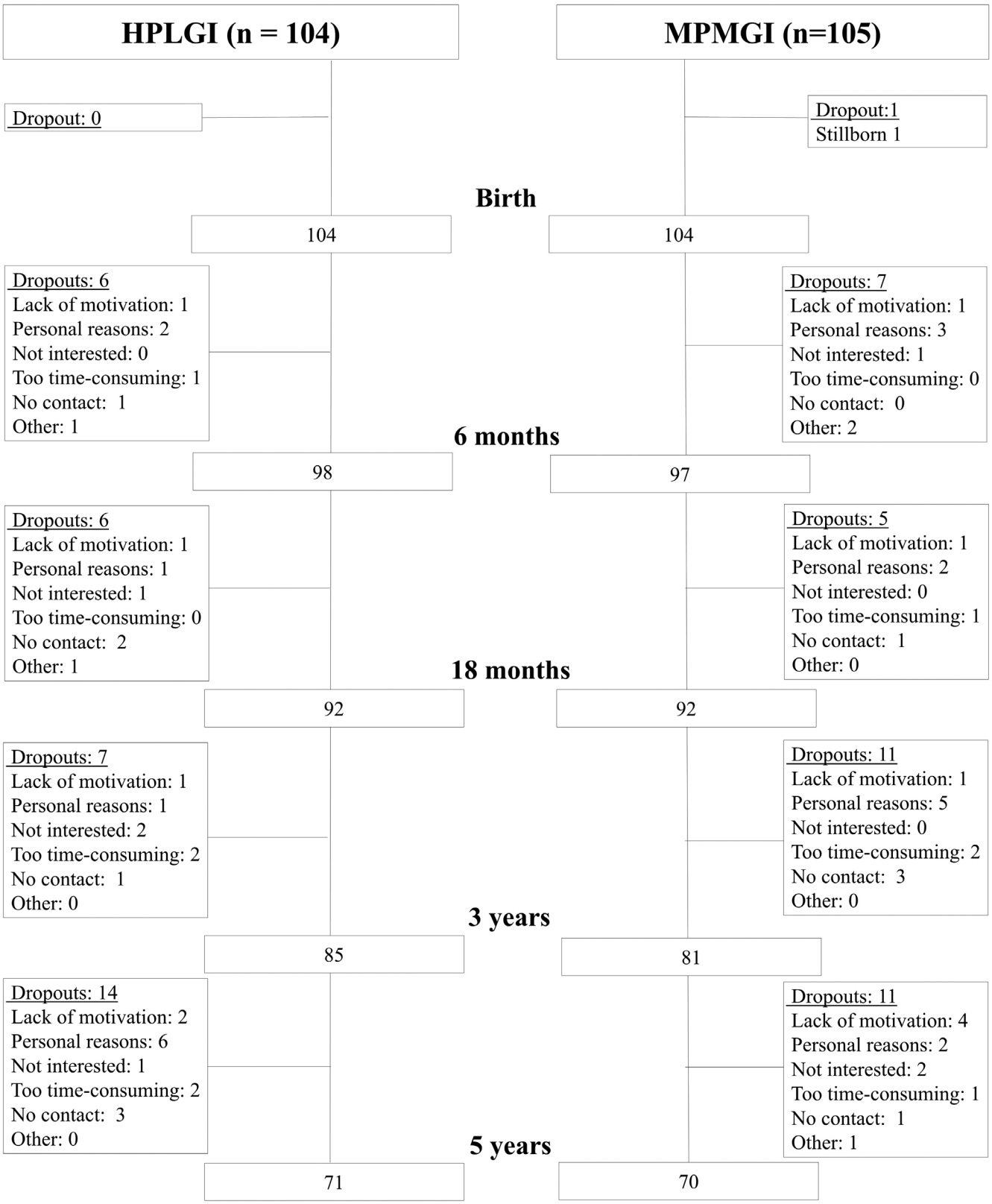

FIGURE 1 Flowchart. HPLGI—high-protein low-glycaemic-index; MPMGI—moderate-protein moderate-glycaemic-index.

compared with the MPMGI group. At 6 months and 3 years of age, there were no significant differences in offspring body composition between the HPLGI diet and MPMGI diet groups (Table 2). We also examined the shifts in the BMI z-score distributions at birth, 6 months, 18 months, 3 years and 5 years of age. The median of the BMI distribution remained relatively similar across time among the HPLGI and MPMGI offspring from birth to 18 months. However, the BMI z-score distribution of offspring in the HPLGI became

TABLE 2 Offspring anthropometry.

	N	HPLGI	*N*	MPMGI	(HPLGI-MPMGI Estimate)	*p*-value
Body weight, kg						
Birth	103	3.56 (0.14)	104	3.57 (0.14)	−0.01 (−0.41:0.38)	0.943
6 months	96	8.28 (0.15)	96	8.37 (0.15)	−0.09 (−0.49:0.31)	0.669
18 months	91	11.70 (0.15)	91	11.83 (0.15)	−0.12 (−0.53:0.29)	0.561
3 years	81	15.24 (0.16)	75	15.28 (0.16)	−0.04 (−0.48: 0.39)	0.848
5 years	71	19.91 (0.16)	69	19.89 (0.17)	0.03 (−0.43:0.48)	0.911
Body height/length, cm						
Birth	103	51.8 (0.30)	104	51.7 (0.30)	0.06 (−0.76:0.89)	0.878
6 months	96	68.9 (0.31)	96	68.7 (0.31)	0.21 (−0.64: 1.05)	0.629
18 months	89	82.8 (0.31)	91	82.9 (0.31)	−0.11 (−0.97: 0.76)	0.808
3 years	81	96.5 (0.32)	75	96.4 (0.33)	0.10 (−0.80:1.00)	0.830
5 years	71	112.6 (0.34)	70	112.9 (0.34)	−0.33 (−1.26:0.60)	0.491
BMI z-score						
Birth	103	−0.21 (0.10)	104	−0.21 (0.10)	0.00 (−0.28:0.29)	0.978
6 months	96	0.15 (0.11)	96	0.33 (0.11)	−0.18 (−0.48:0.11)	0.215
18 months	89	0.78 (0.11)	91	0.86 (0.11)	−0.08 (−0.38:0.22)	0.599
3 years	81	0.57 (0.11)	75	0.66 (0.12)	−0.09 (−0.41:0.22)	0.567
5 years	71	0.24 (0.12)	69	0.20 (0.12)	0.03 (−0.29:0.36)	0.839
Weight z-score						
Birth	103	0.48 (0.10)	104	0.50 (0.10)	−0.02 (−0.28:0.25)	0.897
6 months	96	0.62 (0.10)	96	0.69 (0.10)	−0.07 (−0.34:0.20)	0.615
18 months	91	0.79 (0.10)	91	0.84 (0.10)	−0.04 (−0.32:0.23)	0.761
3 years	81	0.53 (0.10)	75	0.58 (0.11)	−0.05 (−0.34:0.24)	0.745
5 years	71	0.54 (0.11)	69	0.56 (0.11)	−0.02 (−0.33:0.28)	0.879
Length z-score						
Birth	103	1.20 (0.10)	104	1.17 (0.10)	0.03 (−0.26:0.32)	0.839
6 months	96	0.89 (0.11)	96	0.79 (0.11)	0.10 (−0.19:0.40)	0.496
18 months	89	0.78 (0.11)	91	0.84 (0.11)	−0.06 (−0.36:0.24)	0.698
3 years	81	0.20 (0.11)	75	0.21 (0.12)	−0.01 (−0.33:0.31)	0.959
5 years	71	0.60 (0.12)	70	0.69 (0.12)	−0.09 (−0.42:0.24)	0.592
Abdomen circumference, cm						
Birth	98	33.1 (0.31)	95	33.8 (0.31)	−0.65 (−1.51:0.21)	0.142
6 months	96	43.8 (0.31)	94	43.9 (0.31)	−0.04 (−0.91:0.82)	0.919
18 months	90	47.2 (0.32)	90	47.9 (0.32)	−0.64 (−1.52:0.24)	0.157
3 years	79	50.7 (0.34)	71	50.5 (0.35)	0.19 (−0.76:1.14)	0.702
5 years	68	54.0 (0.36)	62	54.0 (0.37)	−0.09 (−1.10:0.91)	0.856
Abdomen skinfold, mm						
6 months	56	10.2 (0.30)	58	10.4 (0.29)	−0.23 (−1.03:0.58)	0.586
18 months	82	8.8 (0.26)	81	8.3 (0.26)	0.48 (−0.23:1.19)	0.187
3 years	74	8.5 (0.27)	66	7.7 (0.28)	0.719 (−0.03:1.47)	0.063
5 years	62	9.1 (0.28)	56	8.1 (0.30)	0.94 (0.14:1.74)	**0.022**
Biceps skinfold, mm						
6 months	56	7.3 (0.24)	58	7.0 (0.23)	0.23 (−0.41:0.88)	0.482
18 months	83	7.3 (0.20)	86	6.6 (0.19)	0.72 (0.19:1.26)	**0.009**
3 years	77	7.0 (0.20)	69	6.5 (0.22)	0.51 (−0.07:1.08)	0.089
5 years	67	6.9 (0.22)	61	6.2 (0.23)	0.69 (0.08:1.31)	**0.029**

TABLE 2 (Continued)

	N	HPLGI	*N*	MPMGI	(HPLGI-MPMGI Estimate)	*p*-value
Triceps skinfold, mm						
6 months	55	9.6 (0.32)	59	10.2 (0.31)	−0.57 (−1.42:0.29)	0.195
18 months	83	10.3 (0.26)	87	9.4 (0.26)	0.90 (0.18:1.62)	**0.015**
3 years	75	10.0 (0.28)	68	9.5 (0.29)	0.45 (−0.32:1.23)	0.258
5 years	65	9.8 (0.29)	59	9.5 (0.31)	0.31 (−0.51:1.14)	0.459
Fat mass, kg (5 years)	61	2.86 (1.71)	57	2.58 (1.32)	0.28 (−0.28:0.84)	0.323
Fat percentage, % (5 years)	61	13.7 (6.05)	57	12.6 (5.55)	1.14 (−0.98:3.27)	0.288
Fat-free mass, kg (5 years)	61	17.1 (1.66)	57	17.4 (1.83)	−0.28 (−0.91:0.36)	0.390

Note: Data are presented as estimated means (SE) and the differences between groups are presented as estimated means (CI). Bold indicates significantly different from the MPMGI group.
Abbreviations: HPLGI, high-protein low-glycaemic-index; MPMGI, moderate-protein moderate-glycaemic-index.

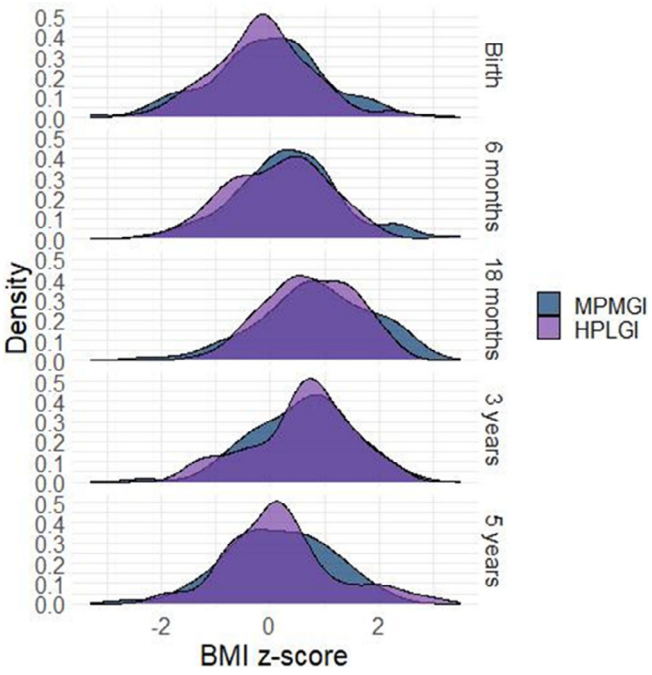

FIGURE 2 Distribution of BMI-for-age z-scores at birth, 6 months, 18 months, 3 years and 5 years of age in the HPLGI and MPMGI maternal diet groups. HPLGI, high-protein low-glycaemic-index; MPMGI, moderate-protein moderate-glycaemic-index.

increasingly left-skewed at 3 years of age ($p = 0.037$), whereas, at 5 years of age, the destitution of BMI z-scores in offspring in HPLGI was right-skewed ($p = 0.037$), indicating that more offspring in the HPLGI group had a BMI z-score above 2 SD, as shown by the length of the tail above 2 SD (Figure 2).

3.2 | Offspring blood biomarkers

At birth, glucose levels were higher in infants of the HPLGI than the MPMGI group ($p = 0.017$) and insulin levels tended to be lower by 27% (log-transformed; $p = 0.056$). At 3 years of age, HPLGI offspring had lower levels of HDL-cholesterol ($p = 0.018$) and 16% (log-transformed; $p = 0.044$) higher levels of TG compared with MPMGI offspring. At 5 years of age, HPLGI offspring had higher levels of total cholesterol ($p = 0.027$) and LDL-cholesterol ($p = 0.003$) compared with MPMGI offspring (Table 3).

4 | DISCUSSION

This unique study found no differences in BMI z-scores, FM, FFM or fat% but a tendency of unfavourable body composition in offspring of the HPLGI group. However, this needs to be further investigated with more advanced techniques for body composition, such as dual-energy x-ray (DEXA) and magnetic resonance imaging (MRI) later in life. Offspring born by women on the HPLGI diet had higher glucose levels and tended to have lower insulin levels at birth compared with MPMGI offspring. At 3 years of age, HPLGI offspring had lower levels of HDL-cholesterol and higher levels of TG, and at 5 years of age, they had higher levels of total cholesterol and LDL-cholesterol, compared with MPMGI offspring. Overall, these data suggest that an HPLGI diet during pregnancy may affect—likely in an unfavourable way—cardiometabolic risk factors and body composition in the offspring during the first 5 years of life. However, at this stage of life, it remains unclear whether the observed differences between the two groups are clinically relevant.

Our findings suggest that an HPLGI diet during pregnancy may worsen offspring metabolic health, despite being generally viewed as beneficial for non-pregnant individuals.[22] One potential explanation is that maternal excessive protein intake could disrupt insulin sensitivity and glucose regulation, altering the metabolic environment during a critical period of organ development which may lead to long-term metabolic alterations in the offspring. However, the specific mechanisms remain unclear and require further investigation. Fetal programming during early gestation, particularly before gestational week 15, might be a crucial period when maternal diet and other environmental factors can impact the development of metabolic pathways in the offspring. Although our study did not specifically focus on this

TABLE 3 Offspring biomarkers.

	N	HPLGI	*N*	MPMGI	(HPLGI-MPMGI Estimate)	*p*-value
Glucose (mmol/L)						
Birth	72	5.58 (0.12)	55	5.15 (0.14)	0.43 (0.08:0.78)	**0.017**
3 years	64	5.05 (0.13)	54	5.03 (0.14)	0.02 (−0.34:0.38)	0.906
5 years	47	5.06 (0.15)	47	5.09 (0.15)	−0.03 (−0.44:0.37)	0.870
Insulin (pmol/L)[a]						
Birth	71	27.1 (22.0:33.4)	56	37.0 (29.0:47.2)	0.73 (0.53:1.01)	0.056
3 years	61	43.4 (34.7:54.4)	53	41.5 (32.6:52.8)	1.05 (0.76:1.45)	0.784
5 years	43	18.2 (13.9:23.8)	40	14.8 (11.2:19.6)	1.23 (0.84:1.79)	0.299
C-peptide (pmol/L)[a]						
Birth	71	302 (264;346)	55	306 (263;357)	0.99 (0.74; 1.33)	0.901
3 years	63	378 (328;436)	53	365 (312;426)	1.04 (0.76;1.41)	0.736
5 years	45	215 (182;255)	40	182 (153;218)	1.18 (0.83;1.69)	0.181
Cholesterol (mmol/L)						
Birth	72	1.61 (0.06)	56	1.67 (0.07)	−0.06 (−0.25:0.12)	0.498
3 years	62	3.77 (0.06)	54	3.75 (0.07)	0.02 (−0.17:0.21)	0.844
5 years	46	3.71 (0.08)	40	3.46 (0.08)	0.25 (0.03:0.47)	**0.027**
HDL (mmol/L)						
Birth	72	0.63 (0.03)	56	0.67 (0.03)	−0.04 (−0.11:0.04)	0.350
3 years	62	1.19 (0.03)	54	1.28 (0.03)	−0.09 (−0.17:-0.02)	**0.018**
5 years	46	1.38 (0.03)	40	1.41 (0.03)	−0.03 (−0.12:0.06)	0.525
LDL (mmol/L)						
Birth	72	0.65 (0.05)	56	0.66 (0.06)	−0.02 (−0.16:0.13)	0.840
3 years	62	2.17 (0.05)	54	2.14 (0.06)	0.03 (−0.12:0.19)	0.689
5 years	46	2.30 (0.06)	40	2.03 (0.07)	0.27 (0.09:0.45)	**0.003**
TG (mmol/L)[a]						
Birth	72	0.53 (0.48;0.58)	56	0.48 (0.43;0.53)	1.10 (0.90;1.26)	0.190
3 years	62	0.85 (0.77;0.94)	54	0.73 (0.65:0.81)	1.16 (1.01;1.34)	**0.044**
5 years	46	0.55 (0.49;0.62)	40	0.49 (0.43;0.55)	1.14 (0.97;1.35)	0.123
IGF-1 (ng/mL)						
Birth	71	55.97 (3.04)	54	56.26 (3.48)	−0.29 (−9.29:8.68)	0.950
3 years	18	68.78 (5.89)	20	78.21 (5.61)	−9.43 (−25.24;6.37)	0.247
5 years	45	95.51 (3.78)	40	93.94 (4.02)	1.57 (−9.19;12.3)	0.776
IGFBP-3 (ug/mL)						
Birth	71	1.70 (0.06)	55	1.72 (0.07)	−0.02 (−0.21;0.17)	0.847
3 years	18	3.04 (0.12)	20	3.39 (0.12)	−0.34 (−0.67;-0.01)	**0.046**
5 years	45	3.86 (0.08)	40	3.85 (0.08)	0.01 (−0.22;0.24)	0.917
CRP (mg/L)[a]						
Birth	72	0.16 (0.13;0.20)	56	0.16 (0.13;0.21)	0.98 (0.60;1.59)	0.891
3 years	60	0.37 (0.29;0.47)	54	0.37 (0.29;0.48)	1.00 (0.60;1.67)	0.998
5 years	46	0.34 (0.26;0.45)	40	0.28 (0.21;0.37)	1.23 (0.68;2.22)	0.321

Note: Data are presented as estimated means (SE), and the differences between groups are presented as estimates (CI).

Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; HPLGI, high-protein low-glycaemic-index; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein 3; LDL, low-density lipoprotein; MPMGI, moderate-protein moderate-glycaemic-index; TG, triglycerides.

[a]Unfitted models were log-transformed and estimates, and 95% CI were back-transformed and expressed as estimated median (95% CI). The differences between groups are presented as a ratio (%). Bold indicates significantly different from the MPMGI group.

window, it is plausible that maternal nutrition or metabolic profile before conception and in early pregnancy plays an important role.[23] There are only a limited number of dietary intervention studies during pregnancy where long-term follow-up of the offspring are performed. However, dietary interventions implemented during pregnancy facilitate improved maternal dietary habits and limit GWG but suggest to not affect offspring obesity during the first 5 years of life.[24] In observational studies with mother–child dyads, the association between pre-pregnancy BMI and GWG with offspring BMI z-score is apparent at birth[25] and may also manifest beyond the initial 5 years of age.[26–28] We did not find an effect of lower GWG with birth weight; however, we believe the lack of difference is greatly caused by the control group managing to limit their GWG while consuming a healthy diet.

A multi-individual meta-analysis demonstrated that maternal obesity is associated with a higher risk of children with overweight or obesity throughout childhood, with a stronger association at later ages. The risk ratio of children being overweight or obese was 2.43 in early childhood (2–5 year) and 4.47 in late childhood (10–18 year), in children born to mothers with obesity compared to mothers with normal body weight. This risk increased further for higher BMI classes of maternal obesity.[26] Moreover, excessive GWG increased the risk of being overweight in early childhood (2–5 year) by 39% and in late childhood (10–18 year) by 72%. Therefore, it seems that pre-pregnancy BMI increases the risk for the offspring having overweight or obesity to a much greater extent than GWG. These observations could potentially explain why we did not find a significant difference in BMI z-score during the first 5 years of life, given our two groups of women were similar in pre-pregnancy BMI. Moreover, our study was limited by the use of indirect measures of body composition in the offspring. As a result, the observed null findings could be influenced by the lower sensitivity of these proxy measures. Future long-term follow-up should incorporate more sensitive and direct assessments, such as DEXA or MRI, to better evaluate fat distribution and metabolic health outcomes in the offspring. However, longitudinal tracking of body composition may be difficult as not all methodologies are appropriate for newborns and young infants, as compared to children aged 3–5 years.

Glycaemic index in maternal diet is positively associated with offspring adiposity and offspring insulin resistance, especially if the fetus is exposed to gestational diabetes mellitus (GDM).[29] Maternal diet during pregnancy alters the intra-uterine environment and can affect the mechanisms regulating glucose homeostasis in the offspring, that is, insulin sensitivity and insulin secretion. Since glucose crosses the placenta while insulin cannot, maternal hyperglycaemia during pregnancy increases glucose levels in the developing fetus, thereby triggering an increase in insulin secretion. In infants born to mothers with GDM, the prevalence of hypoglycaemia in response to hyperinsulinemia at birth is well-documented.[30] Notably, in our study, HPLGI offspring exhibited significantly greater glucose levels and a trend toward decreased insulin levels at birth.

Dietary protein modulates glucose metabolism and insulin secretion in males and non-pregnant females, and the amount and type of protein (and its amino acid composition) may influence relevant metabolic outcomes. Ingestion of protein alone increases secretion of both insulin and glucagon and increases glucose turnover rates (both endogenous glucose production and peripheral glucose uptake) without affecting net glucose levels.[31] Sufficient maternal protein intake during pregnancy is crucial for fetal growth and development. Both excessive and inadequate maternal protein intakes can lead to adverse pregnancy outcomes, including impaired fetal growth.[32] Nevertheless, increased exposure to protein during fetal development is speculated to influence the future offspring protein requirements and appetite regulation, and has been suggested to 'upregulate' offspring protein requirements contributing to calorie overconsumption and weight gain.[33] Additionally, high-protein intake during infancy (from birth to 2 year) is associated with later obesity outcomes,[34–36] suggesting a long-term risk for overweight and obesity in offspring with prolonged exposure to high-protein levels during early life.

There is some uncertainty regarding the physiological and clinical relevance of differences in traditional metabolic risk factors early in life. For example, in studies with breastfed infants compared with formula-fed infants (age < 1 year), those who were exposed to an exogenous provision of cholesterol through breast milk had higher total cholesterol levels compared with formula-fed infants.[37] Yet, in adulthood, total cholesterol levels were lower among individuals who were breastfed for a longer period.[37] It thus seems that offspring with prolonged exposure to breastmilk become adults with a healthier metabolic profile compared with adults who were not breastfed. This observation suggests the presence of a protective mechanism that may confer resistance against unfavourable lipid (and glucose) metabolism in later life. This protective mechanism may arise from preparatory adaptations that occur during infancy; however, the mechanisms are unclear. In our study, we did not find any significant difference in breastfeeding duration between the two groups (Table S2). Offspring under the age of 2 years are advised to consume a high-fat and low-protein diet to mitigate the risk of childhood obesity.[34,36,38] We found that offspring of mothers who consumed the HPLGI diet during pregnancy had higher total and LDL-cholesterol and lower HDL-cholesterol levels between the ages of 3 and 5 years, compared with offspring born to mothers who consumed the MPMGI diet.

Both insulin and insulin-like growth factor (IGF)-1 are involved in receptor-mediated regulation of placental growth and nutrient transport. Dysregulation of this process, as seen in women with diabetes or GDM, leads to elevated plasma amino acid levels, enhancing placental and fetal growth.[39] In our study, women consumed a high-protein diet during pregnancy, which likely increased amino acid availability and other growth-related factors for the placenta and fetus. Therefore, this mechanism may potentially explain the link between maternal protein intake and adverse metabolic outcomes in offspring. Maternal protein intake may therefore help explain the effect of the HPLGI diet on offspring metabolic markers, which would imply that the recommendation for limited protein consumption from infancy to 2 years of age may need to be extended to cover the fetal life as well (i.e. pregnancy).

To follow the HPLGI diet, a simple tool is to reduce carbohydrate intake or increase fat intake. It has been reported that maternal fat intake and lipid metabolism may affect early childhood blood lipid profile and adiposity. Elevated maternal fat intake during pregnancy could

contribute to altered fetal fat accumulation and adverse early childhood outcomes.[40–42] We found the HPLGI group consuming significantly more protein, less carbohydrate and more fat in gestational week 21 (Table 1). However, this difference in macronutrient intake did not lead to significant changes in maternal biomarkers, except for TG, which was lower in the HPLGI group in gestational week 28 (Table S2). Nevertheless, further research is needed to investigate the long-term health outcomes of the maternal diet in the offspring.

Our study is a unique follow-up of offspring born to women who participated in a dietary RCT; however, it is not without limitations. Excluding participants who did not complete the intervention may introduce bias, as those who dropped out could differ significantly from those who completed the study, particularly in terms of motivation, response to the treatment or baseline characteristics. To address this limitation, we conducted a sensitivity analysis, which revealed no significant differences in maternal characteristics. Despite the difference in protein intake and glycaemic index between the two diets, both contained a relatively high amount of protein compared with the average Danish diet (~15 E% protein).[43] Furthermore, although diet composition can influence offspring cardiometabolic health,[34,38] our dataset lacked information on offspring energy intake and macronutrient composition but data on breastfeeding and introduction to solid foods were available. However, given that the women were randomized during pregnancy, it is improbable that there was a systematic difference in offspring diet between the two groups. While our study highlights possible unfavourable effects of an HPLGI diet during pregnancy on offspring metabolic health, the power calculation of the APPROACH study was based on the effect of the intervention on maternal GWG, which is a limitation of the follow-up study. Moreover, over the course of the follow-up study, the dropout was 30%, but compared favourably with other studies which have a dropout rate of 30%–50%.[44–46] Despite these limitations, our study provides valuable insights and highlights the importance of further research to elucidate the complex interplay between maternal diet and offspring health and later risk for cardiometabolic disease. The potentially unfavourable consequences of an HPLGI diet during pregnancy on the metabolic profile of the offspring underscore the need for careful consideration in formulating dietary recommendations for pregnant women.

In conclusion, our study found that an HPLGI diet during pregnancy did not significantly affect offspring BMI z-scores during the first 5 years of life. However, while our data suggest early metabolic changes in offspring, it is premature to conclude that these will lead to adverse long-term health outcomes. Further longitudinal studies are necessary to assess whether these early effects result in metabolic disorders like obesity or insulin resistance in later life. Therefore, our findings should be seen as preliminary and highlighting potential risks that warrant further exploration.

AUTHOR CONTRIBUTIONS

Nina Rica Wium Geiker conceptualized the study and acquired funding. Nina Rica Wium Geiker led the study design and was responsible for data collection. Helle Zingenberg was active in the recruitment process and was involved in initiating the project. Christina Sonne Mogensen led the data analysis. Christina Sonne Mogensen, Nina Rica Wium Geiker and Faidon Magkos drafted the manuscript with all authors providing critical revision of the manuscript for important intellectual content and approval of the final submitted version. Christina Sonne Mogensen has full access to all the data in the study and takes responsibility for the integrity of the data. The corresponding author confirms that all listed authors meet authorship criteria, and no eligible contributors have been omitted. All authors endorsed the final manuscript and accepted accountability for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared.

DATA AVAILABILITY STATEMENT

Data described in the manuscript, code book and analytic code will not be made available because of confidentiality and ethical concerns. The data contain sensitive information requiring careful handling to protect participant privacy.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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