Maternal macronutrient intake during pregnancy is associated with neonatal abdominal adiposity: the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study[1-4]

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Abstract

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L-WC analyzed the data and wrote the first draft of the paper. M-TT and MVF acquired, analyzed MRI images and interpreted the results. L-WC, M-TT, IMA, JYB, and MC contributed to data collection, cleaning, and analysis. PDG, S-MS, Y-SC, FY, KMG, and YSL designed and led the GUSTO study. KMG, MSK, RMvD, MF-FC, and YSL advised on interpretation of results. All authors critically revised the manuscript. MF-FC and YSL had primary responsibility for the final content. L-WC and M-TT are joint first authors; L-WC, MF-FC, and YSL are joint corresponding authors. All authors have read and approved the final manuscript.

Background—Infant body composition has been associated with later metabolic risk, but few studies have examined the association between maternal macronutrient intake and neonatal body composition. Furthermore, most of those studies have used proxy measures of body composition that may not reflect body fat distribution, particularly abdominal internal adiposity.

Objective—We investigated the relation between maternal macronutrient intake and neonatal abdominal adiposity measured using magnetic resonance imaging (MRI) in a multi-ethnic Asian mother-offspring cohort.

Methods—Macronutrient intakes of mothers were ascertained using a 24-h dietary recall at 26-28 weeks gestation. Neonatal abdominal adiposity was assessed using MRI in the second week of life. Mother-offspring dyads with complete macronutrient intake and adiposity information ($n=$ 320) were included in the analysis. Associations were assessed by both substitution and addition models using multivariable linear regressions.

Results—Mothers [mean age: 30 y; 44% Chinese, 38% Malay, 18% Indians] consumed 15.5 $\pm$ 4.3% (mean $\pm$ SD) of their energy intakes from protein, 32.4 $\pm$ 7.7% from fat, and 52.1 $\pm$ 9.0% from carbohydrate. A higher protein, lower carbohydrate/fat diet during pregnancy was associated with lower abdominal internal adipose tissue (IAT) in the neonates [β (95% CI): -0.18 (-0.35, -0.001) mL per 1% protein to carbohydrate substitution and -0.25 (-0.46, -0.04) mL per 1% protein to fat substitution]. These associations were stronger in boys than in girls (*P*-interactions <0.05). Higher maternal intake of animal protein [-0.26 (-0.47, -0.05) mL for fat substitution], but not plant protein, was associated with lower offspring IAT. In contrast, maternal macronutrient intake was not consistently associated with infant anthropometric measurements, including abdominal circumference and subscapular skinfold thickness.

Conclusions—Higher maternal protein intake (at the expense of carbohydrate or fat intake) at 26-28 wk of gestation was associated with lower abdominal internal adiposity in neonates. Optimizing maternal dietary balance might be a new approach to potentially improve offspring body composition.

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Introduction

Overweight and obesity are now epidemic in both developed and developing countries (1). The distribution of adiposity (central or abdominal vs. peripheral fat pattern) may be more closely related to metabolic disease risk than overall adiposity (2). In particular, many studies have reported that visceral adipose tissue (intra-abdominal fat surrounding the internal organs) is directly associated with hypertension, diabetes, and insulin resistance [reviewed in (3)]. Adipocytes from visceral adipose tissue are more metabolically active and insulin-resistant compared with adipocytes from subcutaneous adipose tissue (4). Moreover, the venous blood of visceral adipose tissue drains to the liver directly through the portal

vein, thus providing direct hepatic access to unfavorable and pro-inflammatory adipokines secreted by visceral adipocytes (4,5).

Despite abundant studies in adults, little is known about body fat compartmentalization in infants. A growing body of evidence suggests that early-life factors such as maternal nutrition can influence birth and subsequent child health outcomes, which in turn are associated with obesity and other adverse adult health outcomes [reviewed in (6–8)]. South Asian neonates, despite their smaller size, have been reported to have greater abdominal adiposity than white European neonates, as measured by magnetic resonance imaging (MRI) (9). Other studies using simpler anthropometric measures have reported similar results (10,11). The "thin-fat" phenotype, characterized by smaller body size with preserved body fat content, has been associated with higher insulin resistance (higher cord plasma insulin level) in South Asian neonates compared with European neonates (12,13). A recent Brazilian cross-sectional study reported higher neonatal abdominal visceral fat to be associated with higher insulin resistance (HOMA-IR) in newborns (14). Collectively, these studies suggest that differences in fat distribution at birth may be associated with subsequent metabolic risk.

Maternal macronutrient intake has been studied in relation to birth outcomes in many studies, including ours (15). Few studies, however, have assessed the association between maternal macronutrient intake and offspring body composition, and their results have been inconsistent (16–19). These studies mainly focused on proxies for adiposity such as ponderal index, which may not reflect differences in body composition, let alone its peripheral vs. central distribution. We therefore investigated the influence of maternal macronutrient intake on neonatal abdominal adiposity measured using a gold standard method (MRI) (20–22) in a multi-ethnic Asian mother-offspring cohort. We hypothesized that maternal macronutrient balance during late mid-gestation can affect offspring abdominal fat deposition.

Subjects and Methods

Study design

Data used in this study were derived from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, a mother-offspring cohort in Singapore with detailed assessment of pregnant women and their offspring (23). Participants were pregnant women attending their antenatal care visits (<14 weeks of gestation) at KK Women's and Children's Hospital (KKH) or National University Hospital (NUH), the major public maternity units in Singapore. Recruitment started in June 2009 and ended in September 2010. To be eligible, the pregnant women had to be Singapore citizens or permanent residents between 18 and 50 years old, agree to donate cord blood, cord, and placenta, and intend to deliver in KKH and NUH and to reside in Singapore for the next 5 years. Only Chinese, Malay, and Indian women whose parents and whose husbands' parents were of the same ethnicity were included. Women with serious health conditions such as psychosis and type 1 diabetes mellitus were excluded. The study was granted ethical approval by the institutional review boards of the KKH and NUH. Written informed consent was collected from all women at recruitment. Out of 2034 eligible women, 1247 consented and were recruited into the study.

Subjects

Of the 1247 recruited pregnant women, we excluded those who underwent *in vitro* fertilization or were bearing twins (n= 95). From the remaining 1152, 1127 completed a single 24-h dietary recall and 628 completed a 3-d food diary at 26-28 weeks gestation, and 1087 babies were delivered. At the 32-34 week antenatal visit, mothers were approached regarding MRI scans (to determine abdominal adiposity) in their neonates, of which 478 consented in principle. However, only 379 neonates underwent MRI scan, mainly due to neonates requiring special care (n= 52). All neonates with congenital anomalies or birth defects were excluded at the time of MRI measurement. After further exclusion of 46 unanalyzable datasets due to motion artifacts, complete MRI data were available for 333 neonates in their second week of life [mean ± SD age = 10 ± 3 days]. Finally, 320 mother-child dyads had information on both pregnancy diet and MRI scan and were included in the present analysis. The flow of participants is shown in Supplemental Figure 1. Mothers included in this analysis were younger, more likely to be overweight, had less weight gain until 26-28 weeks gestation, and were less likely to be of Chinese ethnicity and to have a university degree than those not included; maternal energy and macronutrient intake, however, were not different in included and not included participants (Supplemental Table 1; differences in characteristics were assessed using Pearson's χ^2 tests or independent-sample t test). Neonates included in this analysis were less likely to be first-born than those not included (Supplemental Table 1).

Maternal dietary assessment

Maternal dietary intakes were assessed at 26-28 weeks gestation using both a 24-h recall and a 3-d food diary (conducted separately and the data from the two methods were not combined). Clinical research staff (trained by experienced dietitians) conducted the 24-h recall with a 5-step, multiple-pass interviewing method (24) using visual aids (standardized household measuring utensils and food pictures of various portion sizes) to assist women in quantifying their dietary intakes. The 24-h recall was conducted on a weekday or weekend day and the participants were not notified in advance of the 24-h recall interview. The clinical staff also gave instructions on how to complete the 3-d food diary (2 weekdays and 1 weekend day) at home. However, only a subset of participants (n= 197 whose neonates have MRI data) complied with completing the food diaries. Nutrient analysis of the dietary records was performed using nutrient analysis software (Dietplan6, Forestfield Software, UK) and a food composition database of locally available foods (25), with modifications made to inaccuracies found. For food items not found in the database, nutrient information was obtained either from the USDA national nutrient database (26) or food labels.

Assessment of neonatal abdominal adiposity- Magnetic Resonance Imaging (MRI)

Quantification of neonatal abdominal adiposity using MRI has been described in details elsewhere (27). Briefly, non-sedated, fed, and swaddled neonates [mean ± SD age = 10 ± 3 days] who were 5-10 minutes into their sleep were placed in an immobilization bag in supine position within an adult head coil and had their abdomens scanned. T1-weighted water-suppressed axial fast spin echo sequences were acquired (GE Signa HDxt 1.5 TMR scanner, Wisconsin, USA). Superficial subcutaneous adipose tissue (sSAT) has a clear

anatomical outline following the contours of the abdominal image slices. Deep subcutaneous adipose tissue (dSAT) is distinctly separated from sSAT by a fascial plane and is located on the left and right posterior aspect of abdomen. Finally, internal adipose tissue (IAT) is defined as the internal fat contained within the abdominal region (Supplemental Figure 2) and includes intraperitoneal, retroperitoneal, inter-muscular, as well as para-vertebral and intra-spinal fat. IAT in our study is equivalent to visceral fat in other (adult) studies.

Quantification of abdominal adipose tissue compartment volume—The water-suppressed images were processed using in-house semi-automated quantitative analysis software (MATLAB 7.13; The MathWorks Inc., Massachusetts, USA) based on morphological image analysis operations. To optimize segmentation, manual routines were conducted by two trained image analysts who were blinded to all subject information. The volumes occupied by respective segmented voxels from the level of diaphragm to the top of the sacrum from all 34-36 image slices (typical dimension of voxel: 0.4 mm x 0.4 mm x 5.0 mm) were summed to give the total adipose tissue volume for each compartment. Total abdominal volume (TAV) was defined as the volume enclosed by the outermost sSAT. Abdominal adipose tissue compartment volumes were also expressed as percentages of TAV. The mean inter-observer coefficients of variation (CV) were 1.6% for sSAT, 3.2% for dSAT, and 2.1% for IAT.

Assessment of other maternal and infant characteristics

Maternal characteristics—Data on ethnicity, education level, maternal age, and self-reported pre-pregnancy weight were collected from the participants at recruitment visit. During a clinic visit at 26-28 weeks of gestation, information about physical activity, alcohol consumption, and cigarette smoking habits during pregnancy was gathered. At the same clinic visit, maternal weight (SECA weighing scale model 803, SECA Corp., Germany) and height (SECA stadiometer model 213, SECA Corp., Germany) were measured, and weight gain up to 26-28 weeks of gestation was derived by subtracting self-reported pre-pregnancy weight from the weight at 26-28 weeks. Oral glucose tolerance tests (OGTTs) were administered at the same clinic visit; gestational diabetes mellitus (GDM) was defined based on the 1999 World Health Organization standard criteria (28,29).

Infant characteristics—Information on birth weight, gestational age, birth order, and infant sex was abstracted from obstetric records. Gestational age was determined based on dating ultrasound scan in the first trimester. Birth length was measured in triplicate within 72 hours after birth by trained research staff using a mobile measuring mat (SECA model 210, SECA Corp., Germany) and recorded to the nearest 5 mm. Abdominal circumference was measured using a measuring tape (Butterfly brand, China) and recorded to the nearest 1 mm. Triceps and subscapular skinfolds were measured using Holtain skinfold calipers (Holtain Ltd, UK) on the right side of the body and recorded to the nearest 0.2 mm. Anthropometric training and standardization sessions were conducted every 3 months, and observers were trained to obtain measurements that, on average, were closest to the values measured by an expert anthropometrist. Reliability was estimated by inter-observer technical error of measurement (TEM) and coefficient of variation (Supplemental Table 2). BMI and ponderal index were calculated using the formula weight (kg)/ length (m)^2 and weight (kg)/ length

(m)^3, respectively. The infant's total body fat mass was estimated using a validated equation (30) derived from the GUSTO cohort as follows:

Fat mass = −0.022+ (0.307 × weight) − (0.077 × sex) + (0.028 × subscapular skinfold) − (0.019 × gestational age),

where sex = 0 for female, 1 for male

Statistical analysis

Maternal energy and macronutrient intakes ascertained with a 24-h recall were first summarized (means ± SDs) according to maternal and infant characteristics. Differences in energy and macronutrient intakes (expressed as percentages of total energy intake) among the defined categories of these characteristics were assessed using independent-sample *t*-tests or one-way ANOVA tests followed by *post hoc* analysis with Bonferroni adjustment.

Associations between maternal macronutrient intake (assessed using 24-h recall) and neonatal abdominal adiposity were assessed using multivariable linear regression. The regression model was first adjusted for the exact age (in days) of the infants at MRI measurement. The full model was adjusted for potential confounders and determinants of neonatal adiposity, including ethnicity, education status, GDM, birth order, and infant sex as categorical variables, and gestational age, maternal age, height, pre-pregnancy BMI, and gestational weight gain until 26-28 weeks gestation as continuous variables. Missing covariates information [maternal height (*n*= 3); pre-pregnancy BMI (*n*= 29); gestational weight gain (*n*= 30); GDM (*n*= 15)] was imputed with median or the most common category; exclusion of participants with missing covariates information from analysis did not change the associations. Maternal macronutrient intake was modelled both continuously and as tertiles to assess potential dose-response and non-linear associations with neonatal abdominal adiposity. We also assessed the associations of maternal macronutrient intake with birth weight, length, abdominal circumference, triceps and subscapular skinfolds, total body fat, ponderal index, and BMI.

Two types of multivariable regression models (using macronutrient intake assessed by 24-h recall), namely the substitution model and addition model, were used. The substitution model resembles an isocaloric situation and was performed by simultaneously including percentages of energy contribution from the macronutrient of interest (e.g., protein) and another energy-contributing macronutrient (carbohydrate or fat), and total energy intake, in the model. When carbohydrate and total energy intake are kept constant, an increase in percentage of energy intake from protein must be accompanied by a decrease in percentage energy intake from fat. Thus, the effect estimate can be interpreted as the influence of increasing intake of protein at the expense of fat, while keeping total energy intake constant. The addition model was performed according to the macronutrient partition method (31), where all of the macronutrients (in grams), but not total energy intake, were included in the model. Since total energy intake is not kept constant, the addition model represents a non-isocaloric analysis. The coefficients from the addition model can be interpreted as the influence of adding 1 g of a particular macronutrient to maternal diet on neonatal abdominal adiposity, while holding constant the absolute intakes of other macronutrients.

We investigated potential effect modification by ethnicity and infant sex on the associations between maternal macronutrient intake and neonatal abdominal adiposity by including their respective interaction terms in the regression models. We also conducted several sensitivity analyses. First, we repeated the main analyses, but with neonatal abdominal fat volumes expressed as a percentage of total abdominal volume to take into account the variation in abdominal size. Second, we further adjusted the regression models for maternal physical activity, alcohol intake, and cigarette smoking during pregnancy. Third, we further excluded women with type 2 diabetes ($n = 1$), chronic hypertension ($n = 2$), and pregnancy-induced hypertension ($n = 10$) in our analysis. Last, we investigated the associations of maternal macronutrient intake assessed using the 3-d food diary with neonatal abdominal fat ($n = 197$), as the 3-d food diary may be less affected than the 24-h recall by day-to-day variation in dietary intakes of the participants.

All statistical analyses were performed using the statistical software package STATA version 13.1 (StataCorp., Texas, USA).

Results

Table 1 shows the energy intake and percentage of energy intake from protein, fat, and carbohydrate according to maternal and infant characteristics. Study mothers consumed 15.5 ± 4.3% (mean ± SD) of their energy intake from protein, 32.4 ± 7.7% from fat, and 52.1 ± 9.0% from carbohydrate. Chinese mothers had a higher mean energy intake as compared with Malay and Indian mothers. Indian mothers had a higher intake of carbohydrate and a lower intake of fat than Chinese and Malay mothers. Mothers with GDM had a lower fat intake than mothers without GDM. Mothers of first-born children had higher energy and fat intakes than mothers of later-born children. The means ± SDs of neonatal abdominal adiposity were 78.2 ± 21.9 mL for sSAT, 13.4 ± 5.7 mL for dSAT, 22.9 ± 7.6 mL for IAT, and 115 ± 32.5 mL for total abdominal adipose tissue volume.

The associations of maternal macronutrient intake at 26-28 weeks of gestation with neonatal abdominal adiposity are shown in Table 2. After adjustment for potential confounders, higher intake of maternal protein at the expense of carbohydrate was significantly associated with a 0.18 mL lower IAT in the neonates (per 1% energy substitution; $P = 0.048$). Similarly, a higher protein, lower fat diet was significantly associated with 0.25 mL lower neonatal IAT ($P = 0.021$). In the addition model, higher maternal absolute protein intake also tended to be associated with a lower IAT ($P = 0.06$). The association between higher maternal protein intake and lower neonatal internal fat remained unchanged even when fiber, type of carbohydrate, and type of fat were considered. No significant associations were observed between maternal macronutrient intake and sSAT, dSAT, or total abdominal adipose tissue volume.

In the tertile analysis (Figure 1), higher maternal protein intake at the expense of carbohydrate or fat intake was associated with lower neonatal IAT in a dose-response manner. Neonates born to mothers in the highest tertile [median (25th-75th percentile): 18.9 (17.9-21.3) %] of protein intake (at the expense of fat intake) had a 2.10 mL lower IAT ($P =$

0.049), compared with neonates born to mothers in the lowest tertile [median (25th-75th percentile): 11.6 (7.6-13.4) %] (*P*-for-trend= 0.047).

Infant sex modified the associations between maternal macronutrient intake and IAT in both the substitution (*P*-interaction= 0.016 for substitution of protein for fat or carbohydrate) and addition models (*P*-interaction= 0.042 for addition of fat). Maternal macronutrient intake affected IAT deposition to a greater extent in boys than in girls (Supplemental Table 3). Higher maternal protein intake at the expense of carbohydrate or fat intake was significantly associated with a lower IAT in male infants only [β (95% CI): -0.35 (-0.59, -0.10) mL for carbohydrate replacement and -0.43 (-0.72, -0.15) mL for fat replacement; both *P*< 0.01]. Similar results were observed in the addition model (Supplemental Table 3). Furthermore, addition of maternal absolute fat intake was associated with higher total abdominal fat and all abdominal fat subtypes in boys only (Supplemental Table 3).

Significant interactions were also observed between ethnicity and maternal macronutrient intake on neonatal IAT (Supplemental Table 4). A higher protein, lower carbohydrate or fat diet during pregnancy was associated with lower IAT in Chinese and Indian, but not Malay, neonates (*P*-interaction= 0.048 for replacement of carbohydrate and 0.06 for replacement of fat). Furthermore, higher maternal protein intake at the expense of carbohydrate or fat intake was associated with lower dSAT in Indian neonates only (*P*-interaction= 0.038 for replacement of carbohydrate and 0.05 for replacement of fat). Similar trends were observed in the non-isocaloric addition models (Supplemental Table 4).

Table 3 shows the associations of maternal plant and animal protein intakes with IAT. Higher maternal animal protein intake was associated with lower IAT in both the substitution and addition models. Again, the associations were stronger in boys. Maternal plant protein intake was not associated with offspring IAT, but its range of exposure was narrower: mean ± SD of energy contribution= 6.1% ± 2.1% vs. 9.3% ± 5.0% for animal protein.

Maternal macronutrient intake was not consistently associated with neonatal measurements, including abdominal circumference, triceps and subscapular skinfolds, ponderal index, BMI, total body fat, birth weight, and weight at MRI measurement day (Supplemental Table 5). Nonetheless, higher maternal protein intake was associated with a lower birth length (β= -0.06 cm for both carbohydrate and fat replacement, *P*< 0.05).

Sensitivity analyses

Expressing neonatal abdominal fat volumes as % of total abdominal volume yielded similar results (Supplemental Table 6). When we further adjusted for maternal physical activity, cigarette smoking and alcohol consumption during pregnancy in the multiple regression models, the associations persisted (Supplemental Table 7). Excluding women with chronic or pregnancy-induced disease did not change the results substantially and the conclusions remained the same. When we repeated the analyses with maternal macronutrient intake assessed by 3-d food diary, similar results were also observed (Supplemental Table 8), although confidence intervals were wider, probably owing to the smaller sample size (n= 197).

Discussion

In this Asian multi-ethnic mother-offspring cohort study, higher maternal protein intake at the expense of carbohydrate or fat intake was associated with lower abdominal IAT in the newborns. The associations were stronger in boys than in girls and stronger in Chinese than in Malay infants. The 5th-95th percentile range of percentage energy intake from protein of mothers in our study (9.4%-22.5%) largely falls within the recommended percentage energy contribution from protein intake for adults [10-35% by Institute of Medicine (32) and 10-20% by Nordic Nutrition Recommendations (33)], indicating that insufficient or excessive protein intake is rare in this population. To the best of our knowledge, ours is the first study to demonstrate an impact of maternal macronutrient intake on neonatal abdominal adiposity assessed by a gold-standard method (MRI).

Comparison with previous studies

Previous human studies have largely relied on simple anthropometric measures or estimates of adiposity distribution. Several cohort studies in UK and Australia have assessed the association of maternal protein intake during late pregnancy with ponderal index at birth (17–19). Despite having comparable median protein intakes (15% energy intake in the UK study and 16% energy intake in the Australian studies), reported results have been conflicting. One of the Australian studies (n= 557) (18) reported no association between maternal protein intake during late pregnancy and birth ponderal index, as we observed in our study. In contrast, higher maternal protein intake was associated with a higher ponderal index in the UK study (n= 538) (17) and with a lower ponderal index in another Australian study (n= 1040) (19). Ponderal index is only a proxy for overall adiposity, however, and does not reflect body fat distribution. Indeed, a recent study suggests that both ponderal index and BMI at birth are poor predictors of newborn whole-body adiposity, as measured by air displacement plethysmography (34).

In concordance with our results, higher maternal protein intake and protein:carbohydrate ratio were associated with lower fetal abdominal fat assessed using ultrasound in an Australian study (n= 179) (16). Similarly, several animal studies have reported that low maternal protein diet (8% of energy intake from protein vs. 20% in the control diet) led to increased visceral adiposity in the offspring (35–37).

Potential mechanisms

The biological mechanisms underlying an inverse relationship between maternal protein intake and neonatal IAT are not well established. Animal studies suggest that protein restriction during pregnancy results in increased expression of genes encoding lipogenic enzymes such as fatty acid synthase (FAS) and glycerol-3-phosphate dehydrogenase (G3PDH) in visceral adipose tissue of the offspring, implicating lipogenesis in protein restriction-induced visceral adiposity (36). Furthermore, protein restriction during pregnancy has been reported to upregulate adipocyte differentiation factors such as MAP-kinase phosphatase-1 (MKP-1) in the offspring visceral adipose tissue (36). Angiogenic factors such as leptin are also upregulated by *in utero* protein restriction, whereas antiangiogenic factors such as F-spondin are downregulated, which may in turn promote adipose tissue

expansion (36,38). Furthermore, maternal protein restriction also increases the rate of preadipocyte proliferation in the offspring (39). Our results suggest that variation in protein intake within normal physiologic range can influence neonatal abdominal adiposity; whether this is mediated by changes in gene expression should be investigated in future studies.

Sex and ethnic differences

We observed that sex and ethnicity modified the associations between maternal macronutrient intake and neonatal abdominal adiposity. The association of higher maternal protein intake and lower offspring IAT was stronger in boys. This observation is in keeping with the results of many animal studies, which have reported greater influence of low maternal protein intake on visceral adiposity (35), altered pancreatic islet mitochondrial function (40), and insulin resistance and hyperinsulinaemia (41) in male offspring. Moreover, it has been suggested that body fat distribution is subjected to stricter genetic control in women than in men [reviewed in (42)], which may partly explain the lesser influence of maternal protein intake on the body composition of female offspring.

The association between higher maternal protein intake and lower IAT in offspring was not observed in our Malay participants, while higher maternal protein intake was associated with lower dSAT only in Indian participants. These observations require confirmation, however, owing to smaller sample sizes of Malay and Indian participants. If the observed ethnic differences in associations are indeed real, they may be partly due to the inherent differences in body composition and dietary intakes among the ethnic groups. We observed that Malay and Indian neonates tended to have higher sSAT and dSAT but lower IAT volumes, as compared with Chinese neonates (27). Similar results were reported in Singaporean adults, where Malay and Indian men seemed to accumulate more dSAT with increasing % body fat, as compared with Chinese men (43).

Plant vs. animal protein

We observed that higher intake of maternal animal protein, but not plant protein, was associated with lower IAT of the offspring. Animal proteins contain most essential amino acids and generally provide a greater protein supply per energy intake and better muscle anabolic response, as compared with plant proteins (44,45). In overweight and obese adults, whey protein (animal source) preloads before meals for 12 weeks were found to be more beneficial than soy protein (plant source) preloads (greater increase in lean mass and decrease in body fat mass) (46). However, in a prospective cohort study involving 684 Danish mother-child pairs, high maternal animal protein intake during late pregnancy (mean intake in the highest quartile= 72 g/d compared with 36 g/d in the lowest quartile) has been associated with higher risk of offspring overweight 20 years later, indicating that excessive animal protein intake may have long-term harmful consequences for the offspring (47).

Strengths and limitations

Our study has several strengths. First, we used a gold-standard method (multi-slice volume MRI) for assessing neonatal abdominal adiposity (20). Compared with computed tomography scan, the other gold standard, MRI scan does not involve radiation. MRI measurement in neonates is challenging, and no previous study has achieved as large a

sample size (n> 300) as ours; this enabled us to investigate potential interactions with infant sex and ethnicity. Furthermore, we were able to differentiate between deep subcutaneous and superficial subcutaneous adipose tissues with the MRI images.

One limitation of our study is that maternal nutritional intake was measured only once during late mid-gestation. Influences of maternal macronutrient intake on neonatal adiposity may be trimester-specific, but we are unable to assess such differences. However, some studies in Asian population suggest that, on average, the changes in energy-adjusted macronutrient intakes during pregnancy were small (48,49). Our single 24-h recall may not have captured an individual's usual dietary intake due to day-to-day variation in food intake. However, the association between maternal macronutrient intake and neonatal adiposity was very similar in a subset of participants who completed a 3-d food diary. The mothers included in the current studies had different socio-demographic background from those not included, indicating that our results may be more applicable to younger and less educated women in a developed country. However, because our exposure is maternal macronutrient intake, and that maternal macronutrient intake did not differ significantly between included and not included participants in the current study, we do not expect selection bias to affect our observation. Moreover, the results for maternal macronutrient intake and other neonatal measurements such as birth weight and skinfold thickness in this subpopulation (limited by completion rate of MRI scan) of our cohort are in agreement with the previously published results in a larger population (15). Finally, as in any observational study residual confounding may have affected our results and causality cannot be claimed.

Conclusion

In conclusion, we found that a higher maternal protein, lower carbohydrate/fat diet at 26-28 wk of gestation was associated with lower abdominal internal adipose tissue in their neonates. Pending replication of the results by other independent studies, optimizing maternal dietary balance during pregnancy might be a new approach to potentially improve the offspring body composition. Furthermore, the lack of association between maternal macronutrient intake and various proxy measures of adiposity at birth indicated that accurate body fat distribution measurement in early life may provide more valuable insights on offspring metabolic risk.

1   Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used:

[2]

dSAT	deep subcutaneous adipose tissue
FAS	fatty acid synthase
G3PDH	glycerol-3-phosphate dehydrogenase
GDM	gestational diabetes mellitus
GUSTO	Growing Up in Singapore Towards healthy Outcomes
IAT	internal adipose tissue
KKH	KK Women's and Children's Hospital
MKP-1	MAP-kinase phosphatase-1
MRI	magnetic resonance imaging
NUH	National University Hospital
OGTT	oral glucose tolerance tests
sSAT	superficial subcutaneous adipose tissue
TAV	total abdominal volume
USDA	U.S. Department of Agriculture

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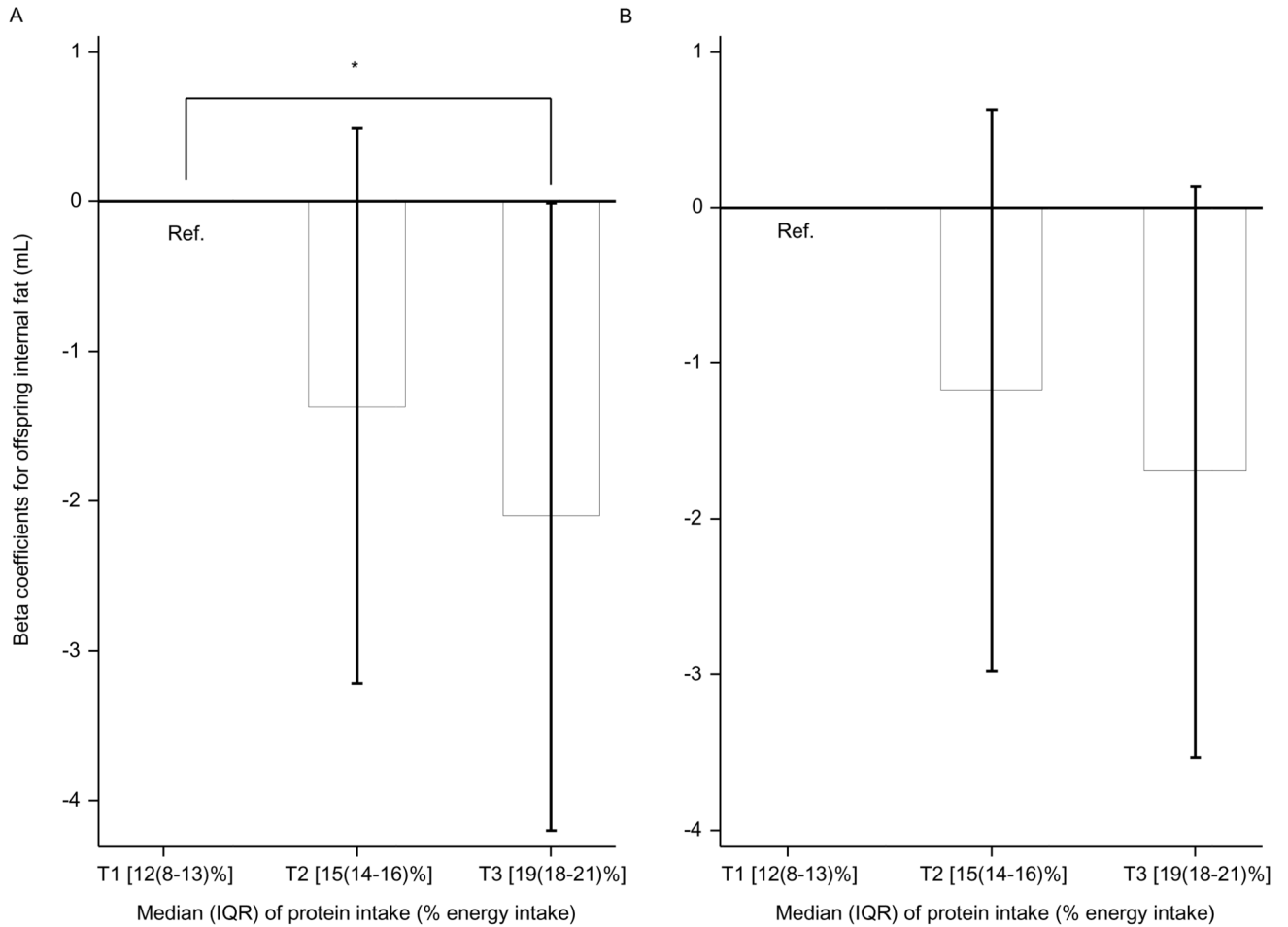
Figure 1.
Associations of protein to fat (A) or protein to carbohydrate (B) substitutions in maternal diets with neonatal abdominal internal fat by tertile of maternal protein energy intake in the GUSTO study (n= 320). Values on the x axes are medians (IQRs), n= 106 (T1, lowest) or 107 (T2 and T3). Bars are regression coefficients from multiple linear regressions for the associations of second and third tertiles of protein to fat/carbohydrate substitution and neonatal internal fat, as compared with the first (lowest) tertile. Capped vertical lines are 95% confidence intervals of the respective regression coefficients. Regressions were adjusted for age at MRI measurement, ethnicity, maternal age, height, pre-pregnancy BMI, pregnancy weight gain until 26-28 weeks gestation, education status, gestational diabetes mellitus, infant gender, gestational age, and birth order.
*P= 0.049; P-trend= 0.047

Table 1

Energy intake and energy contribution from protein, fat, and carbohydrate intakes from a single 24-h recall at 26-28 wk of gestation according to maternal and infant characteristics in the GUSTO study[1,2]

	n	%	Energy intake		Energy from protein		Energy from fat		Energy from carbohydrate	
			kcal/d	P	% total energy	P	% total energy	P	% total energy	P
Total population	320	100	1851 ± 625		15.5 ± 4.3		32.4 ± 7.7		52.1 ± 9.0	
Maternal characteristics										
Ethnicity				0.003		0.08		<0.001		<0.001
Chinese	141	44.1	1982 ± 584		16.0 ± 4.7		33.3[a] ± 7.3		50.7[a] ± 8.7	
Malay	123	38.4	1770[b] ± 680		15.5 ± 4.1		32.9[a] ± 7.5		51.7[a] ± 8.3	
Indian	56	17.5	1699[b] ± 539		14.5 ± 3.6		28.9[b] ± 8.3		56.6[b] ± 9.9	
Education status				0.018		0.39		0.024		0.013
Primary/secondary	135	42.2	1743[a] ± 658		15.4 ± 4.3		31.4[a] ± 8.0		53.1[a] ± 9.3	
Post-secondary	124	38.8	1896[b] ± 573		15.9 ± 4.5		33.8[b] ± 7.7		50.2[b] ± 9.0	
University	61	19.1	1999[b] ± 620		15.0 ± 4.0		31.4[a] ± 6.7		53.6[a] ± 7.6	
Maternal age, y				0.12		0.24		0.81		0.98
18-24	61	19.1	1840 ± 733		14.7 ± 3.5		32.9 ± 8.4		52.4 ± 9.1	
25-29	108	33.8	1750 ± 576		15.5 ± 3.6		32.6 ± 7.2		51.9 ± 8.0	
30-34	85	26.6	1962 ± 603		16.2 ± 5.5		31.8 ± 7.3		52.0 ± 9.0	
35	66	20.6	1885 ± 611		15.5 ± 4.3		32.2 ± 8.5		52.3 ± 10.5	
Maternal height, cm				0.34		0.50		0.28		0.21
<158	164	51.3	1819 ± 650		15.4 ± 4.1		31.9 ± 7.9		52.7 ± 9.1	
158	156	48.8	1885 ± 599		15.7 ± 4.6		32.8 ± 7.5		51.4 ± 8.8	
Pre-pregnancy BMI, kg/m²				0.26		0.37		0.81		0.98
<18.5	41	12.8	1830 ± 689		15.2 ± 3.8		33.2 ± 7.1		51.6 ± 8.6	
18.5-24.9	182	56.9	1886 ± 597		15.3 ± 4.1		32.4 ± 7.9		52.3 ± 8.8	
25.0-29.9	64	20.0	1869 ± 551		16.3 ± 5.1		31.7 ± 7.4		52.0 ± 9.4	
30.0	33	10.3	1652 ± 800		15.9 ± 4.5		32.3 ± 8.4		51.8 ± 9.9	
Weight gain until 26 weeks gestation, kg				0.06		0.31		0.86		0.74
<8.6	172	53.8	1790 ± 620		15.3 ± 4.3		32.4 ± 7.8		52.2 ± 9.1	

	n	%	Energy intake		Energy from protein		Energy from fat		Energy from carbohydrate	
			kcal/d	P	% total energy	P	% total energy	P	% total energy	P
8.6	148	46.3	1922 ± 626		15.8 ± 4.3		32.3 ± 7.6		51.9 ± 8.8	
Gestational diabetes mellitus				0.08		0.10		0.010		0.22
Yes	48	15.0	1710 ± 582		16.6 ± 5.0		29.6 ± 7.7		53.7 ± 10.0	
No	272	85.0	1876 ± 630		15.3 ± 4.2		32.8 ± 7.6		51.8 ± 8.8	
Infant characteristics										
Infant sex				0.90		0.99		0.76		0.80
Male	173	54.1	1855 ± 655		15.5 ± 4.2		32.2 ± 7.5		52.2 ± 8.7	
Female	147	45.9	1846 ± 590		15.5 ± 4.4		32.5 ± 8.0		52.0 ± 9.3	
Birth weight, g				0.68		0.43		0.70		0.84
<2500	26	8.1	1793 ± 747		16.5 ± 6.6		31.8 ± 8.0		51.7 ± 10.7	
2500	294	91.9	1856 ± 614		15.5 ± 4.1		32.4 ± 7.7		52.1 ± 8.8	
Birth length, cm				0.15		0.06		0.94		0.39
<48.4	156	48.8	1799 ± 633		16.0 ± 4.7		32.3 ± 7.4		51.7 ± 8.7	
48.4	164	51.3	1901 ± 615		15.1 ± 3.9		32.4 ± 8.0		52.5 ± 9.3	
Birth order				0.028		0.07		0.034		0.35
First-born	121	37.8	1953 ± 679		15.0 ± 3.8		33.5 ± 7.0		51.5 ± 8.3	
Non-first-born	199	62.2	1789 ± 583		15.9 ± 4.6		31.7 ± 8.1		52.5 ± 9.4	
Gestational age				0.07		0.67		0.25		0.76
<37 weeks	19	5.9	1608 ± 580		16.2 ± 7.1		31.1 ± 4.5		52.7 ± 8.2	
37 weeks	301	94.1	1867 ± 626		15.5 ± 4.1		32.4 ± 7.9		52.1 ± 9.0	

[1] Values are means ± SDs, unless otherwise stated.

[2] Labelled means in a column without a common letter differ ($P < 0.05$).

Table 2

Associations of maternal macronutrient intake at 26-28 wk of gestation and neonatal abdominal adiposity in the GUSTO study ($n= 320$)[1-3]

	Superficial subcutaneous fat (mL)		Deep subcutaneous fat (mL)		Internal fat (mL)		Total abdominal fat (mL)	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Substitution models (isocaloric)								
Substitute protein for carbohydrate	-0.23 (-0.78, 0.33)	-0.38 (-0.87, 0.12)	0.01 (-0.14, 0.15)	-0.02 (-0.16, 0.11)	-0.10 (-0.29, 0.08)	-0.18 (-0.35, -0.001) *	-0.32 (-1.14, 0.50)	-0.57 (-1.31, 0.16)
Substitute protein for fat	-0.33 (-0.99, 0.34)	-0.52 (-1.11, 0.08)	-0.01 (-0.19, 0.16)	-0.07 (-0.23, 0.10)	-0.17 (-0.39, 0.06)	-0.25 (-0.46, -0.04) *	-0.51 (-1.48, 0.47)	-0.83 (-1.71, 0.05)
Substitute fat for carbohydrate	0.10 (-0.23, 0.43)	0.14 (-0.16, 0.44)	0.02 (-0.07, 0.11)	0.04 (-0.04, 0.13)	0.06 (-0.05, 0.17)	0.07 (-0.03, 0.18)	0.18 (-0.30, 0.67)	0.25 (-0.19, 0.69)
Addition models (non-isocaloric)								
Addition of protein	-0.05 (-0.17, 0.06)	-0.10 (-0.20, 0.01)	0.004 (-0.03, 0.03)	-0.01 (-0.03, 0.02)	-0.01 (-0.05, 0.02)	-0.04 (-0.07, 0.001)	-0.07 (-0.23, 0.10)	-0.14 (-0.29, 0.01)
Addition of fat	0.07 (-0.05, 0.18)	0.09 (-0.01, 0.19)	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.03 (-0.01, 0.07)	0.03 (-0.002, 0.07)	0.11 (-0.06, 0.27)	0.14 (-0.01, 0.29)
Addition of carbohydrate	0.002 (-0.04, 0.04)	0.01 (-0.02, 0.05)	-0.003 (-0.01, 0.01)	-0.002 (-0.01, 0.01)	0.004 (-0.01, 0.02)	0.01 (-0.01, 0.02)	0.002 (-0.05, 0.06)	0.02 (-0.03, 0.07)

*
P< 0.05

[1]Values are β (95% CI) derived from multiple linear regressions and expressed for per 1% energy substitution of a macronutrient for another macronutrient in the substitution model and per 1 g increase in absolute macronutrient intake in the addition model.

[2]Model 1 was adjusted for age at MRI measurement.

[3]Model 2 was further adjusted for ethnicity, maternal age, height, pre-pregnancy BMI, pregnancy weight gain until 26-28 weeks gestation, education status, gestational diabetes mellitus, infant gender, gestational age, and birth order.

Table 3

Associations of maternal plant and animal protein intakes at 26–28 wk of gestation and neonatal abdominal internal fat in the GUSTO study[1,2]

	All (*n*=315)	Male (*n*=169)	Female (*n*=146)	*P*-interaction (sex x nutrient)
Substitution models (isocaloric)				
Substitute protein for fat				
Animal protein	−0.26 (−0.47, −0.05) *	−0.47 (−0.76, −0.17) **	−0.09 (−0.40, 0.22)	0.013
Plant protein	0.13 (−0.30, 0.56)	−0.12 (−0.67, 0.43)	0.31 (−0.38, 1.00)	0.35
Substitute protein for carbohydrate				
Animal protein	−0.15 (−0.33, 0.02)	−0.34 (−0.59, −0.09) **	−0.01 (−0.27, 0.25)	0.018
Plant protein	0.24 (−0.20, 0.68)	0.01 (−0.56, 0.57)	0.39 (−0.33, 1.11)	0.50
Addition models (non-isocaloric)				
Animal protein	−0.04 (−0.08, −0.001) *	−0.07 (−0.12, −0.01) *	−0.02 (−0.07, 0.04)	0.07
Plant protein	0.04 (−0.05, 0.12)	−0.01 (−0.12, 0.10)	0.05 (−0.09, 0.19)	0.68

*$P <$ 0.05; ** $P <$ 0.01

[1] Values are β (95% CI) derived from multiple linear regressions and expressed for per 1% energy substitution of animal or plant protein intakes for fat or carbohydrate intakes in the substitution model and per 1 g increase in absolute animal or plant protein intakes in the addition model.

[2] Regressions were adjusted for age at MRI measurement, ethnicity, maternal age, height, pre-pregnancy BMI, pregnancy weight gain until 26–28 weeks gestation, education status, gestational diabetes mellitus, infant gender, gestational age, and birth order.