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Optimal protein intake during pregnancy for reducing the risk of fetal growth restriction: the Japan Environment and Children's Study

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Abstract

Clinical trials show that protein supplement increases infant size in malnourished populations; however, epidemiological studies in high-income countries have reported mixed results. Although these findings suggest a non-linear relationship between maternal macronutrient intake and fetal growth, this relationship has not been closely examined. We assessed the association between maternal protein intake and fetal growth among 91 637 Japanese women with singletons in a nation-wide cohort study using validated FFQ. The respondents answered the FFQ twice, once during early pregnancy (FFQ1; 16·3 (SD 6·0) weeks), and second during mid-pregnancy (FFQ2, 28·1 (SD 4·1) weeks). Daily energy intake and percentage energy from protein, fats and carbohydrates were 7477 (SD 2577) kJ and 13·5 (SD 2·0), 29·5 (SD 6·5) and 55·3 (SD 7·8) %, respectively, for FFQ1, and 7184 (SD 2506) kJ and 13·6 (SD 2·1), 29·8 (SD 6·6) and 55·3 (SD 7·9) %, respectively, for FFQ2. The average birth weight was 3028 (SD 406) g, and 6350 infants (6·9 %) were small for gestational age (SGA). In both phases of the survey, birth weight was highest and the risk of SGA was lowest when the percentage energy from protein was 12 %, regardless of whether isoenergetic replacement was with fat or carbohydrates. Furthermore, when protein density in the maternal diet was held constant, birth weight was highest when 25 % of energy intake came from fat and 61 % came from carbohydrates during early pregnancy. We found maternal protein intake to have an inverse U-curve relationship with fetal growth. Our results strongly suggest that the effect of protein on birth weight is non-linear, and that a balanced diet fulfilling the minimum requirement for all macronutrients was ideal for avoiding fetal growth restriction.

Key words: Protein: Macronutrients: Maternal diet: Fetal growth: Small-for-gestational age

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Abbreviations: JECS, Japan Environment and Children's Study; SGA, small for gestational age.

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Adequate nutrition during pregnancy is crucial for fetal development. The theory of the fetal origins of adult disease has renewed interest in nutrition during pregnancy. A study of survivors of the Dutch famine demonstrated that low birth weight due to maternal malnutrition increased infant susceptibility to CVD and type 2 diabetes in adulthood[1,2]. Among the nutrient groups assessed, proteins have emerged as an important factor related to birth weight.

Studies on protein intake during pregnancy suggested that while fulfilling the minimum protein requirement may help prevent fetal growth restriction, a higher protein intake may be more harmful than beneficial. A rodent model has repeatedly demonstrated that protein deprivation can hamper fetal growth[3–6] and cause permanent changes in the structure of tissues and organs and physiological functions in offspring[1,3,6–8]. In low- and middle-income countries, as well as in populations where malnutrition is common, protein supplementation has been shown to reduce the risk of low birth weight and small for gestational age (SGA) births[9–11]. However, randomised interventional studies using supplementation with excessive protein (>20 % of energy as protein) have repeatedly shown that increased protein intake decreased fetal growth[12,13], especially in high-income countries.

Based on evidence that both high and low protein intake may reduce fetal growth, current recommendations suggest a maternal dietary protein intake of 10–20 % of the total energy intake[14]. However, the ‘optimal’ protein intake for pregnant women is unknown. The interventional studies on this topic have thus far been two-armed and therefore unsuitable for detailed calculation. Most previous epidemiological studies have proposed nothing more complex than a linear association between protein intake and birth outcomes with inconsistent results. We were able to find only two studies, one from New Zealand with 504 women[15] and another from Australia with 1040 women[16], which suggested a non-linear relationship between maternal macronutrient intake and fetal growth. However, while both studies found that a quadratic model fit better than a linear model, the studies were relatively small and had insufficient power to produce conclusive results. Furthermore, most research on maternal macronutrients has focused on protein, and only a few studies have examined the importance of the other two macronutrients, that is, carbohydrates and fats.

Using data from a nationwide cohort study involving nearly 100 000 women and the cubic-spline model, we evaluated the association between maternal macronutrient intake and fetal growth.

Methods

Study sample

This study was based on data obtained from the Japan Environment and Children’s Study (JECS), a Japan-wide prospective cohort study of pregnant women, their spouses and their children[17,18]. Pregnant women in any of the fifteen study regions throughout Japan were recruited from January 2011 to March 2014 either at their first antenatal visit at the participating, local health care institution or at a local government office issuing the so-called Mother and Child Health Handbook, a booklet issued to all expecting mothers in Japan enabling them to receive municipal health care services for their pregnancy, delivery and childcare. Women who were unable to participate in the survey or had difficulty filling out the questionnaire in Japanese were excluded. During pregnancy, the participants were asked to respond to two surveys covering demographics, life style, behaviour and medical history; one questionnaire was administered at recruitment (MT1) and the other was administered later during the pregnancy (MT2). Both surveys included a FFQ. Birth characteristics and medical information were collected separately from the medical records (Dr0m).

For this study, we used the dataset for birth outcomes ‘jecs-ag-20160424’, which was created in April 2016 and revised in October 2016. From the 104 102 births, we excluded multiple pregnancies, preterm deliveries before 28 weeks, post-term deliveries after 42 weeks, births with missing background characteristics and births from heavily obese women (BMI >35 kg/m^2). In total 91 637 (90 %) subjects were enrolled (Fig. 1). Among these, 79 578 (87 %) underwent gestational weight gain measurement at 6–14 weeks and had answered the first FFQ (daily total energy intake of 2092–18828 kJ); 82 250 (89 %) underwent a gestational weight gain measurement at 20–28 weeks and had answered the second FFQ (daily total energy intake of 2092–18828 kJ).

FFQ

Our FFQ, listing 172 food and beverage item, asked about respondents’ habitual consumption of the listed food items using three portion sizes and nine frequency categories[19]. The FFQ has been previously validated using 3-d dietary records and blood samples[20,21] in adults, but not specifically in pregnant women. The intake of energy was calculated by food group using a food composition table developed for the FFQ based on the Standardized Tables of Food Composition developed in Japan (2010 edition). The first FFQ (FFQ1) was administered during early pregnancy and asked about respondents’ diet over the past year while the second FFQ (FFQ2) was administered during mid pregnancy and asked about respondents’ diet during pregnancy (from conception to the answering date).

Protein, fat and carbohydrate density was calculated as a percentage of the energy intake from that nutrient; for example, protein density was calculated using the formula: (protein intake (g) reported in FFQ × 4 (kJ/g)/energy intake (kJ) reported in FFQ).

Demographic data

Maternal socio-demographic data collected from the responses to the second questionnaire were categorised by annual household income (<2 million yen, 2–4 million yen, 4–6 million yen, 6–8 million yen, >8 million yen and no answer); maternal education (university education or higher, 2-year college, vocational school, high school or lower) and smoking status

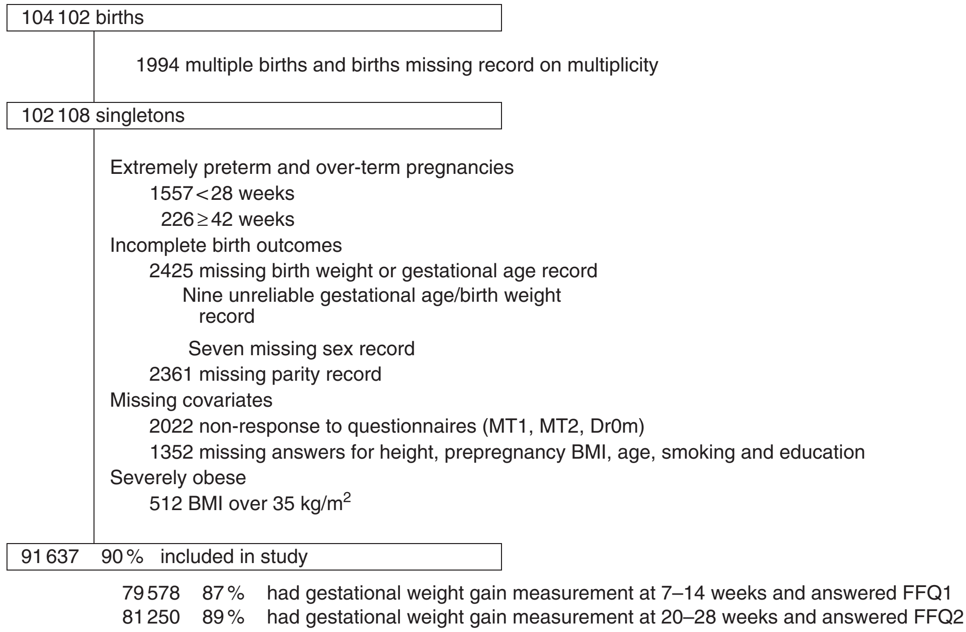

Fig. 1. Population flow chart.

(never smoked, previously smoked but stopped before pregnancy, previously smoked but stopped because of pregnancy and current smoker). Pre-pregnancy BMI was calculated from height and pre-pregnancy weight (either self-reported or measured) and categorised as under 18·5, 18·5–25 or 25 kg/m^2 or higher. Weight measurements at the antenatal check-up visit at 7–14 and 20–28 weeks were collected from the Mother-Child Health Handbook and used to calculate gestational weight gain during early and mid-pregnancy.

Other baseline characteristics retrieved from the medical records were maternal age (<25, 25–34, ≥35 kg/m^2) and parity (0, 1 or more). Data on birth outcomes (gestational age and birth weight) were also obtained from the medical records. SGA was defined as a birth weight below the tenth percentile of the normal population at each day of gestation and was stratified by sex and parity using the Japanese birth weight reference chart[22]. The same chart was used to calculate the birth weight *z*-score for each infant.

Ethics

The JECS protocol was approved by the review board for epidemiological studies of the Ministry of the Environment and by the Ethics Committees of all the participating institutions. The JECS protocol was conducted in accordance with the Helsinki Declaration and other nationally valid regulations, and written informed consent was obtained from each participant.

Statistics

We calculated the average birth weight and proportion of SGA of infants based on the characteristics of the study population and the average dietary profile of the respondents to the two FFQ.

Next, we used the cubic spline model to analyse the association between maternal macronutrient intake and birth outcomes, namely, birth weight and risk of SGA. In the restricted cubic spline, all data points are used to estimate the dose–response association between the continuous independent variable and the outcome, thus allowing non-linear associations. The OR (for SGA) and change in birth weight, as well as their accompanying 95 % CI, were calculated with four knots placed at the 5th, 50th, 85th and 95th percentiles of the density of each macronutrient.

As our primary exposure of interest was maternal protein intake, our main analysis focused on the association of protein density in the maternal diet as assessed by FFQ1 (of the pre-pregnancy period to the first trimester) with birth weight and the risk of SGA. To evaluate the effect of protein relative to other macronutrients in an isoenergetic diet, a substitution model using the multivariate nutrient density method was employed[23].

Next, we created a model in which both energy and protein density were constant in order to observe the association between carbohydrate density and fetal growth when isoenergetic replacement was with fat, and conversely the association between fat density and fetal growth when isoenergetic replacement was with carbohydrates.

Due to the possibility that some women may have modified their diet after becoming pregnant, we conducted a sensitivity analysis using the maternal diet as assessed in FFQ2 (covering diet from conception to the second trimester).

Maternal age, parity, education, income, prepregnancy BMI, height, smoking status and infant sex were treated as potential confounders in all our analyses, and adjustments were made for recruitment site, total energy intake as reported in the FFQ, and gestational weight gain and age at the time of the survey (which also reflected energy intake and contained fewer measurement errors than the total energy value reported in the FFQ).

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All statistical analyses were conducted using the statistical software package Stata 13 (Stata Corp). A P-value <0·05 was considered to be statistically significant when performing hypothesis tests. The spline curve was constructed using the ‘mkspline’ command in STATA. As this was a secondary analysis of a large cohort study, sample size calculation was not conducted before analysis.

Results

Table 1 shows the birth outcomes according to the characteristics of the study population. The average birth weight was 3028 (SD 406) g, and 6350 (6·9 %) births were SGA. The proportion of SGA births was highest among women who were shorter, thinner or continued to smoke during pregnancy. The proportion of SGA births was also higher among women from a lower income or educational level, older women, primiparae and women with low gestational weight gain until 20–28 weeks.

Table 2 shows the nutritional characteristics. The mean daily energy intake and percentage energy from protein, fats and carbohydrates were 1787 (7477 (SD 2577) kJ and 13·5 (SD 2·0), 29·5 (SD 6·5) and 55·3 (SD 7·8) %, respectively, for FFQ1, and 7184 (SD 2506) kJ and 13·6 (SD 2·1), 29·8 (SD 6·6) and 55·3 (SD 7·9) %, respectively, for FFQ2. The mean gestational weight gain for measurements when the two questionnaire were answered were 0·5 (SD 2·0) and 11·1 (SD 1·6) kg, respectively.

The cubic-spline model demonstrated a dose–response relationship between maternal protein density as reported in FFQ2 and birth outcomes after adjusting for maternal

Table 1. Birth weight and fetal growth by characteristic (*n* 91 637)
(Numbers and percentages; mean values and standard deviations)

			Birth weight (g)		SGA	
	n	%	Mean	SD	*n*	%
Maternal age (years)						
<25	10 382	11·3	3026	389	703	6·8
25–34	59 120	64·5	3033	399	4076	6·9
≥35	22 135	24·2	3019	429	1571	7·1
Birth order						
First child	36 891	40·3	2996	406	2669	7·2
Not first child	54 746	59·7	3050	404	3681	6·7
Height (cm)						
130–155	28 951	31·6	2956	399	2661	9·2
156–160	33 618	36·7	3030	398	2206	6·6
161–183	29 068	31·7	3098	408	1483	5·1
Prepregnancy BMI (kg/m^2)						
<18·5	14 839	16·2	2927	387	1526	10·3
18·5–24·9	67 452	73·6	3038	397	4355	6·5
≥25	9346	10·2	3122	459	469	5·0
Annual household income (millions of yen)						
<2	4798	5·2	3014	418	376	7·8
2 to <4	29 484	32·2	3027	409	2088	7·1
4 to <6	28 349	30·9	3031	401	1872	6·6
6 to <8	13 677	14·9	3031	404	938	6·9
≥8	9273	10·1	3032	406	627	6·8
No response	6056	6·6	3026	406	449	7·4
Highest maternal education						
High school or less	33 152	36·2	3027	411	2389	7·2
Vocational school	22 469	24·5	3026	406	1599	7·1
2-year college	16 172	17·6	3029	405	1075	6·6
University or higher	19 844	21·7	3033	397	1287	6·5
Smoking status						
Never smoked	53 003	57·8	3027	403	3646	6·9
Stopped before pregnancy	21 991	24·0	3048	405	1361	6·2
Stopped because of pregnancy	12 443	13·6	3037	412	823	6·6
Current smoker	4200	4·6	2912	407	520	12·4
Gestational weight gain measured at 7–14 weeks (kg)*						
<0	26 776	29·2	3021	397	1835	6·9
0 to <2	37 315	40·7	3027	403	2651	7·1
≥2	16 657	18·2	3059	413	1020	6·1
Gestational weight gain measured at 20–28 weeks (kg)†						
<4	25 901	28·3	2976	409	2311	8·9
4 to <7	34 828	38·0	3027	395	2361	6·8
≥7	21 675	23·7	3103	403	1002	4·6
Infant sex						
Male	46 942	51·2	3071	411	3242	6·9
Female	44 695	48·8	2983	395	3108	7·0

SGA, small for gestational age.
* Among 80 748 with weight measurement at 7–14 weeks.
† Among 82 404 with weight measurement at 20–28 weeks.

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Table 2. Dietary profile of pregnant women during pregnancy
(Mean values and standard deviations)

	FFQ1* (*n* 80 748)		FFQ2† (*n* 82 404)	
	Mean	SD	Mean	SD
Gestational age at survey (weeks)	16·3	6·0	28·1	4·1
Daily energy intake (kJ)	7475·1	2575·7	7183·1	2505·0
Daily protein intake (g)	61·2	25·6	58·9	25·1
Energy from protein (%)	13·5	2·0	13·6	2·1
Daily fat intake (g)	59·9	28·4	58·2	27·9
Energy from fat (%)	29·5	6·5	29·8	6·6
Daily carbohydrates intake (g)	243·4	80·2	233·7	77·0
Energy from carbohydrates (%)	55·3	7·8	55·3	7·9

* FFQ1 was administered during early pregnancy and asked about respondents' diet over the previous year.
† FFQ2 was administered during mid pregnancy and asked about the respondents' diet during pregnancy.

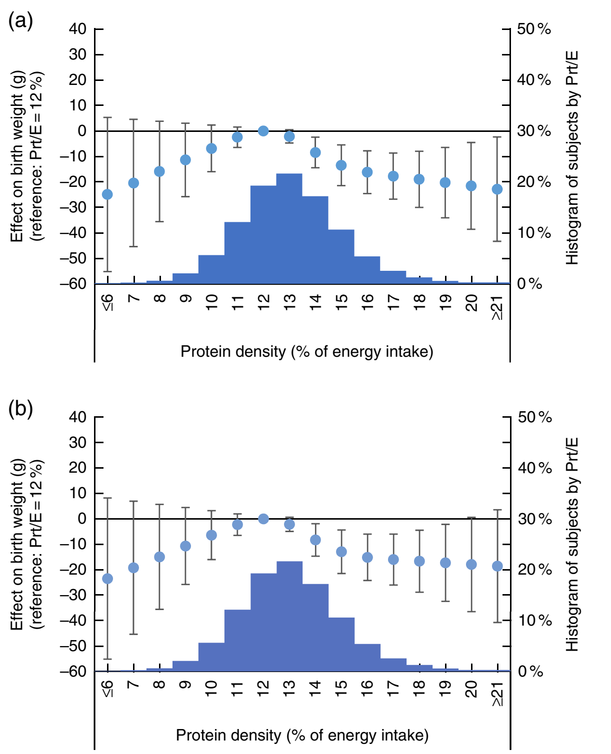

Fig. 2. Association between dietary protein density as assessed in FFQ1 and birth weight. (a) Analysis with isoenergetic replacement of protein with carbohydrate. (b) Analysis with isoenergetic replacement of protein with fat. Prt/E, protein energy percentage. Histogram displays number of subjects by dietary protein density as assessed in FFQ1. Estimated difference in birth weight compared with women who had highest birth weight is shown in circle, with associated 95 % CI shown as whiskers.

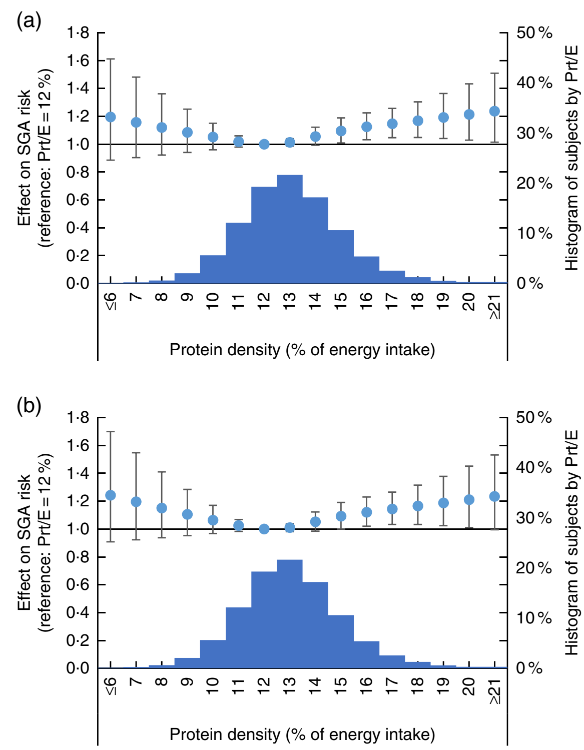

Fig. 3. Association between dietary protein density as assessed in FFQ1 and risk of small for gestational age (SGA) birth. (a) Analysis with isoenergetic replacement of protein with carbohydrate. (b) Analysis with isoenergetic replacement of protein with fat. Prt/E, protein energy percentage. Histogram displays number of subjects by dietary protein density as assessed in FFQ1. Estimated OR of SGA compared with women who had lowest risk of SGA is shown in circle, with associated 95 % CI shown as whiskers.

characteristics and energy intake. The accompanying histograms showing the distribution of protein density are shown in Fig. 2 (for birth weight) and Fig. 3 (for risk of SGA). The reference level of the spline models was set at a protein density of 12 %. Fig. 2 shows that birth weight was highest when percentage energy from protein was 12 %, regardless of whether isoenergetic replacement was with fat or carbohydrates. Women whose diet contained a protein density above 14 % showed a significantly lower birth weight than those whose protein density was 12 %. Similarly, Fig. 3 shows a U-curve relationship between protein density and the risk of SGA; the latter was lowest when percentage energy from protein was 12 %, regardless of whether isoenergetic replacement was with fat or carbohydrates. Women whose diet contained a protein density above 15 % showed a significantly higher risk of SGA compared with those whose protein density was 12 %. The shape of the association between dietary protein density and birth outcomes was similar to that of the maternal diet as assessed in FFQ1 (online Supplementary Appendix S1).

Fig. 4(a) shows the association between dietary carbohydrate density with birth weight while Fig. 4(b) shows the association between dietary fat density with birth weight. The

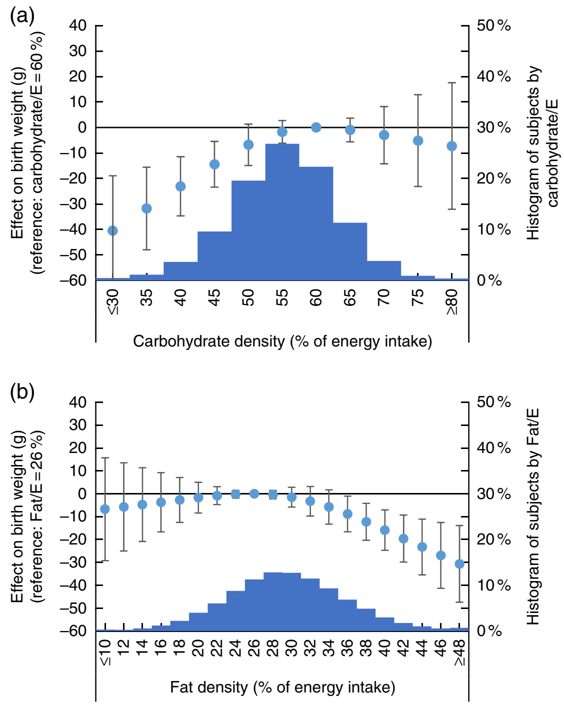

Fig. 4. Association between dietary fat and carbohydrate density as assessed in FFQ1 and birth weight. (a) Analysis with isoenergetic replacement of fat with carbohydrate. (b) Analysis with isoenergetic replacement of carbohydrate with fat. Fat/E, fat energy percentage; carbohydrate/E, carbohydrate energy percentage. Histogram displays number of subjects by dietary protein density as assessed in FFQ1. Estimated difference in birth weight compared with women who had highest birth weight is shown in circle, with associated 95 % CI shown as whiskers.

accompanying histograms show the macronutrient density distribution for each nutrient. All models were adjusted for maternal characteristics, energy intake and protein intake. Fig. 4(a) shows that women whose diet had fat density of 25 % had highest birth weight, with women whose diet had fat density over 35 % having significantly lower birth weight. Fig. 4(b) shows that women whose diet contained a carbohydrate density of 59 % had highest birth weight, with women whose diet had carbohydrate density below 47 % having significantly lower birth weight. On the other hand, no significant difference in birth weight by carbohydrate or fat density of the diet was observed for FFQ2 (online Supplementary Appendix S2).

For both questionnaires, 12 % remained the protein density with highest birth weight in crude analysis not adjusting for possible confounders, and after excluding 2949 women who were diagnosed who developed gestational diabetes during pregnancy (results not shown).

Discussion

Among Japanese women, the association between protein intake and risk of fetal growth restriction described a U-shaped curve, suggesting that both high and low protein intake can increase its risk. Our study demonstrated that fetal growth peaked when protein density in the maternal diet was 12 %. This association was consistent for dietary reporting both in early pregnancy (covering diet over the past year) and in mid-pregnancy (covering diet during pregnancy). We also found that when protein intake was constant, birth weight decreased if <29 % of the energy intake derived from fat or more than 59 % derived from carbohydrates.

Most previous epidemiological studies have failed to consider anything more complex than a linear association between protein intake and birth outcomes. Their findings were also inconclusive. Studies conducted in the UK[8], Australia[16,24] and Spain[25] reported that higher protein density in the maternal diet was associated with a linear increase in birth weight; however, studies in the USA demonstrated that a higher protein intake was associated with reduced fetal growth[12,13,26]. Other studies[27–30] failed to find any significant linear association. The present study suggests that the relationship is not linear but U-shaped; hence the discrepancies with previous studies may have resulted in part from differences in the mean protein intake in the population (e.g., women in the USA generally have a high protein intake)[26]. Such interpretations have been proposed in smaller studies[12,15] but have not been confirmed.

We believe our study is the first to calculate the optimal protein density for the maternal diet during pregnancy. The cubic-spline model and a sufficient sample size allowed us to express the association between protein intake and fetal growth in a nonlinear fashion. We found that while an increase in protein density up to 12 % was associated with increased fetal growth and reduced SGA, any further increase in protein density significantly reduced fetal growth. Our findings did not conflict with those of interventional studies showing that while balanced protein supplementation (10–20 % of energy as protein) promoted fetal growth, especially in malnourished populations[9–11,31], supplementation with excessive protein (>20 % of energy as protein) decreased fetal growth, especially in populations with a diet already high in protein density[12,13]. Our results further suggested that the protein requirement was quite low, as we found that women who reported a diet containing a protein density of 14 % in early pregnancy had a significantly lower birth weight and birth weight *z*-score than those who reported a protein density of 12 %.

Few studies have examined the influence of maternal carbohydrate and fat intake, compared with that of protein, on fetal growth. A cohort study in the UK found that low carbohydrate intake in early pregnancy was associated with lower methylation levels in DNA sequences regulating an imprinted gene associated with fetal growth but failed to find any association with birth weight[32]. Animal studies showed that a high maternal fat intake may increase adult body weight[33]; however, studies of birth weight have produced inconsistent findings[33] as seen, for instance, in the conflicting reports of increased birth weight in mice[34] and reduced birth weight in rats[35,36]. Our study demonstrated that lower fat and higher carbohydrate density in early-pregnancy significantly decreased risk of fetal growth. However, as these findings lack the support of previous epidemiological or experimental studies, the general validity of these findings needs to be weighed against the results of future studies.

Strengths and limitations

The chief strength of our study is its large subject pool, which enabled us to examine the non-linear relationship between nutritional intake and birth outcomes. In addition, assessment of nutritional status was conducted twice during pregnancy, with the replication of the results providing greater reassurance of their reliability. However, there are several limitations. First, dietary assessments conducted during early pregnancy covered the previous year, which included the prepregnancy period in addition to early pregnancy. However, previous studies on the replication of nutritional assessment have shown that while absolute intake may change from prepregnancy to pregnancy (due to morning sickness)[37] and the intake of certain micronutrients (such as folate) may change due to antenatal education, overall dietary preferences do not change[38]. Second, the reported total energy intake later in pregnancy (mean: 7184 kJ/d) was lower than in early pregnancy (7477 kJ/d), suggesting the possibility that women under-reported their energetic intake later in pregnancy possibly out of concern for social norms[39] or reduced their intake in compliance with the strict national gestational guidelines[40], which discourage excessive energetic intake during pregnancy. However, the adjustment for total energy intake in our analyses, the absence of any discernible reason for under-reporting macronutrient consumption disproportionately, the consistency of the association between macronutrient intake and fetal growth in the two FFQ, all suggest that this problem may not have had a significant influence on our results. Third, the association between macronutrients and the outcomes may have been affected by the intake of micro-nutrients correlating with the intake of macronutrients. However, the evidence from previous epidemiological and interventional studies using protein was consistent with our results. Similarly, while we cannot deny the fact there may be residual confounding due to unmeasured confounders, the stableness of the estimates which did not change much from the crude analysis to the adjusted analysis, as well as after excluding women with gestational diabetes suggest our results are likely to be robust. We thus believe that our interpretation of the inverse U-curve relationship of maternal dietary protein density with fetal growth is valid. Fourth, our sample population was limited to Japanese women, who generally have a different dietary culture than their Western counterparts and also eat less on the whole. Recent statistics (2010–2011) from the Japan National Health and Nutrition Survey based on 3-d dietary records[41] show that consumption of both protein and total energy among pregnant women is very low[42], with an average pregnant woman eating only 6966 (SD 1845) kJ/d with a protein density of 13·8 (SD 4·3) %, comparable to what we found in our population (7184 (SD 2506) kJ/d and 13·6 (SD 2·1) %, respectively). Therefore, while our findings on the association between macronutrients and fetal growth is likely generalisable to the Japanese population or other populations with generally low energy intake, they may not be directly applicable to those who have a higher energy. Ideally, studies similar to the present one would be conducted in populations with different dietary cultures. Finally, while our study was able to calculate the 'optimal' ratio of the three nutrients to reduce risk of fetal growth restriction, it is much too early to interpret this as the 'optimal' maternal diet. The observed effect of protein intake on birth weight in this study is 10–20 g and quite small, thus if a different and more important outcome shows a different pattern with protein intake, such findings also need to be incorporated when deciding on what the true nutritional requirement is. Our study focused only on birth outcomes as the long-term outcomes of the cohort participants are not yet available, and mainly on fetal growth restriction as other birth outcomes such as neonatal deaths (<0·1 %), stillbirths (0·3 %) or macrosomia (0·6 % had birth weight over 4 kg) were extremely rare in the Japanese population. Future rigorous research using adequate databases should be conducted to estimate the influence of maternal nutrition on multiple outcomes, to provide the full picture of nutritional recommendations during pregnancy.

Conclusion

In conclusion, we found that protein intake from conception to the second trimester showed an inverse U-curve relationship with fetal growth. Our results strongly suggest that the association between protein intake and birth weight is nonlinear, and that a balanced diet fulfilling the minimum requirement for all macronutrients is ideal for avoiding fetal growth restriction.

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S. Y. and N. M. initiated the idea; N. M. designed the study; the JECS study group collected the data; N. M. analysed the data, wrote the paper and had primary responsibility for the final content. K. Kato, M. S., E. S., M. T., A. S., T. K. and K. Kusuhara critically reviewed the draft and provided important intellectual content to the interpretation of the results. All the authors have read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711451800291X

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