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Associations of Maternal Carbohydrate Intake During Pregnancy and Adolescent Adiposity and Metabolic Health

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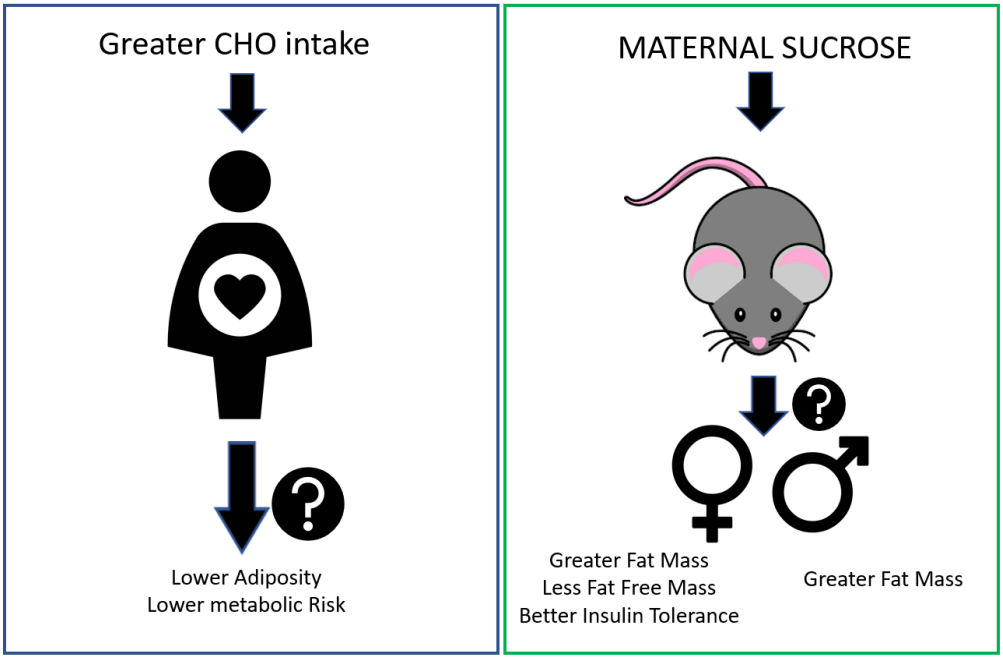
COMMITTEE MEMBERS:

WEI PERNG PHD, MPH

DAVE BRIDGES PHD

KAREN PETERSON DSC

Abstract



Maternal diet during gestation is known to affect offspring phenotypes. The majority of dietary studies in pregnancy look at restriction of protein or exposure to high fat diet. Few concentrate on the critical macronutrient for fetal growth: carbohydrates (CHO). This study analyses both human and animal data for evidence of developmental programming of adiposity and dysmetabolism in adolescent offspring.

In 237 mother-child birth pairs in the human ELEMENT birth cohort, mother's dietary carbohydrate intakes were assessed for association with child adiposity and metabolic health outcomes in peripuberty. Mothers with greater intakes of total and net CHO during pregnancy gave birth to children who had lower adiposity and lower metabolic risk during the peripubertal period. After accounting for maternal age, child age, sex, and pubertal status, children of women in the 4th vs 1st quartile of total CHO intake in the first trimester had a 0.12-unit lower BMIz score (95% CI -0.55, 0.31, p=0.10). Children of mothers in the 4th quartile of total CHO intake also had a 0.07 unit (95% CI 0.28, 0.13, p=0.13) lower metabolic risk z score in peripuberty. Measures of c-peptide followed a similar trend, such that the 4th quartile of total CHO intake in the first trimester was associated with a 0.31-unit lower c-peptide score (95% CI -0.72, 0.11, p=0.05) compared to those whose mothers were in the 1st quartile. These associations were not attributed to delivery method, child's diet in peripuberty, or nutrient substitution with protein or fat.

This was further modeled in animals by exposing pregnant mice to 10% sucrose water or tap water during gestation. Sucrose exposed dams gave birth to offspring who had greater fat mass than dams exposed to water. Female pups of sucrose-exposed dams also had less fat free mass and better insulin tolerance in young adulthood. The mechanism for these effects is yet to be elucidated, but is not attributable to maternal weight during pregnancy, offspring food intakes, or offspring feeding efficiency. Further is necessary to highlight the mechanisms underlying these divergent results to two model systems.

1. Introduction

The incidence of obesity has markedly increased in the last three decades,[1]now pervading pediatric populations, with nearly 24 percent of children classified as overweight or obese worldwide in 2013.[2] In parallel with the trends in childhood obesity, metabolic illnesses that have historically been confined to adult populations now afflict children and adolescents. For example, diagnoses of high blood pressure, non-alcoholic fatty liver disease (NAFLD), sleep apnea, cardiovascular risk, and type 2 diabetes have increased among children and adolescents in recent decades.[3] The younger age of onset of such metabolic conditions not only increases economic and healthcare burden, but also has the potential to adversely affect the health of future generations.[4]

Gestation is a time when nutrition has the power to affect offspring phenotype and health in future life. Carbohydrates and their metabolic regulation are crucial to successful pregnancy. Glucose is the principal substrate provided by the mother that fuels the growth of the fetus.[5, 6] It is well established that maternal diet during pregnancy can influence metabolic phenotype in the offspring.[7,8]Studies designed to evaluate the role of the gestational environment in offspring metabolic health have largely focused on low protein intake or increased fat intake during pregnancy; leaving the role of carbohydrate intake in gestation largely unstudied.[7, 9, 10] To date, the few studies relating maternal carbohydrate intake to offspring health have focused on health outcomes during infancy or early childhood. In a study of 320 mother-child pairs in the Growing Up in Singapore Towards health Outcomes (GUSTO) cohort, Chen et al. examined associations of maternal macronutrient intake at 26-28 weeks' gestation with offspring adiposity at birth.[11] The researchers found that higher ratio carbohydrate-to-protein intake during pregnancy was related to greater neonatal adiposity – an association that was driven primarily by sugar intake.[11] Some studies have also used the glycemic index as a proxy for the physiological effects of carbohydrate intake. For example, Scholl et al. found that greater consumption of low glycemic index foods during pregnancy was correlated with greater prevalence of small for gestational age infants in a prospective study of 1,802 mother-infant pairs in Camden, NJ.[12] Taken together, these studies indicate the relevance of both carbohydrate quantity and quality on offspring health outcomes.

Animal studies provide insight on mechanisms linking maternal carbohydrate intake to offspring health. In rodent models, maternal high carbohydrate intake during pregnancy has been related to hypertension, poor

glycemic control, inflammation, and increased adiposity in offspring.[7, 13, 14, 15, 16] Some studies attribute these changes with an upregulation in appetitive signals and inflammation in the in the neonate.[15, 16, 17] A more recent study identifies pancreatic islet size increases alongside exposure to a high carbohydrate diet *in utero*, possibly explaining both changes in metabolism and body fat storage in the offspring.[18] Together, these findings indicate a lasting influence of maternal carbohydrate intake on offspring metabolic health. However, there exist limitations in the current literature.

Current literature demonstrates a few crucial limitations. In human studies, many analyses of maternal diet in pregnancy fail to measure intake over the duration of gestation, many rely on a single dietary questionnaire administered in mid or late pregnancy. The inclusion of additional measures of dietary intake during gestation may be more informative as maternal dietary patterns could vary during the pregnancy, and timing of dietary changes captured in additional questionnaires may occur concomitantly with critical periods of development in the fetus, affecting organ and tissue development as it occurs. The outcomes of studies that examine maternal diet in pregnancy often focus on infancy and early childhood. The exclusion of later periods of life, and critical ones such as adolescence, may result in limited knowledge of the more lasting consequences of maternal diet on child health. This is surprising, as adolescence is a life stage that is not only thought to be a sensitive period for development of obesity-related disease,[7] but is also a time when many metabolic risk factors (e.g., obesity status,[14, 19] lipid profile,[9] blood pressure[20]) may be set for life.[6, 21] Therefore, identification of modifiable early-life determinants of obesity and metabolic risk during adolescence is critical.

Animal models have also evaluated the effect of high carbohydrate diets in pregnancy. These studies utilize numerous methods to deliver high carbohydrate to developing animals; from exposure of the mother to sucrose water, oral gavage of sweetened milk substitutes.[15] The use of non-protein matched control diets,[14] making interpretation of the results difficult, as it is known that protein restriction is a factor in fetal growth restriction, and later adult obesity.[5] There is also inconsistencies in the practices of maintaining pups on control diets or exposing them to further dietary insult, further complicating the analysis of the original maternal insult. Some studies utilize the reducing of litters to better correct for number of pups and abundance of dam's milk in

lactation. This ultimately means many mechanisms are proposed to explain a variety of phenotypes; making recommendations for improving dietary practices difficult to generate.

2. Specific Aims

To address the gaps in the literature, I examined associations of maternal carbohydrate intake during pregnancy with adiposity and metabolic health outcomes of adolescent offspring in the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT). This was then paralleled in an animal model. Because carbohydrates are necessary for accretion of tissue in the fetus and exposure to high levels of carbohydrates may program metabolic systems for greater presence of CHO, it is expected that carbohydrate intakes during pregnancy will be associated with greater metabolic health risk and higher adiposity in adolescence. This will be evaluated in the following specific aims:

1. Examine the association of maternal carbohydrate intake during pregnancy with offspring adiposity according to body mass index (BMI) z score, waist circumference, skinfold thickness, and metabolic health as indicated by glycemia (fasting blood glucose and insulin), leptin, lipid profile (total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL], and triglycerides), and blood pressure (systolic blood pressure [SBP], and diastolic blood pressure [DBP]).

2. Investigate whether the relationship between maternal carbohydrate intake during pregnancy and offspring adiposity and health is modified by the child's own carbohydrate intake during peri-puberty.

3. Examine the effects of maternal exposure to high carbohydrate diet during pregnancy on offspring adiposity as indicated by indirect fat mass measurement, and metabolic health in a mouse model.

3. Methods

3.1 Human epidemiological methods

Study population

Participants are part of the ongoing Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) cohort in Mexico City. Pregnant women in their first trimester were recruited in public maternity hospitals

between the years 1997 and 2004 to be included as mother-child pairs in the study. From 2011 to 2012, 250 children of these mothers in the ELEMENT cohort were enrolled in the study to evaluate peri-pubertal outcomes. Mother's consent and child's assent were obtained. These children were between the ages of 8 and 14 and they completed anthropometric evaluation, a fasted blood draw, and questionnaires administers by an interviewer. All protocols were approved by the institutional review boards of the Mexico National Institute of Public Health and the University of Michigan.

Dietary assessment

Dietary intake for mothers was collected during each trimester through use of a semi-quantitative food frequency questionnaire (FFQ) based on the 2006 Mexican Health and Nutrition Survey.[22] The FFQ was administered during an interview and is designed to represent the previous month's usual intake. The FFQ is composed of a list of 104 foods, validated as representative for the typical Mexican diet in 1983.[23] Dietary information for the children was collected via self-reported semi-quantitative FFQ, developed in close relation to the 2006 Mexican health and nutrition survey.[22] For children between the ages of 7 and 11, the FFQ was administered with the help of the mother.[24] For children ages 12 and older, the FFQ was completed by the child.

Frequency of consuming a food was reported using a scale ranging from "never" to "6 times a day." Nutrient content for the foods were verified by two of the following food database sources: Instituto Nacional de Salud Publica 2002, the United Stated Department of Agriculture, and the Mexican National Institute of Nutrition and Medical Services Salvador Zubiran.[24]The kilo-calories (kcals) for one portion size of the food was multiplied by its frequency of consumption and all foods were summed, to create a usual daily kcal intake. The food groups are total-energy adjusted using the residuals method. This analysis utilized 3 carbohydrate variables, total carbohydrate, net carbohydrate, and sugar. The net carbohydrate value was calculated as fiber intake subtracted from total carbohydrate intake and was then energy adjusted. The sugar variable represents total sugar consumed, and therefore does not differentiate between sugar from natural sources, such as fruits and vegetables, and added sugars supplied in processed foods.

Adiposity

Indicators of child adiposity was carried out by trained research staff. Weight was measured in kilograms on a digital scale. Height was measure in centimeters using a calibrated stadiometer. Waist circumference was measured using a non-stretchable measuring tape. The skinfold thicknesses of both sub-scapular and triceps were taken in millimeters using calibrated skin calipers. Each of these measures was completed twice, with the average of the two measures being using in the analysis. Using weight and height data collected, age and sex-specific Body Mass Index (BMI) z-scores were calculated for each participant using the WHO references [25] to indicate total body size and overall adiposity. Indicators of visceral adiposity include sub-scapular skinfold thickness and waist circumference. Triceps skinfold thickness indicates subcutaneous adiposity.[26]

Metabolic Health

After an 8 hour fast, blood was collected from children by a trained research assistant. The fasted blood sample provided measures of glycemia, such as glucose, and C-peptide. Blood lipids were deduced from this sample including total cholesterol (mg/dL), triglyceride (mg/dL), and both HDL and LDL cholesterol (mg/dL). Systolic and Diastolic Blood pressure (mmHg) was assessed in the seated position by a research assistant in duplicate, with the average of the two used in the analysis. A summary risk variable (MetRiskz)was calculated using an aver of rive-internally standardized z scores for waist circumference, fasting blood glucose, fasting C-peptide (as a surrogate for insulin secretion), the ratio of triglyceride to HDL content, and the average of blood pressure measures.[27]

Covariates

Upon recruitment, mothers provided information including current age, reproductive history, lifestyle factors, and information on socioeconomic status. During the adolescent research visit, a pediatrician examined each child and assigned a tanner stage of 1(no pubertal development) to 5(fully developed) based on testicles, breasts, and pubic hair.[27]

Data analysis

Data were analyzed using SAS ® software version 9.4. I first conducted a univariate analysis to interrogate the distributions of the variables of interest, which include: mother's carbohydrate intake during each trimester of pregnancy and offspring adiposity and metabolic outcomes (table 1). Then I proceeded with bivariate analyses to

identify potential confounding variables to be included in later multivariable models. This was achieved by looking at adiposity (BMIz score) and metabolic risk (MetRiskz score) distributions in relation across categories of sociodemographic variables (table 2), and maternal and perinatal characteristics (table 3). Variables with a p trend less than 0.2 were considered for inclusion in further models.

I examined associations between child outcomes and quartiles of trimester-specific maternal carbohydrate intake (total CHO, net CHO, and sugar intake). The use of quartiles enabled discovery of non-linear associations. After primary analysis I utilized multivariable linear regression to assess the associations between maternal CHO intakes during pregnancy with the offspring adiposity and metabolic health outcomes using four models. The first was the unadjusted association between maternal intakes and child outcomes. The second, included maternal intakes, maternal age at enrollment, child's age, and child's sex. The third added child's pubertal status, and the fourth included an interaction term between child's carbohydrate intake in peri-puberty and maternal CHO intake in pregnancy to deduce if the associations between maternal intake and child outcomes were mediated by child diet. Tables reflect beta estimates with 95% confidence intervals (CIs). Because inclusion of the interaction term did not precipitously alter the beta estimates, results are shown for model 3. Analysis stratified by sex was evaluated, but was found not to differ, results are therefore not stratified by sex.

We also conducted further sensitivity analyses. First, we evaluated maternal carbohydrate intake using current recommendations for carbohydrate consumption. We coded trimester-specific intakes of total CHO into a three-level variable, to reflect the acceptable macronutrient distribution ratios (AMDR) for carbohydrate; 45-65 percent of energy[28], as well as a two-level variable for the sugar recommendation of less than 10 percent of energy from added sugar.[29] Sugar intakes in ELEMENT are a reflection of total sugar intakes, and therefore are not a direct measure of this recommendation, but it is the closest approximation available in the ELEMENT dietary data. To attempt to parse apart the effects of added sugar from naturally occurring sugar, we conducted trimester-specific multivariable analyses using quartiles of sugar sweetened beverage and fruit intakes with child BMIz and MetRiskz scores. We also used nutrient substitution models to evaluate potential confounding by protein or fat intake. Here, we examined the effect of replacing total carbohydrate intake by a matched kcal portion of either fat or protein.

Finally, we further explored the impact of adjustment for additional covariates – namely, delivery method, and number of household possessions (an indicator of socioeconomic status) in lieu of maternal education.

3.2 Basic science mouse model

Animals

Male and Female NON/ShiLtJ mice (stock # 002423) were purchased from Jackson Laboratory (Bar Harbour, ME).[30] This strain is characterized by low plasma insulin levels and are commonly used in studies for obesity outcomes.[30] Mice are maintained on 12-hour light-dark cycle in a humidity and temperature-controlled facility in accordance with institutional animal care and use committee (IACUC) policy. Addition of sucrose to water was approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC).

Breeding

Virgin female NON/ShiLtJ mice were bred with male NON/ShiLtJ in monogamous pairs. Females carried their first litter to parturition and nursed pups for 3 weeks to allow for development of maternal instinct and proper mammary gland function. Prior to the second mating of these females, half (n=8) were exposed to 10 percent (w/v) sucrose in water *ad libitum* for two weeks, whereas the other half (n=8) received water *ad libitum*. Food intake, and liquid intake were measured weekly. Body weight was measured, and MRIs were taken of dams during each week of exposure and of pregnancy. After two weeks of sucrose exposure, males were reintroduced for breeding as monogamous pairs. The offspring of the second pregnancy were enrolled in the study. After post-natal day (PND) 3, litters were standardized to 4 pups, 2 females and 2 males. The pups that were kept were the closest to the mean weight for each sex at PND 3. Due to mis-assignment as male or female, future litters are to be sexed and reduced at PND 4. At birth, water bottles were taken away and regained access to automated water supply. Litters nursed normally until weaning, and food was weighed weekly. Due to a lower than expected number of litters born during the first round of breeding, a second cohort has begun exposure to increase sample size.

Offspring

On PND 21, mice were weaned by sex and had *ad libitum* access to chow and water. Offspring were maintained in this way into young adulthood (PND 109). Food and water intake was measured weekly. Total body weight, fat mass, and lean mass was measured indirectly through weekly Echo MRI scan beginning PND 21. Once pups reached adolescence (PND 50), pups were subjected to an insulin tolerance test (ITT) to assess insulin sensitivity. This was conducted after a 6-hour by intraperitoneal injection of Humulin (0.75uL/kg body weight). The day before sacrifice, PND 108, pups were subjected to a two-hour preference test where in the first two hours of the dark cycle they were denied water, and in the following two hours were provided two bottles; one filled with 10% sucrose, the other water. Volume consumed of both water and sucrose was recorded and analyzed for evidence of differential sweet taste preference. Following taste preference testing, animals were fasted for 16 hours and sacrificed. At sacrifice, blood glucose levels were assessed by glucometer, and serum was collected by cold centrifugation of whole blood at 12000 rpm for twenty minutes. Liver, inguinal white adipose tissue, and gonadal white adipose tissue were collected and snap frozen using liquid nitrogen and maintained in -80 degrees F.

Data analysis

Statistical analyses and data visualization were completed using R version 3.3.2. Two-way ANOVA was utilized to compare effects of maternal treatment group, sex, and their interaction. The current analysis represents seven total litters born to date (sucrose n=2, water n=5). Due the small sample size of the current treatment groups, the current analysis reflects only preliminary results. Resulting p-values are from a two-way ANOVA following the sacrifice of the animals at PND 109. A p-value of 0.05 or less is significant. Upon completion of additional litters in the study, mixed linear modeling will be employed to assess the significance of treatment group, sex, and control for collinearity of repeated measures.

4. Results

4.1 Human epidemiological results

Energy-adjusted carbohydrate(CHO) intakes in pregnancy are shown in **Table 1**, values are shown as total CHO, net CHO (total CHO minus fiber), and sugar intakes for each trimester. Total CHO intakes meet the recommendation of 175 gram per day during pregnancy for each trimester.[6] Net CHO

intakes and total CHO demonstrate similar variance. Sugar intakes in all three trimesters show the greatest variance, with as little as 10.7 grams per day and as many as 108.4 grams.

Table 2 displays the associations between maternal and child participant characteristics and child BMIz and MetRiskz scores. Delivery method appeared to be associated with both BMI and MetRiskz scores (p= 0.06, 0.10 respectively); with vaginal birth being associated with lower risk. For this reason, we evaluated including birth method in the multivariate model. The inclusion of birthing method failed to change z score estimates, and as a result was excluded in the final models. Child's age was associated with differential adiposity and metabolic risk, and for this reason was included in the model. Pubertal status was associated with differential metabolic risk. With those males and females who had not begun puberty having lower metabolic risk z scores. For this reason, pubertal status was included in the model.

Carbohydrate intakes in each trimester was assessed for maternal and child characteristics in **Table 3**. Although higher parity in the first trimester was associated with greater total CHO intakes (p=0.004), it was not considered for inclusion in the model, as it was not associated with CHO intakes in later trimesters.

Adiposity measures were assessed, and the trend of greater intakes of total and net CHO in the first trimester was associated with lower adiposity in peripuberty. characteristics. **Table 4** shows the maternal intakes of carbohydrate during each trimester in quartiles with indicators of adolescent adiposity. In the first and third trimesters, greater intakes of total CHO are associated with lower BMIz, waist circumference, and skinfold thicknesses. The same trend appears for net carbohydrate intakes and for total CHO in the third trimester, with sub-scapular to triceps skinfold thickness ratio reaching statistical significance (p=0.05). Sugar; however, does not have a linear association. Redistribution of sugar intakes into quintiles did not clarify the directionality and strength of the trend, so further analyses were left as quartiles. Analyses using SSB and fruit intake did not result in consistent trends for either BMIz or MetRiskz outcomes and are therefore not shown. This analysis failed to clarify the effect of sugar intakes one offspring health and adiposity outcomes.

Associations of Glycemia and adipokine (leptin) levels with maternal intakes in CHO are shown in **Table 5**. Total and net CHO intakes in the first and third trimesters were associated with lower c-peptide

levels and c-peptide IR. The trends in glucose and leptin levels were inconsistent, as were those associations with all glycemia measures and sugar intakes.

Blood lipid levels and their associations with maternal carbohydrate intakes are shown in **Table 6**. Although in the second trimester greater total and net CHO intakes appear to be associated with greater total cholesterol in peripuberty, large CIs indicate these estimates are not a sensitive reflection of the true association. The same trend appears in the second trimester for LDL cholesterol, with u-shaped associations and wide confidence intervals.

Table 7 shows associations with metabolic risk and blood pressure. There appear to be no significant trends in direction or magnitude for blood pressure and maternal CHO intakes in pregnancy. In MetRiskz scores, there is a non-significant trend in the first and third trimesters for greater total and net CHO intakes conferring lower risk z scores. However, these associations failed to reach statistical significance.

Sensitivity analyses

Evaluation of sugar and total carbohydrate intake recommendations were completed and are shown in **Table 8**. For both BMIz and MetRiskz scores, consuming more sugar than is recommended is associated with having lower z scores. These associations failed to reach statistical significance. The associations of BMIz in relation to the AMDR for carbohydrate were inconsistent in direction. This is evidenced by the lowest BMIz score being associated with below recommended intakes in the first and third trimester, and with greater than recommended intakes in the second trimester. Similar inconsistencies are evident in MetRiskz; the lowest risk was associated with greater than recommended intakes of CHO in the first, and second trimester, and with lower than recommended intakes in the third trimester. Neither the associations for BMIz nor MetRiskz were significant. The study of nutrient substitution shown in **Table 9** suggests that substitution of either protein or fat for total carbohydrate was neither protective nor detrimental for child adiposity and metabolic risk, as the CI were very large.

4.2 Basic Science Results.

Dams who had *ad libitum* access to sucrose and chow consumed fewer kcals from chow (figure 1A), but more kcals overall (figure 1B); with the remaining energy coming from sucrose. The large differences in macronutrient intakes came from carbohydrate intakes, with the greatest differences in

refined carbohydrates between groups (figure 2A and 2B, respectively). Maternal body weight did not differ by exposure group (figure 3) and the mean differences in gestational weight gain were not significantly different (11.3g±2.70 and 11.4g±1.94 for sucrose and water respectively). Fed blood glucose differed between treatment groups in dams as shown in figure 4 ($p=0.02$). Dams who were exposed to sucrose treatment had greater fed blood glucose than those in the water group.

Offspring outcomes were heavily influenced by sex and were therefore separately analyzed. After PND 30, male and female bodyweights diverged, with males weighing more than females (figure 5A). Beginning PND 80, males from the sucrose group developed greater bodyweight than their water treated counterparts. By the time of sacrifice, bodyweight was not statistically different by treatment as demonstrated in figure 5B ($p=0.24$, but was by sex, $p=0.001$). Offspring fat mass begins to diverge between groups at PND 50, with both male and female sucrose groups having greater fat mass than those exposed to water (figure 6). Fat mass as measured at sacrifice by both inguinal and gonadal fat pats normalized to bodyweight were not significantly different between treatment groups ($p=0.60$ and $p=0.73$, respectively). Offspring fat free mass differed in trend by sex. Female sucrose exposed mice had lower fat free mass than water exposed females, and this persisted from PND 30 to sacrifice. In males, sucrose exposed mice had greater fat free mass than those exposed to water beginning PND 70, which became more divergent over time (figure 7). As muscle mass was not evaluated in sacrifice, no p value is available for this measure. Insulin tolerance was improved in females exposed to sucrose (figure 8A), but not males (figure 8B).

Offspring food intake patterns also differed by sex, with males of both sucrose and water groups eating similar cumulative number of kcals and females exposed to sucrose consuming fewer kcals than those exposed to water (figure 9). Feeding efficiency was explored as a possible mechanism for differing body compositions between groups and sexes (figure 10). It was found that in general, males have greater feeding efficiency ($p=0.03$) than females and that group assignment did not explain the differences in feeding efficiency ($p=0.17$). After exposure to a dark cycle sucrose preference test, it was found that there was no difference in preference to sucrose based on maternal exposure group or sex of offspring (figure 11).

5.Discussion

Here, we showed that maternal carbohydrate intakes were related to offspring phenotype in two models with diverging results. In humans, we found that higher intake of total carbohydrates and sugar during pregnancy was associated with lower adiposity and metabolic risk among 237 mother-child pairs in Mexico City. In the rodent model, we found that maternal sucrose exposure during pregnancy resulted in greater adiposity in both males and females, greater body weights in adult males, and reduced fat free mass in females throughout the life course.

Findings from the human epidemiological study

There are many factors that could contribute to the reduced adiposity associated with greater carbohydrate intakes. One of the most likely contributors to these associations could have been measures of maternal physiology; including maternal pre-pregnancy BMI and a reliable measure of maternal glycemia in pregnancy. Both weight status and hyperglycemia have been found to be associated with nutrient sensing in development, which resulted in a larger birth weights in those infants exposed to both gestational diabetes and obesity.[31] It is possible that maternal glycemia could have confounded the analysis because we did not have information on gestational diabetes(GD) collected in ELEMENT. If this were true, women who had GD may still have been diagnosed by their physician and counseled in executing a carbohydrate-controlled diet, which is known to result in more healthful outcomes for children at birth. The presence of this scenario without the availability of the GD variable to control for in the analysis may have led to a masking of the effects of a carbohydrate controlled dietary counseling intervention. Gestational weight gain is much more complex than simply the weight gained during the course of pregnancy. There is evidence that individual macronutrients may have the propensity to accrete gestational fat tissue.[8] Whisner and colleagues found that carbohydrate intakes in pregnant adolescents were positively associated with an increase in abdominal fat.[8] This effect was driven by added sugar intakes. If more gestational weight gain occurred as a result of carbohydrates intakes, it is possible that there was more abundant substrate (adipose tissue) for maternal gluconeogenesis, providing more glucose to the fetus.

Another plausible explanation of these results could be that exposure to high carbohydrate diet *in utero* programmed the offspring for a metabolic efficiency in metabolizing glucose. This could manifest in

greater utilization of glucose by lean mass during adolescence resulting controlled blood glucose levels without additional fat tissue deposition, as seen in the ELEMENT cohort with lower glycemia and less adiposity. There is also the possibility that the effects of fetal programming changes overtime and may result in a worsening of phenotype as the children age. Programming may occur many way, one such proposed method is through the microbiome, whose diversity can vary greatly on many factors, including birthing method. It has been noted that the odds of overweight and obesity in childhood are increased in those who were delivered via cesarean section.[32] This is usually thought to be a result of unique flora associated with either colonization by vaginal or skin related micro-organisms during the birthing process. In the present study, it is not evident that the associations are attributable to the proxy measure we have available, delivery method.

In any longitudinal analysis, there is potential bias and error. In this study, there are two factors that may be the result of error in measurement. Firstly, capturing complex physiological processes can be difficult. This is true of puberty as it is measured in the ELEMENT analyses. As the pubertal transition is a phenomenon that occurs on a continuous spectrum, it can hard to categorize children into one of five stages. Thus, our results may still be vulnerable to residual confounding in tempo of sexual maturation that was not captured by Tanner stage. Another difficult area to accurately measure in subjects longitudinally is that of the habitual diet. The accuracy of the estimates based on analysis of the questionnaire depend on participants to accurately recall and report usual intakes, which can be a daunting task. This may result in participants underreporting their nutrient intakes. It may also manifest as reporting bias, in that participants may overreport foods they deem to be healthful and underreport foods they see as nutrient poor. If reporting bias was present, and was systemic in nature, it could not be corrected for in linear regression. Furthermore, food lists and options included food frequency questionnaires may not be exhaustive, and therefore could miss portions of the individual's usual diet in the analysis. In general, though FFQs are imperfect and are subject to recall and reporting biases, they are one of the best measures available to deduce habitual diet, especially after total energy intake adjustment of nutrients.[33] The U-shaped associations may have been driven by women who reported the lowest intakes of total carbohydrate also being among those who reported the lowest energy intake. The frequencies of persons reporting both lowest intakes of total carbohydrate and of energy were assessed

for each trimester and they did not outnumber individuals in other intake quartiles; therefore, this is unlikely to be what contributed to U-shaped associations.

After examination of current literature, no other study to date has demonstrated that greater maternal carbohydrate intakes in pregnancy is associated with lower adiposity and metabolic risk parameters. Somewhat similar studies that have focused on glycemic index found that mothers who consumed more carbohydrate low in glycemic index were more likely to deliver children that were small for gestational age.[12] The lack of metabolic dysregulation and adiposity may be a result of the child's *in utero* programmed environment matching the environment they live in daily, one that is rich in carbohydrates. If this is the case, it would take a mismatch of environment to stimulate disease phenotypes and obesity.[34] Still other studies have published findings discordant to these, such as Chen and colleagues, who found that increased carbohydrate intakes in maternal diet during pregnancy was found to be associated with both greater BMI in childhood, but also earlier BMI peak velocity.[34] Inconsistencies in the literature about the effects of high carbohydrate diets in pregnancy with offspring health, and a lack of evidence for the microbiome, nutrient substitution, and sugar intake as a mechanism demonstrates a need for further study. It is still unclear what the true associations for this exposure are, and what biological mechanisms underpin them.

Findings from the animal model

In the animal model, the results are a better reflection of the current literature. Toop et al found similar associated of body weight in early postnatal rats who were prenatally exposed to 10 percent sucrose. Pups were weaned onto chow diet and no differences in body weight were present at 3 weeks between males or females of groups. Unlike this study; however, once these pups reached 12 weeks of age, the lack of differences in body weight persisted including in gonadal fat masses in.[35] Toop and colleagues also found a reduction in relative pancreas weight, which merits further analysis as the mechanism of action at play in the current study. Further evidence for pancreatic alteration driving changes in the offspring was offered when Zhang and colleagues,[18] found that prenatal sucrose exposure in rats increased islet area in offspring which did not result in changes in insulin resistance measure by HOMA-IR. This would likely mean that timing of the exposure *in utero* is related to organogenesis, and more specifically, timing of exposure specific to the development of the pancreas. Because the sample

size is small, analysis of animal tissues collected; including serum, adipose tissue, and liver has not been completed. As a result of the preliminary findings, the next cohort of pups will undergo additional analysis of the pancreas, both at PND 4 in those pups who will be sacrificed to standardize milk supply and at PND 109 at sacrifice. This may perhaps provide better evidence for the mechanism whereby adiposity is greater with sucrose exposure and differing tolerance to insulin is seen when stratified by sex.

Strengths and Limitations of the study

The current study has several strengths, namely the duality in models for assessing the effects of carbohydrates *in utero*. Few studies evaluate the same exposure in both humans and animals. In the human analysis, trimester-specific associations allow for more detailed understanding of critical periods within gestation. The use of multiple adiposity outcomes in children also strengthens this analysis. Subcutaneous and central measures of adiposity having been included allow for more sensitivity and may even facilitate inference of compartmentalization of fat tissue during development. The use of multiple measures of metabolic health, encompassing the whole range of metabolic risk- from hypertensive markers, to lipid markers, and glycemia- to the use of the MetRiskz score provided a detailed picture of adolescent metabolic health. Another benefit is the location of the sample, Mexico City. Mexico is one of the most heavily burdened nations in the world with both adult and childhood obesity.[37,38] Studying the etiology of obesity in a nation that is heavily burdened may elucidate what makes certain nations or individuals a higher risk for development of obesity and comorbid illness and provide optimal opportunity for intervention and prevention.

It is well-established that murine models are highly valuable in understanding the etiology of obesity as a disease,[39] especially as this method enables correction for varying environmental exposures constant across treatment groups and controls for genetic heterogeneity usually observed in human obesity. The use of 10 percent sucrose water was meant to model the habitual consumption sugar sweetened beverages in humans, and therefore represents a physiological dose of high carbohydrate exposure. This study was designed to protein-match control and experimental groups to control for confounding by protein restriction resulting in intrauterine growth restriction (IUGR). Protein restriction was not evident, as the sucrose group consumed 16.7±0.35 percent of energy from protein, and the water group consumed 24±0.00 percent of energy from protein. The sucrose group, although it had lower intake

of protein, did not reach the level of protein intake characteristic of restriction in IUGR studies, usually between 6-8 percent of energy from protein.[40,41] As the timing of exposure being contained to only gestation, and not lactation, allowed us to narrow the timepoint of dietary programming to only effects having happened in the womb, and not confounded by offspring exposure to sucrose through use of water bottles and mother's milk. The ability to monitor weekly changes in both fat and lean masses elucidated differences in body composition before changes in weight were noted.

Despite the careful design and execution of this study, there still exist limitations. In the human analysis, measures of maternal glycemia and of pre-gravid BMI would have been valuable tools. The inclusion of maternal glycemia and therefore gestational diabetes status may have helped to elucidate if the associations of higher carbohydrate intake relating to lower adiposity and metabolic risk z scores was at least, in part, attributable to hyperglycemia. As in any epidemiological study, it is possible that the method of recruiting mothers in health centers may have introduced selection bias, as those who choose to participate in a research study on health may be more invested in their health and take greater care in executing health behaviors characteristic of a healthful pregnancy. The composition of the sugar variable, being representative of total sugar and not added sugar, also created difficulty in comparing this sample to the recommendation for sugar intake given by the Institute of Medicine. Most importantly, a noted limitation of the human analysis is a potential lack of generalizability. The ELEMENT population is largely comprised of low to middle socioeconomic status and Hispanic individuals and therefore confines the generalizability of results to women and children of similar background and financial status.

There are limitations in the animal model as well, as murine models are not a perfect microcosm of human physiology. Because of the level of control of the design, the results are not widely applicable to humans directly. The findings may only become applicable if the driving factor for changes in offspring phenotype turn out to be conserved metabolic systems in both humans and mice. The analysis of individual mechanisms in a mouse model also fail to fully capture the complex milieu of human obesity. Most notably, the low birth rates experienced after the first round of exposure limit the statistical power to observe the true effects manifested in the offspring. Thankfully, with continued breeding and further analysis of offspring tissues, this may be remedied.

The reasons for conflicting results in the two models could be attributable to many things. First, the human analysis analyzed carbohydrate intakes as a whole, whereas in the animal model the effects of refined sugar only was evaluated. The opposing directions of the associations with body composition could be a reflection of the quality of the carbohydrate exposure. There is also the possibility that there exist differences in underlying genetic, physiologic, or environmental factors between the two organisms that were not either held constant in the animal design or were not adjusted for in the multivariable model in the human analysis.

The results of this analysis are valuable in both models, as the murine model will aid in elucidating targets for treatment and prevention, and the human model demonstrates the synergistic effects that the biological mechanism exerts in a very specific population, adolescents in Mexico City. Both are informative and incomplete without the other. In the future, more stringent research is merited to further isolate the reason for departure of results in these two models.

6. Conclusion and Public Health Relevance

It is clearly evidenced in the current study that maternal carbohydrate intakes in pregnancy can potently alter both adiposity and metabolic health parameters in mice and in human children. The effects on offspring in the mouse model are not attributable to maternal body weight, offspring food intakes, feeding efficiency, or preference to hyperpalatable sucrose. The associations of lower adiposity and metabolic risk in human children is not mediated by child intake of carbohydrate in peripuberty and is not explained by maternal adherence to pertinent carbohydrate recommendations.

These findings add to the body of evidence demonstrating the long-term influence of maternal diet in pregnancy, specifically with respect to the differing carbohydrate types and the timing of their consumption, on offspring adiposity and metabolic risk in adolescence. Given the importance of maternal diet during pregnancy, the findings from the present analysis, and in conjunction with the scant and discrepant existing literature, indicate the need for additional research in this area. Further study is required to replicate findings in human epidemiologic studies, and to elucidate mechanisms driving the detected associations, in order to provide expectant mothers with realistic and attainable recommendations to optimize child health outcomes with maternal carbohydrate intake.

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8. Tables and Figures

Table 1 Distribution of total energy adjusted carbohydrate intake (grams/day) during pregnancy among 237 ELEMENT mothers.

	N	Mean ± SD	Percentile					
			5th	25th	50th	75th	95th	Max
1st trimester								
Total carbohydrate intake (g/day)	228	266.9 ± 33.0	212.2	245.3	266.1	288.5	322.4	363.2
Net carbohydrate intake (g/day)	228	243.6 ± 31.0	194.4	223.2	243.1	265.9	293.9	343.0
Sugar intake (g/day)	228	34.2 ± 16.4	12.3	22.2	31.6	44.6	62.2	89.2
2nd trimester								
Total carbohydrate intake (g/day)	235	269.8 ± 33.5	212.2	248.7	268.3	293.4	323.5	379.1
Net carbohydrate intake (g/day)	235	246.9 ± 31.6	194.0	226.0	246.0	267.9	299.9	351.9
Sugar intake (g/day)	235	36.4 ± 17.8	11.9	23.3	33.8	46.2	71.4	89.3
3rd trimester								
Total carbohydrate intake (g/day)	236	269.7 ± 35.1	213.7	244.6	269.6	293.8	333.4	366.7
Net carbohydrate intake (g/day)	236	247.4 ± 33.1	196.9	223.5	245.6	269.7	305.9	337.0
Sugar intake (g/day)	236	37.3 ± 18.6	10.7	25.1	34.1	46.4	71.9	108.4

Totals are energy adjusted using the residuals method

Table 2 Distribution of BMI z-score and metabolic risk phenotype risk z-score ("MetRisk z-score") across characteristics of 237 ELEMENT mother-child pairs.

	BMI z-score				MetRisk z-score			
	N	Mean ± SD		*P*[a]	*N*	Mean ± SD		*P*[a]
Overall	237	0.87	± 1.24		235	0.00	± 0.63	
Maternal characteristics								
Age at enrollment				0.99				0.89
15-24 y	87	0.95	± 1.33		87	0.05	± 0.59	
25-34 y	117	0.75	± 1.16		117	-0.08	± 0.60	
35-44 y	32	1.10	± 1.25		32	0.16	± 0.75	
Marital status				0.73				0.24
Married	168	0.85	± 1.22		166	-0.3	± 0.65	
Single	69	0.92	± 1.27		69	0.07	± 0.56	
Maternal education				0.93				0.80
< 10 years	85	0.87	± 1.24		84	-0.01	± 0.60	
10 - 12 years	116	0.88	± 1.27		115	0.002	± 0.64	
≥ 13 years	34	0.89	± 1.15		34	0.03	± 0.64	
Parity				0.23				0.32
0	84	0.92	± 1.26		83	0.03	± 0.60	
1 to 2	139	0.91	± 1.19		138	0.01	± 0.62	
≥ 3	14	0.21	± 1.38		14	-0.22	± 0.78	
Smoking during pregnancy				0.97				0.17
Yes	3	0.85	± 0.62		3	-0.50	± 0.14	
No	234	0.87	± 1.24		232	0.01	± 0.63	
Delivery method				0.06				0.10
C-section	101	1.05	± 1.26		101	0.08	± 0.64	
Vaginal	134	0.75	± 1.21		132	-0.06	± 0.61	
Delivery weight				0.23				0.40
<3100 grams	111	0.97	± 1.19		109	0.04	± 0.59	
>3100 grams	125	0.78	± 1.27		125	-0.03	± -0.66	
Child characteristics								
Sex				0.86				0.99
Male	112	0.89	± 1.19		111	0.00	± 0.65	
Female	125	0.86	± 1.28		124	0.00	± 0.61	
Child's age				0.11				**<0.0001**
<10 y	124	0.99	± 1.21		123	-0.15	± 0.64	
10 to 12 y	63	0.78	± 1.25		62	0.09	± 0.57	
> 12 y	50	0.69	± 1.27		50	0.26	± 0.57	

Carbohydrate intake				0.57				0.96
Q1 (lowest)	59	1.09	± 1.40		59	0.02	± 0.64	
Q2	59	0.65	± 1.30		59	-0.07	± 0.59	
Q3	59	0.89	± 1.25		57	0.06	± 0.66	
Q4 (highest)	59	0.87	± 0.94		59	-0.01	± 0.62	
Physical activity (h/day)				0.58				0.17
Q1 (lowest)	33	0.88	± 1.27		33	0.15	± 0.66	
Q2	70	0.93	± 1.36		70	-0.08	± 0.64	
Q3	59	0.91	± 1.20		57	0.03	± 0.61	
Q4 (highest)	75	0.78	± 1.14		75	-0.02	± 0.60	
Time spent watching TV (h/day)				0.68				0.17
Q1 (lowest)	55	0.93	± 1.04		53	-0.14	± 0.52	
Q2	57	0.92	± 1.25		57	0.05	± 0.70	
Q3	59	0.76	± 1.27		59	0.02	± 0.66	
Q4 (highest)	66	0.88	± 1.36		66	0.05	± 0.60	
Pubertal status: Males[a]				0.92				**0.01**
Prepubertal	56	0.92	± 1.28		55	-0.16	± 0.59	
Pubertal	52	0.90	± 1.11		52	0.15	± 0.68	
Pubertal status: Females[b]				0.65				**0.01**
Prepubertal	85	0.82	± 1.31		84	-0.10	± 0.64	
Pubertal	40	0.93	± 1.24		40	0.22	± 0.46	

[a] Represents a test for linear trend where an ordinal indicator is entered into the model as continuous variable, with the exception of binary variables (Wald test).
[b] Puberty was defined as Tanner stage 3-5 (vs. 1-2) for breast (girls), testicular (boys), and pubic hair (both) development.

Table 3 Distribution of total carbohydrate intake during pregnancy across characteristics of 237 ELEMENT mother-child pairs.

	1st trimester			2nd Trimester			3rd Trimester		
	N	Mean ± SD	*P*[a]	*N*	Mean ± SD	*P*[a]	*N*	Mean ± SD	*P*[a]
Overall	228	266.9 ± 33.0		235	269.8 ± 33.5		236	269.7 ± 35.1	
Maternal characteristics									
Age at enrollment			0.41			0.80			0.15
15-24 y	84	268.7 ± 33.3		86	269.0 ± 31.5		86	273.4 ± 35.2	
25-34 y	111	266.0 ± 33.5		116	270.2 ± 34.6		117	268.8 ± 34.7	
35-44 y	32	263.6 ± 30.3		32	270.4 ± 36.1		32	263.4 ± 36.5	
Marital status			0.99			0.47			0.22
Married	165	266.9 ± 33.2		166	268.8 ± 32.9		168	267.9 ± 33.8	
Single	63	266.9 ± 32.8		69	272.3 ± 35.0		68	274.1 ± 38.0	
Maternal education			0.94			0.91			0.80
< 10 years	81	265.0 ± 33.9		84	268.5 ± 35.5		85	268.2 ± 36.0	
10 - 12 years	112	269.1 ± 31.8		115	271.6 ± 30.4		115	270.8 ± 34.3	
≥ 13 years	33	261.9 ± 34.5		34	265.8 ± 39.0		34	268.9 ± 36.2	
Parity			**0.004**			0.31			0.99
0	79	260.9 ± 28.9		83	272.1 ± 34.0		83	270.6 ± 36.9	
1 to 2	135	268.0 ± 33.8		138	269.2 ± 34.0		139	268.6 ± 34.1	
≥ 3	14	290.5 ± 37.5		14	262.5 ± 24.3		14	275.4 ± 35.2	
Smoking during pregnancy			0.55			0.35			0.58
Yes	3	255.6 ± 25.1		3	251.8 ± 36.1		3	280.8 ± 54.0	
No	225	267.1 ± 33.1		232	270.1 ± 33.5		233	269.5 ± 34.9	
Delivery type			0.11			0.90			0.17
C-Section	98	263.0 ± 32.9		99	269.6 ± 34.0		101	266.1 ± 34.3	
Vaginal	129	269.9 ± 33.1		134	270.1 ± 33.4		133	272.5 ± 35.6	

[a] Represents a test for linear trend where an ordinal indicator is entered into the model as continuous variable, with the exception of binary variables (Wald test).

Table 4 Associations between trimester-specific maternal carbohydrate intake and offspring indicators of adiposity during peripuberty

Quartile of intake (median g/day)	β (95% CI) in offspring adiposity[a]			
	BMIz[b]	WC	SS+TR	SS:TR
1st trimester				
Total carbohydrate				
Q1 (228.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	0.29 (-0.14, 0.73)	3.33 (-0.21, 6.87)	3.83 (-0.25, 7.92)	0.06 (-0.02, 0.14)
Q3 (275.8)	0.36 (-0.07, 0.80)	2.62 (-0.90, 6.14)	2.63 (-1.43, 6.69)	0.003 (-0.08, 0.08)
Q4 (306.9)	-0.12 (-0.55, 0.31)	-0.25 (-3.76, 3.27)	-0.25 (-4.30, 3.81)	-0.03 (-0.12, 0.05)
P-difference[c]	0.10	0.13	0.16	0.18
Net carbohydrate				
Q1 (206.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (233.2)	0.32 (-0.12, 0.76)	3.58 (0.03, 7.14)	4.01 (-0.09, 8.11)	0.07 (-0.01, 0.15)
Q3 (251.6)	0.17 (-0.27, 0.60)	1.22 (-2.31, 4.75)	1.14 (-2.93, 5.20)	-0.01 (-0.09, 0.07)
Q4 (282.0)	-0.11 (-0.55, 0.32)	0.09 (-3.44, 3.61)	-0.40 (-4.46, 3.66)	-0.04 (-0.12, 0.04)
P-difference[c]	0.26	0.20	0.17	0.07
Sugar				
Q1 (16.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (26.5)	-0.18 (-0.62, 0.26)	-1.08 (-4.65, 2.49)	-1.94 (-6.04, 2.17)	-0.01 (-0.09, 0.08)
Q3 (36.0)	-0.18 (-0.62, 0.26)	-1.31 (-4.90, 2.28)	0.50 (-3.64, 4.63)	-0.02 (-0.10, 0.07)
Q4 (54.8)	0.004 (-0.45, 0.44)	-1.03 (-4.65, 2.58)	-1.00 (-5.16, 3.16)	-0.04 (-0.12, 0.05)
P-difference[c]	0.76	0.81	0.61	0.68
2nd trimester				
Total carbohydrate				
Q1 (232.9)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	-0.25 (-0.69, 0.19)	-1.81 (-5.37, 1.75)	-2.74 (-6.88, 1.40)	-0.05 (-0.13, 0.04)
Q3 (279.7)	0.04 (-0.40, 0.49)	1.50 (-2.09, 5.10)	-1.26 (-5.45, 2.92)	0.03 (-0.05, 0.12)
Q4 (308.7)	-0.33 (-0.78, 0.12)	-2.73 (-6.37, 0.90)	-2.23 (-6.46, 2.00)	-0.03 (-0.11, 0.06)
P-difference[c]	0.33	0.11	0.68	0.28
Net carbohydrate				
Q1 (213.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.7)	0.07 (-0.52, 0.37)	-0.55 (-4.15, 3.05)	-0.59 (-4.75, 3.58)	0.004 (-0.08, 0.09)
Q3 (254.5)	-0.05 (-0.50, 0.40)	0.77 (-2.86, 4.40)	-1.67 (-5.87, 2.52)	0.05 (-0.04, 0.13)
Q4 (284.4)	-0.19 (-0.65, 0.27)	-2.07 (-5.77, 1.64)	-1.26 (-5.55, 3.03)	0.01 (-0.07, 0.10)
P-difference[c]	0.93	0.51	0.92	0.60
Sugar				
Q1 (16.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (29.1)	-0.01 (-0.46, 0.44)	-0.03 (-3.67, 3.61)	-0.47 (-4.67, 3.74)	0.02 (-0.06, 0.11)

Q3 (39.8)	-0.17 (-0.62, 0.28)	-1.35 (-5.00, 2.30)	-0.81 (-5.03, 3.41)	-0.03 (-0.11, 0.06)
Q4 (58.6)	-0.03 (-0.50, 0.44)	-1.64 (-5.43, 2.15)	-0.61 (-4.99, 3.77)	-0.04 (-0.12, 0.05)
P-difference[c]	0.90	0.80	0.99	0.55
3rd trimester				
Total carbohydrate				
Q1 (230.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (255.7)	0.11 (-0.34, 0.56)	1.79 (-1.87, 5.45)	2.37 (-1.84, 6.58)	0.09 (0.01, 0.17)
Q3 (280.6)	-0.16 (-0.59, 0.28)	0.45 (-3.12, 4.02)	-0.003 (-4.11, 4.10)	0.05 (-0.03, 0.13)
Q4 (311.7)	-0.41 (-0.85, 0.04)	-1.82 (-5.46, 1.82)	-2.33 (-6.52, 1.86)	-0.02 (-0.10, 0.06)
P-difference[c]	0.14	0.29	0.21	**0.05**
Net carbohydrate				
Q1 (212.6)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.4)	0.09 (-0.36, 0.53)	1.60 (-2.02, 5.23)	1.19 (-2.99, 5.37)	0.06 (-0.03, 0.14)
Q3 (258.4)	-0.02 (-0.46, 0.41)	1.64 (-1.87, 5.15)	1.52 (-2.52, 5.57)	0.04 (-0.04, 0.12)
Q4 (288.5)	-0.37 (-0.81, 0.06)	-1.40 (-4.91, 2.12)	-2.24 (-6.29, 1.81)	-0.02 (-0.10, 0.07)
P-difference[c]	0.22	0.32	0.30	0.32
Sugar				
Q1 (18.4)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (30.4)	-0.57 (-1.01, -0.13)	-3.97 (-7.55, -0.39)	-4.27 (-8.40, -0.15)	-0.10 (-0.18, -0.01)
Q3 (38.8)	-0.44 (-0.88, 0.01)	-2.56 (-6.17, 1.05)	-1.78 (-5.94, 2.38)	-0.02 (-0.10, 0.07)
Q4 (57.7)	-0.44 (-0.89, 0.01)	-3.45 (-7.12, 0.22)	-4.07 (-8.30, 0.16)	-0.04 (-0.13, 0.04)
P-difference[c]	0.07	0.12	0.13	0.14

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status

[b] Body Mass Index z score (BMIz) is calculated according to the WHO growth reference for children ages 5-19.

WC: waist circumference (cm)

SS: Sub-scapular skinfold thickness (mm) TR: Triceps Skinfold thickness (mm)

SS + TR: the sum of sub-scapular and triceps skinfolds in mm

SS:TR: the ratio of sub-scapular to triceps skinfold thickness

[c] P-difference is the result of a Wald Chi Square test

Table 5 **Associations** between trimester-specific maternal carbohydrate intake and offspring biomarkers of glycemia during peripuberty

Quartile of intake (median g/day)	β (95% CI) in offspring measures of glycemia[a] Glucose (mg/dL)	C-peptide (ng/mL)	CP- IR[b]	Leptin (ng/mL)
1st trimester				
Total carbohydrate				
Q1 (228.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	1.40 (-1.95, 4.75)	0.26 (-0.15, 0.68)	0.07 (-0.05, 0.19)	1.14 (-1.90, 4.19)
Q3 (275.8)	2.58 (-0.73 (5.90)	0.20 (-0.21, 0.61)	0.07 (-0.05, 0.18)	2.06 (-0.95, 5.08)
Q4 (306.9)	-0.30 (-3.63, 3.03)	-0.31 (-0.72, 0.11)	-0.07 (-0.19, 0.05)	-1.03 (-4.06, 1.99)
P-difference[c]	0.38	**0.05**	0.08	0.13
Net carbohydrate				
Q1 (206.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (233.2)	2.99 (-0.34, 6.32)	0.46 (0.05, 0.88)	0.14 (0.02, 0.25)	1.61 (-1.44, 4.65)
Q3 (251.6)	0.24 (-3.07, 3.56)	0.08 (-0.33, 0.49)	0.01 (-0.10, 0.13)	1.24 (-1.79, 4.28)
Q4 (282.0)	-1.08 (-4.40, 2.23)	-0.29 (-0.70, 0.12)	-0.07 (-0.18, 0.05)	-0.91 (-3.94, 2.12)
P-difference[c]	0.13	**0.01**	**0.01**	0.23
Sugar				
Q1 (16.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (26.5)	4.51 (1.21, 7,81)	0.31 (-0.11, 0.73)	0.11 (-0.00, 0.23)	-1.31 (-4.36, 1.75)
Q3 (36.0)	3.17 (-0.17, 6.51)	0.14 (-0.28, 0.57)	0.05 (-0.07, 0.16)	-0.29 (-3.38, 2.79)
Q4 (54.8)	0.93 (-2.41, 4.27)	-0.04 (-0.46, 0.39)	-0.00 (-0.12, 0.12)	-1.13 (-4.22, 1.97)
P-difference[c]	0.06	0.43	0.22	0.90
2nd trimester				
Total carbohydrate				
Q1 (232.9)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	2.23 (-1.14, 5.59)	0.14 (-0.29, 0.56)	0.07 (-0.05, 0.19)	-2.12 (-5.19, 0.95)
Q3 (279.7)	0.28 (-3.10, 3.67)	0.12 (-0.31, 0.55)	0.03 (-0.09, 0.15)	0.31 (-2.77, 3.39)
Q4 (308.7)	3.74 (0.31, 7.16)	-0.26 (-0.69, 0.17)	-0.04 (-0.16, 0.08)	-2.66 (-5.78, 0.46)
P-difference[c]	0.14	0.23	0.33	0.18
Net carbohydrate				
Q1 (213.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.7)	3.41 (0.04, 6.79)	0.29 (-0.13, 0.72)	0.07 (-0.05, 0.19)	-1.39 (-4.48, 1.70)
Q3 (254.5)	1.93 (-1.44, 5.30)	0.14 (-0.29, 0.56)	-0.01 (-0.13, 0.11)	0.63 (-2.45, 3.72)
Q4 (284.4)	4.38 (0.94, 7.82)	-0.12 (-0.56, 0.31)	-0.04 (-0.16, 0.09)	-2.32 (-5.47, 0.84)
P-difference[c]	0.09	0.22	0.31	0.27
Sugar				
Q1 (16.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (29.1)	-0.63 (-4.04, 2.79)	0.33 (-0.09, 0.76)	0.11 (-0.01, 0.23)	-0.36 (-3.48, 2.76)

Q3 (39.8)	1.62 (-1.83, 5.06)	-0.05 (-0.48, 0.38)	0.04 (-0.08, 0.16)	-1.16 (-4.30, 1.99)
Q4 (58.6)	1.34 (-2.22, 4.89)	-0.16 (-0.60, 0.29)	-0.00 (-0.12, 0.12)	-0.79 (-4.04, 2.45)
P-difference[c]	0.56	0.11	0.17	0.95
3rd trimester				
Total carbohydrate				
Q1 (230.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (255.7)	0.43 (-3.03, 3.88)	0.18 (-0.24, 0.61)	0.05 (-0.07, 0.16)	1.83 (-1.24, 4.91)
Q3 (280.6)	-0.35, -3.73, 3.04)	-0.15 (-0.57, 0.27)	-0.05 (-0.16, 0.07)	2.11 (-0.90, 5.13)
Q4 (311.7)	0.76 (-2.70, 4.21)	-0.42 (-0.85, 0.01)	-0.10 (-0.22, 0.02)	-2.52 (-5.60, 0.56)
P-difference[c]	0.94	**0.04**	0.10	**0.01**
Net carbohydrate				
Q1 (212.6)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.4)	-3.77 (-7.16, -0.38)	-0.27 (-0.69, 0.16)	-0.10 (-0.22, 0.02)	1.51 (-1.57, 4.58)
Q3 (258.4)	-0.58 (-3.88, 2.71)	-0.38 (-0.79, 0.03)	-0.11 (-0.23, 0.00)	2.24 (-0.75, 5.23)
Q4 (288.5)	-0.93 (-4.23, 2.37)	-0.54 (-0.96, -0.13)	-0.15 (-0.26, -0.03)	-1.63 (-4.62, 1.36)
P-difference[c]	0.15	**0.05**	0.06	0.08
Sugar				
Q1 (18.4)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (30.4)	-2.54 (-5.93, 0.86)	-0.48 (-0.90, -0.05)	-0.14 (-0.26, -0.02)	-3.10 (-6.18, -0.02)
Q3 (38.8)	-0.17 (-3.58, 3.23)	-0.21 (-0.64, 0.21)	-0.07 (-0.19, 0.05)	-1.38 (-4.47, 1.71)
Q4 (57.7)	-0.89 (-4.38, 2.59)	-0.41 (-0.84, 0.03)	-0.12 (-0.24, 0.00)	-1.58 (-4.74, 1.58)
P-difference[c]	0.43	0.10	0.07	0.23

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status

[b] CP-IR: C-peptide associated insulin resistance score

[c] P-difference is the result of a Wald Chi Square test

Table 6 Associations between trimester-specific carbohydrate intakes and offspring lipid profile during peripuberty

Quartile of intake (median g/day)	β (95% CI) in offspring measures of blood lipids[a] Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
1st trimester				
Total carbohydrate				
Q1 (228.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	5.03 (-4.77, 14.83)	-2.57 (-18.77, 13.62)	1.58 (-2.66, 5.82)	3.97 (-4.05, 11.99)
Q3 (275.8)	7.41 (-2.28, 17.11)	9.89 (-6.13, 25.91)	1.95 (-2.25, 6.15)	3.48 (-4.45, 11.42)
Q4 (306.9)	4.11 (-5.63, 13.84)	7.11 (-8.98, 23.20)	3.56 (-0.65, 7.78)	-0.88 (-8.85, 7.09)
P-difference[b]	0.58	0.54	0.36	0.59
Net carbohydrate				
Q1 (206.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (233.2)	1.34 (-8.38, 11.05)	1.85 (-14.23, 17.94)	2.08 (-2.16, 6.32)	-1.12 (-9.07, 6.84)
Q3 (251.6)	10.84 (1.17, 20.52)	16.77 (0.75, 32.79)	1.52 (-2.70, 5.74)	5.97 (-1.95, 13.89)
Q4 (282.0)	2.24 (-7.43, 11.92)	10.61 (-5.41, 26.62)	3.21 (-1.01, 7.43)	-3.09 (-11.01, 4.83)
P-difference[b]	0.17	0.28	0.44	0.18
Sugar				
Q1 (16.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (26.5)	3.54 (-6.17, 13.25)	5.16 (-10.93, 21.25)	2.52 (-1.68, 6.73)	-0.01 (-8.02, 7.99)
Q3 (36.0)	10.85 (1.04, 20.67)	15.93 (-0.34, 32.20)	4.60 (0.35, 8.86)	3.07 (-5.03, 11.16)
Q4 (54.8)	6.74 (-3.09, 16.57)	10.97 (-5.32, 27.27)	1.03 (-3.23, 5.29)	3.51 (-4.59, 11.62)
P-difference[b]	0.21	0.43	0.14	0.78
2nd trimester				
Total carbohydrate				
Q1 (232.9)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	-11.16 (-20.91, -1.41)	-9.49 (-25.68, 6.70)	-0.81 (-5.12, 3.49)	-8.45 (-16.45, -0.45)
Q3 (279.7)	3.54 (-6.27, 13.34)	11.07 (-5.21, 27.35)	-0.53 (-4.86, 3.80)	1.86 (-6.19, 9.90)
Q4 (308.7)	-3.46 (-13.38, 6.45)	9.32 (-7.14, 25.78)	-0.04 (-4.42, 4.34)	-5.28 (-13.42, 2.85)
P-difference[b]	**0.03**	0.06	0.95	**0.05**
Net carbohydrate				
Q1 (213.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.7)	-13.66 (-23.35, -3.97)	-4.62 (-20.93, 11.69)	-4.42 (-8.69, -0.15)	-8.31 (-16.35, -0.27)
Q3 (254.5)	4.58 (-5.10, 14.26)	10.71 (-5.58, 27.00)	0.32 (-3.95, 4.58)	2.13 (-5.90, 10.15)
Q4 (284.4)	-3.53 (-13.42, 6.36)	13.44 (-3.20, 30.08)	-1.90 (-6.26, 2.46)	-4.32 (-12.52, 3.89)
P-difference[b]	**0.003**	0.10	0.10	0.06
Sugar				
Q1 (16.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (29.1)	-5.12 (-15.09, 4.84)	-0.87 (-17.32, 15.58)	0.23 (-4.10, 4.56)	-5.17 (-13.32, 2.97)

Q3 (39.8)	-2.46 (-12.51, 7.59)	-11.89 (-28.48, 4.70)	-0.21 (-4.57, 4.16)	0.13 (-8.09, 8.34)
Q4 (58.6)	2.11 (-8.27, 12.49)	2.46 (-14.67, 19.58)	1.78 (-2.73, 6.28)	-0.16 (-8.64, 8.33)
P-difference[b]	0.52	0.36	0.85	0.50
3rd trimester				
Total carbohydrate				
Q1 (230.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (255.7)	5.73 (-4.29, 15.76)	16.68 (0.21, 33.16)	-2.10 (-6.42, 2.22)	4.50 (-3.67, 12.67)
Q3 (280.6)	-1.84 (-11.67, 7.98)	6.51 (-9.63, 22.66)	-1.83 (-6.06, 2.40)	-1.32 (-9.33, 6.69)
Q4 (311.7)	-2.16 (-12.19, 7.87)	-2.57 (-19.05, 13.91)	2.62 (-1.70, 6.94)	-4.27 (-12.44, 3.91)
P-difference[b]	0.40	0.11	0.14	0.23
Net carbohydrate				
Q1 (212.6)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.4)	-0.06 (-10.05, 9.93)	21.80 (5.60, 38.01)	-4.19 (-8.46, 0.09)	-0.23 (-8.38, 7.92)
Q3 (258.4)	-3.02 (-12.73, 6.70)	-2.93 (-18.68, 12.83)	-1.53 (-5.68, 2.63)	-0.90 (-8.82, 7.02)
Q4 (288.5)	-2.35 (-12.08, 7.37)	5.89 (-9.88, 21.67)	1.30 (-2.86, 5.46)	-4.83 (-12.77, 3.10)
P-difference[b]	0.91	**0.02**	0.10	0.65
Sugar				
Q1 (18.4)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (30.4)	1.91 (-7.97, 11.79)	7.54 (-8.83, 23.91)	1.98 (-2.32, 6.29)	-1.58 (-9.65, 6.48)
Q3 (38.8)	-6.83 (-16.73, 3.08)	-2.86 (-19.28, 13.55)	1.32 (-2.99, 5.63)	-7.57 (-15.66, 0.51)
Q4 (57.7)	-5.54 (-15.67, 4.59)	-7.31 (-24.10, 9.48)	2.57 (-1.84, 6.98)	-6.65 (-14.92, 1.62)
P-difference[b]	0.26	0.37	0.75	0.21

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status

HDL: High density Lipoprotein

LDL: Low density Lipoprotein

[b] P-difference is the result of a Wald Chi Square test

Table 7 Associations between trimester specific intakes of carbohydrate in pregnancy and offspring blood pressure and metabolic risk in peripuberty

	β (95% CI) in offspring measures of blood pressure and metabolic risk[a]		
Quartile of intake (median g/day)	**SBP**	**DBP**	**MetRiskz[b]**
1st trimester			
Total carbohydrate			
Q1 (228.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	1.89 (-1.61, 5.39)	1.18 (-1.37, 3.72)	0.17 (-0.05, 0.38)
Q3 (275.8)	-1.23 (-4.71, 2.25)	0.25 (-2.28, 2.78)	0.15 (-0.06, 0.36)
Q4 (306.9)	-1.52 (-4.99, 1.96)	-0.12 (-2.65, 2.41)	-0.07 (-0.28, 0.15)
P-difference[c]	0.27	0.72	0.13
Net carbohydrate			
Q1 (206.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (233.2)	1.90 (-1.61, 5.42)	1.25 (-1.30, 3.80)	0.25 (0.03, 0.46)
Q3 (251.6)	-1.50 (-4.98, 1.99)	-0.56 (-3.09, 1.97)	0.07 (-0.14, 0.29)
Q4 (282.0)	-0.75 (-4.23, 2.73)	0.16 (-2.37, 2.68)	-0.05 (-0.26, 0.17)
P-difference[c]	0.33	0.59	0.07
Sugar			
Q1 (16.3)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (26.5)	-1.83 (-5.32, 1.67)	-0.55 (-3.07, 1.98)	0.10 (-0.11, 0.32)
Q3 (36.0)	-2.15 (-6.67, 0.37)	-2.22 (-4.77, 0.32)	0.04 (-0.18, 0.26)
Q4 (54.8)	-0.78 (-4.32, 2.76)	-1.28 (-3.84, 1.28)	-0.002 (-0.22, 0.22)
P-difference[c]	0.43	0.43	0.81
2nd trimester			
Total carbohydrate			
Q1 (232.9)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (258.7)	-2.13 (-5.66, 1.41)	-1.25 (-3.80, 1.31)	-0.05 (-0.27, 0.17)
Q3 (279.7)	-1.81 (-5.39, 1.76)	-0.86 (-3.44, 1.72)	0.04 (-0.18, 0.26)
Q4 (308.7)	-0.05 (-3.66, 3.56)	0.84 (-1.77, 3.44)	0.04 (-0.18, 0.27)
P-difference[c]	0.53	0.36	0.84
Net carbohydrate			
Q1 (213.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.7)	-1.96 (-5.52, 1.59)	-1.55 (-4.10, 1.01)	0.05 (-0.17, 0.27)
Q3 (254.5)	-0.68 (-4.26, 2.90)	-0.71 (-3.29, 1.87)	0.08 (-0.14, 0.30)
Q4 (284.4)	0.14 (-3.52, 3.80)	0.77 (-1.86, 3.40)	0.11 (-0.11, 0.34)
P-difference[c]	0.67	0.31	0.74
Sugar			
Q1 (16.7)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)

Q2 (29.1)	1.12 (-2.44, 4.69)	0.72 (-1.87, 3.32)	0.05 (-0.17, 0.27)
Q3 (39.8)	0.44 (-3.14, 4.03)	0.72 (-1.88, 3.32)	-0.06 (-0.28, 0.17)
Q4 (58.6)	3.25 (-0.47, 6.97)	1.07 (-1.63, 3.77)	0.04 (-0.19, 0.27)
P-difference[c]	0.33	0.95	0.80
3rd trimester			
Total carbohydrate			
Q1 (230.2)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (255.7)	0.54 (-3.06, 4.13)	0.62 (-1.99, 3.24)	0.15 (-0.07, 0.37)
Q3 (280.6)	2.67 (-0.84, 6.18)	1.21 (-1.34, 3.76)	0.05 (-0.17, 0.26)
Q4 (311.7)	-1.37 (-4.95, 2.20)	-0.40 (-3.00, 2.21)	-0.13 (-0.35, 0.09)
P-difference[c]	0.17	0.65	0.12
Net carbohydrate			
Q1 (212.6)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (235.4)	0.80 (-2.76, 4.36)	0.61 (-1.98, 3.20)	0.01 (-0.21, 0.23)
Q3 (258.4)	3.67 (0.23, 7.11)	2.00 (-0.50, 4.50)	0.02 (-0.19, 0.23)
Q4 (288.5)	-0.33 (-3.77, 3.11)	0.28 (-2.23, 2.78)	-0.12 (-0.33, 0.10)
P-difference[c]	0.12	0.45	0.58
Sugar			
Q1 (18.4)	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)
Q2 (30.4)	-0.30 (-3.87, 3.26)	0.55 (-2.03, 3.12)	-0.18 (-0.40, 0.04)
Q3 (38.8)	-0.79 (-4.39, 2.80)	0.22 (-2.38, 2.81)	-0.09 (-0.31, 0.12)
Q4 (57.7)	-0.08 (-3.73, 3.58)	-0.50 (-3.14, 2.14)	-0.17 (-0.40, 0.05)
P-difference[c]	0.95	0.88	0.31

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status

[b] MetRiskz: a cumulative z score calculated by taking the average of 5 internally- standardized z-scores for waist circumference, blood glucose, c-peptide, triglyceride/(high density lipoprotein), and (systolic +diastolic blood pressure)/2

SBP: Systolic Blood Pressure (mmHg)

DBP: Diastolic Blood Pressure (mmHg)

[c]P-difference is the result of a Wald Chi Square test

Table 8 Associations of maternal Intakes during pregnancy in relation to nutritional recommendations and child adiposity and metabolic risk in peripuberty

Sugar Recommendations	β (95% CI)[a]	
Trimester 1 *(N)*	BMIz[b]	MetRiskz[c]
Sugar<10% energy *(142)*	0.00 (reference)	0.00 (reference)
Sugar>10% energy *(66)*	-0.12 (-0.49, 0.25)	-0.03 (-0.22, 0.15)
P-difference[d]	0.52	0.74
Trimester 2 *(N)*		
Sugar<10% energy (*160)*	0.00 (reference)	0.00 (reference)
Sugar>10% energy *(59)*	-0.16 (-0.54, 0.21)	-0.07 (-0.26, 0.11)
P-difference[d]	0.39	0.44
Trimester 3 *(N)*		
Sugar<10% energy (*149*)	0.00 (reference)	0.00 (reference)
Sugar>10% energy (*72)*	-0.09 (-0.44, 0.27)	-0.07 (-0.24, 0.10)
P-difference[d]	0.63	0.42
AMDR Recommendations[e]		
Trimester 1 *(N)*		
<45% energy *(64)*	-0.06(-0.47, 0.36)	0.03 (-0.18, 0.23)
45-65% energy *(54)*	0.00 (reference)	0.00 (reference)
>65% energy *(90)*	0.06 (-0.38, 0.39)	-0.05 (-0.24, 0.14)
P-difference[d]	0.73	0.45
Trimester 2 *(N)*		
<45% energy *(67)*	-0.28 (-0.68, 0.12)	-0.08 (-0.28, 0.12)
45-65% energy *(64)*	0.00 (reference)	0.00 (reference)
>65% energy *(88)*	-0.42 (-0.79, -0.05)	-0.19 (-0.38, -0.01)
P-difference[d]	0.44	0.25
Trimester 3 *(N)*		
<45% energy *(61)*	-0.41 (-0.80, -0.01)	-0.14 (-0.33, 0.06)
45-65% energy *(76)*	0.00 (reference)	0.00 (reference)
>65% energy *(84)*	-0.18 (-0.55, 0.18)	-0.12 (-0.30, 0.06)
P-difference[d]	0.33	0.94

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status
[b]Body Mass Index z score (BMIz) is calculated according to the WHO growth reference for children ages 5-19.
[c] MetRiskz: a cumulative z score calculated by taking the average of 5 internally- standardized z-scores for waist circumference, blood glucose, c-peptide, triglyceride/(high density lipoprotein), and (systolic plus diastolic blood pressure) divided by 2.
[d] P-difference is the result of a Wald Chi Square test
[e] AMDR: acceptable macronutrient distribution range

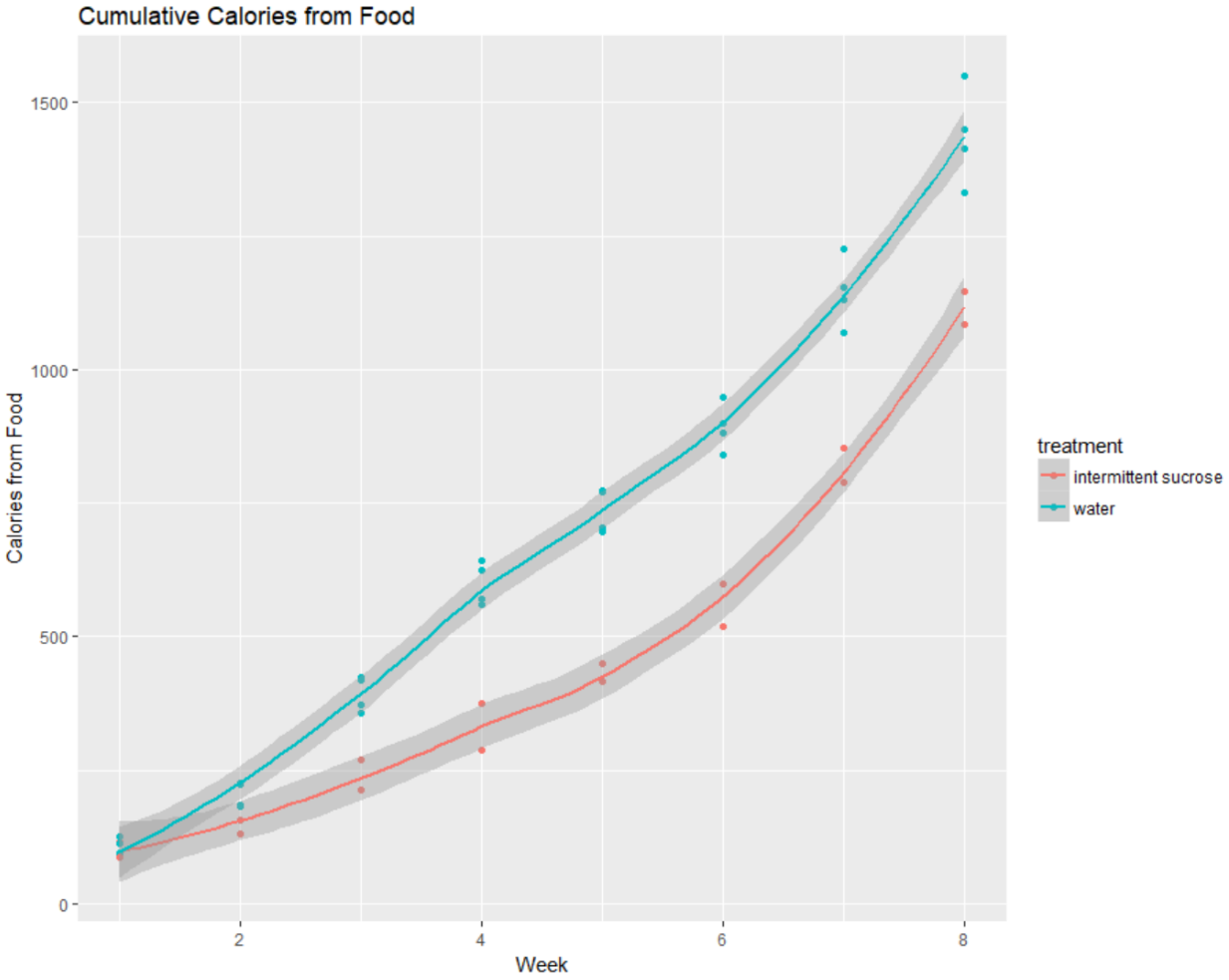
Table 9 Associations of maternal raw protein and fat intakes substituted for raw total carbohydrate intakes with metabolic risk and adiposity in peripuberty

	β (95% CI)[a]	
	BMIz[b]	MetRiskz[c]
Trimester 1		
Protein	11.40 (-18.40, 41.21)	-3.06 (-18.17, 12.05)
Fat	-10.87 (-40.87, 19.12)	2.18 (-13.03, 17.38)
Trimester 2		
Protein	5.92 (-23.92, 35.77)	-7.89 (-22.55, 6.78)
Fat	-6.43 (-36.81, 23.94)	7.23 (-7.72, 22.19)
Trimester 3		
Protein	-13.14 (-38.71, 12.42)	-14.22 (-26.61, -1.83)
Fat	12.15 (-14.28, 38.58)	12.28 (-0.52, 25.07)

[a] Model is adjusted for maternal carbohydrate intake, child sex, child age, and pubertal status

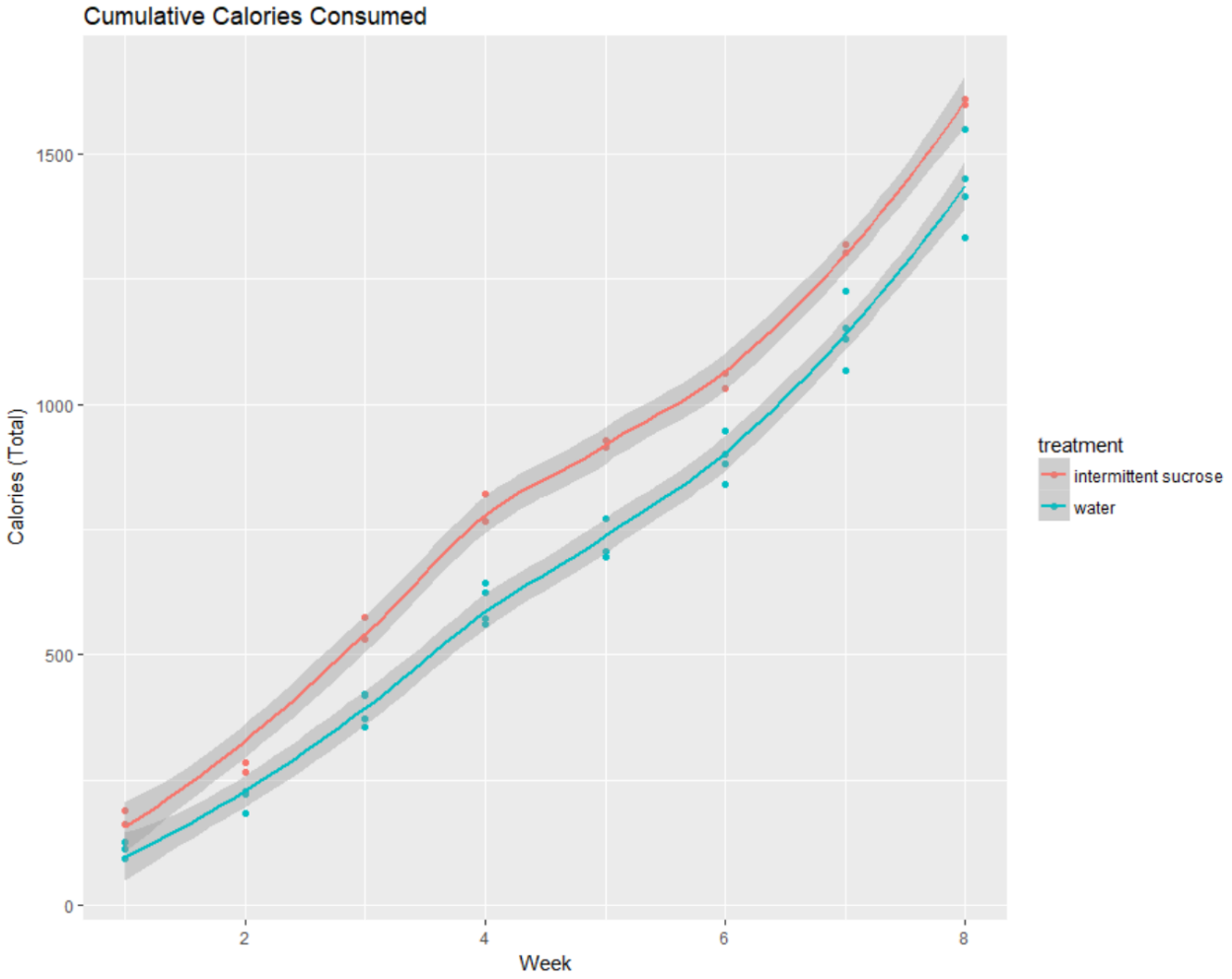
[b]Body Mass Index z score (BMIz) is calculated according to the WHO growth reference for children ages 5-19.
[c] MetRiskz: a cumulative z score calculated by taking the average of 5 internally- standardized z-scores for waist circumference, blood glucose, c-peptide, triglyceride/(high density lipoprotein), and (systolic plus diastolic blood pressure) divided by 2.

Figure 1 A



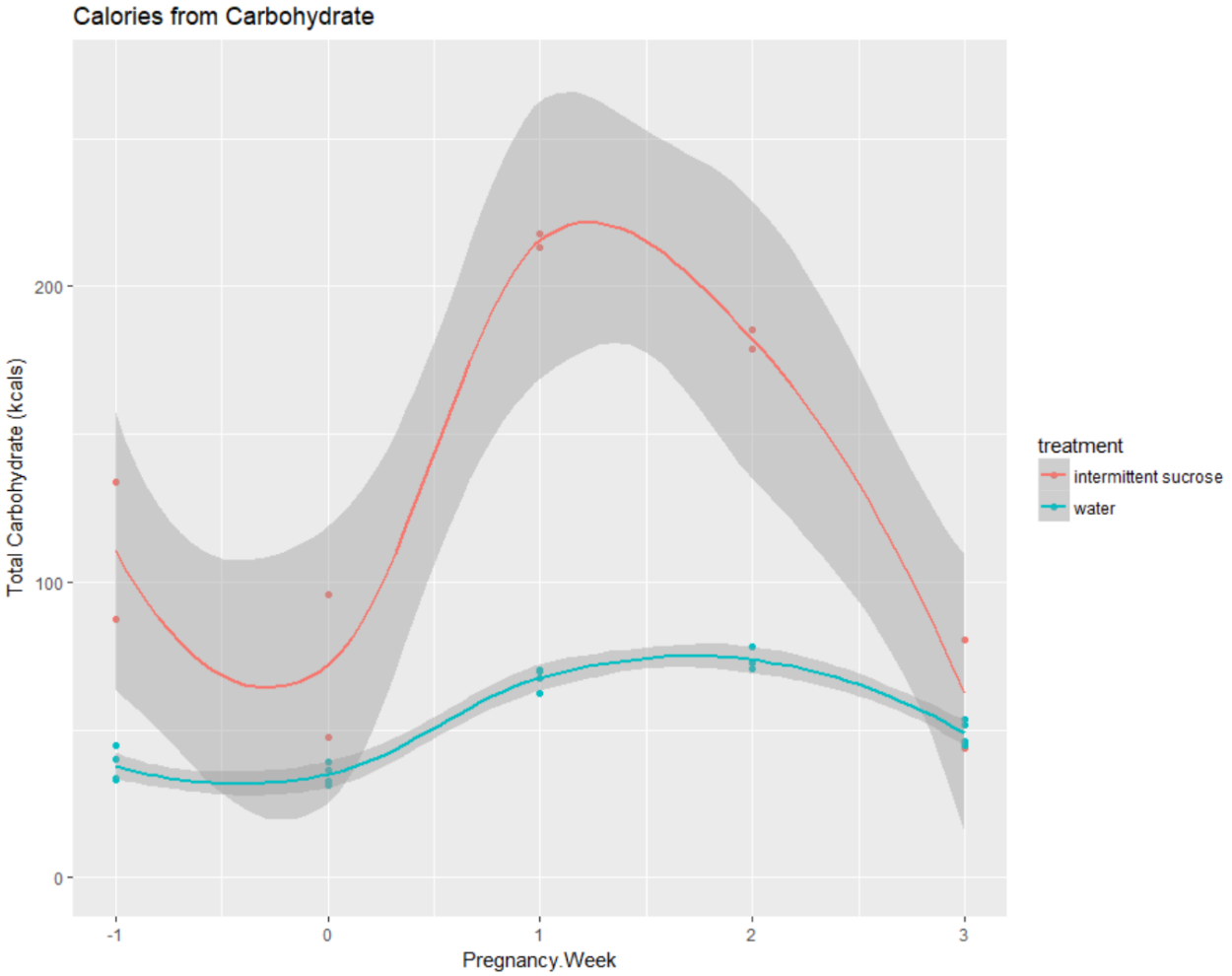
Cumulative food intake of dam is shown in kcals. Dams randomized to sucrose exposure consumed fewer kcals from food than those randomized to water exposure.

Figure 1 B



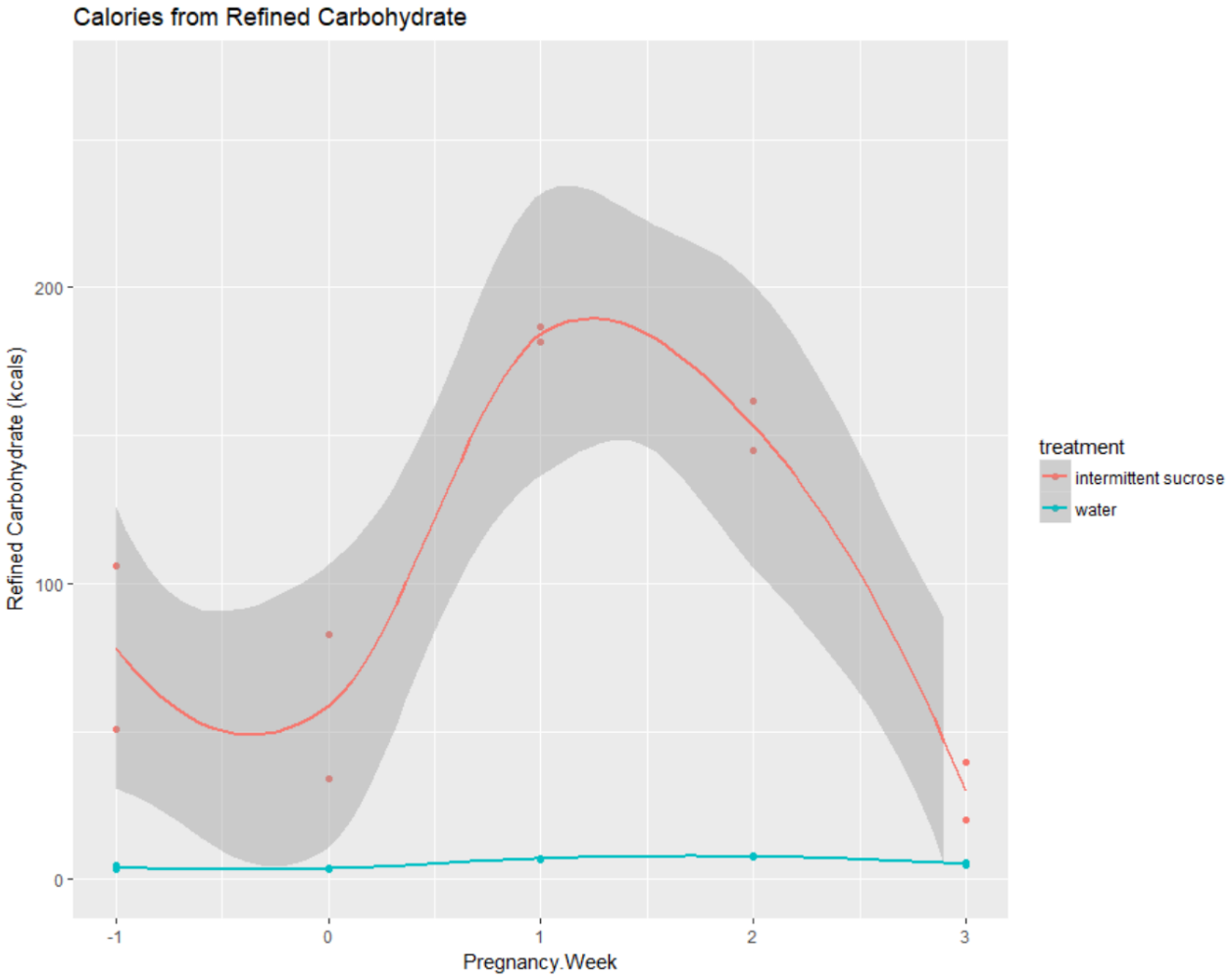
Cumulative food intake of dams is shown in kcals. Dams randomized to sucrose exposure consumed more kcals than those exposed to water, the additional kcals are from sucrose water consumption.

Figure 2 A



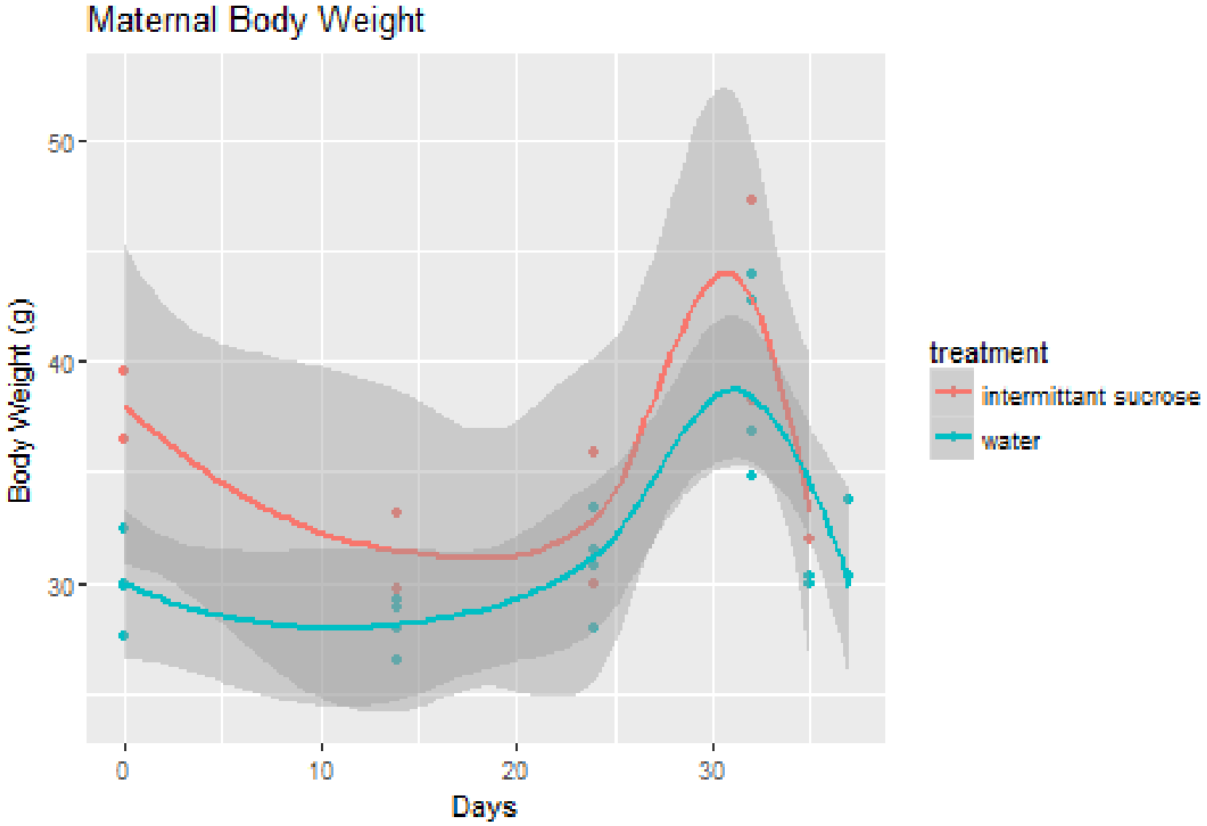
Sucrose exposed dams consumed significantly more calories from carbohydrate than did the water exposed dams.

Figure 2 B



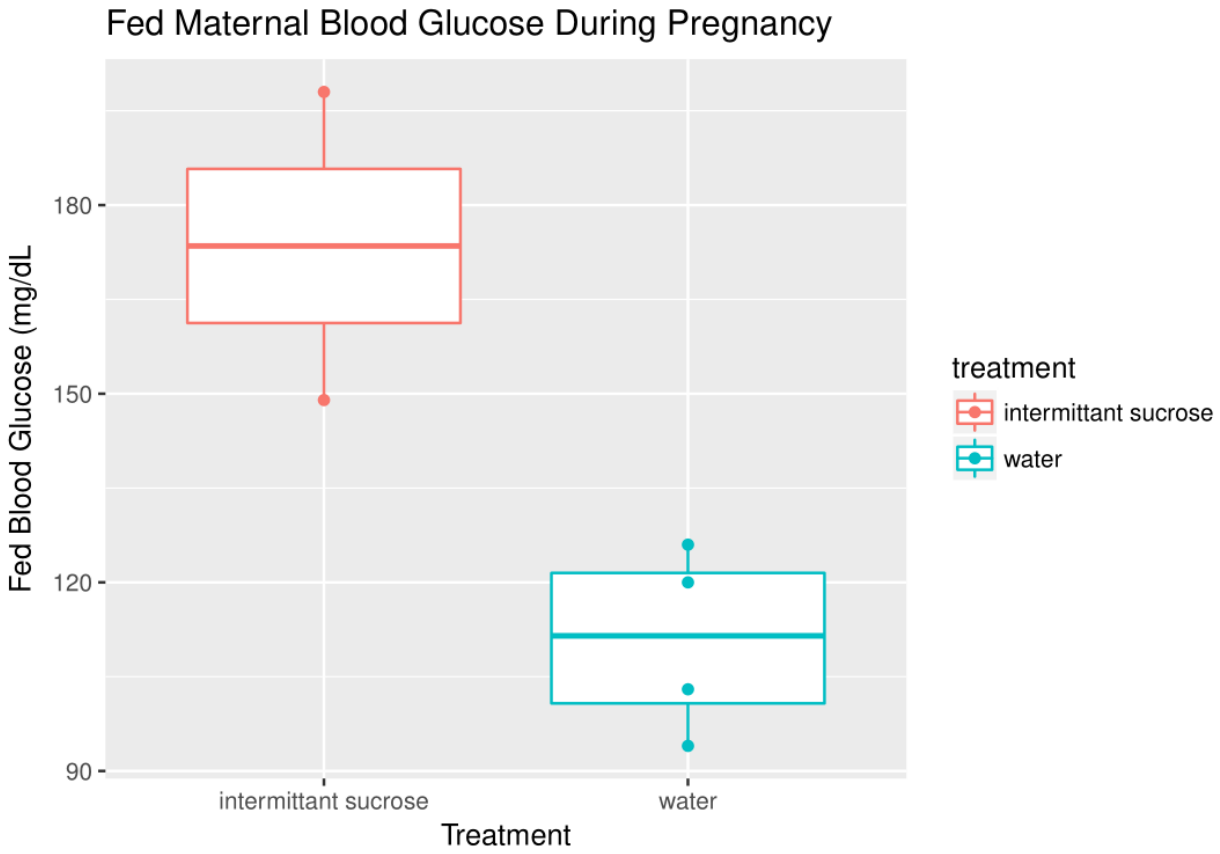
Sucrose exposed dams consumed significantly more calories from refined carbohydrate than did the water exposed dams.

Figure 3



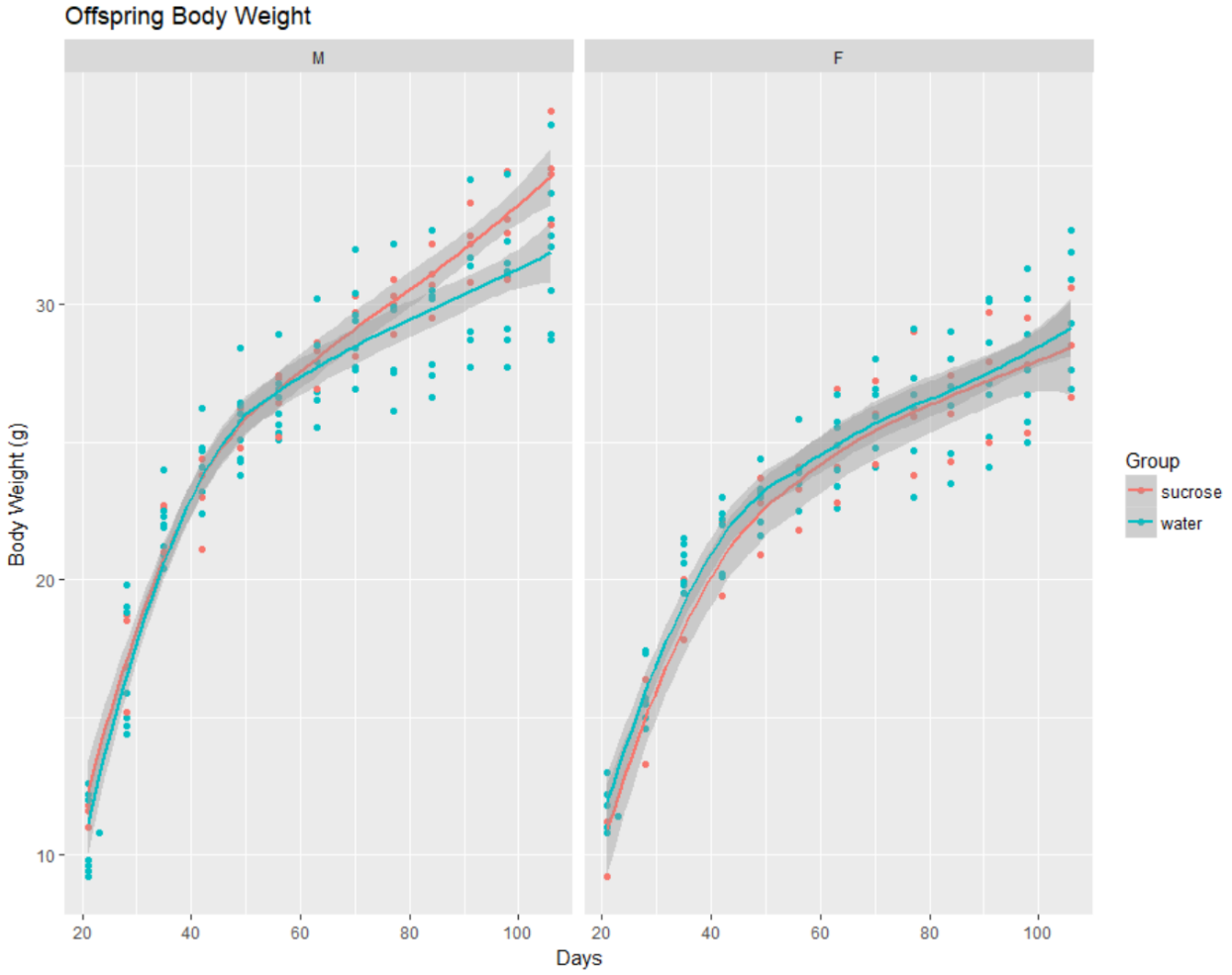
Maternal body weight is displayed in grams over days of exposure. Sucrose exposed dams have greater body weight, but confidence intervals overlap, meaning the differences are not statistically significant.

Figure 4



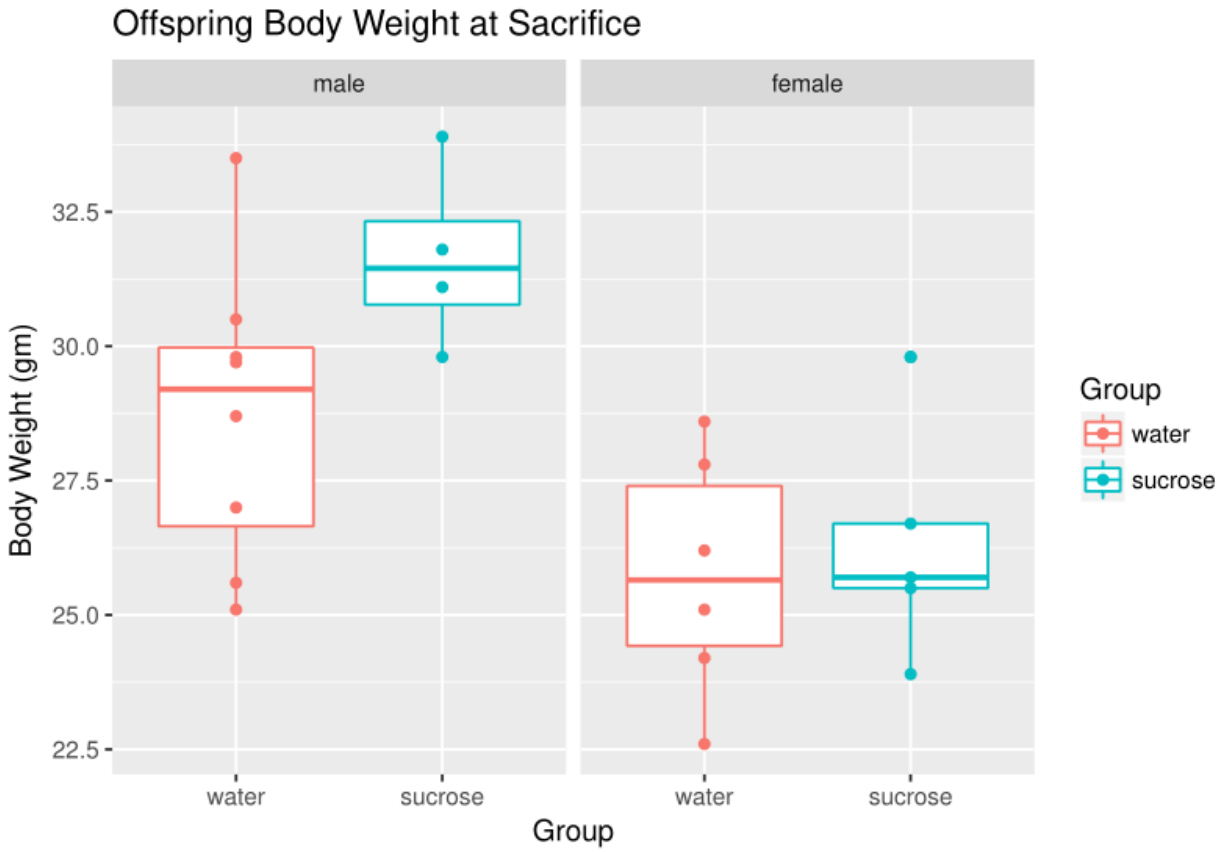
Fed blood glucose was taken during the third week of pregnancy via tail clip and read with a glucometer. Results are in mg/dL.

Figure 5 A



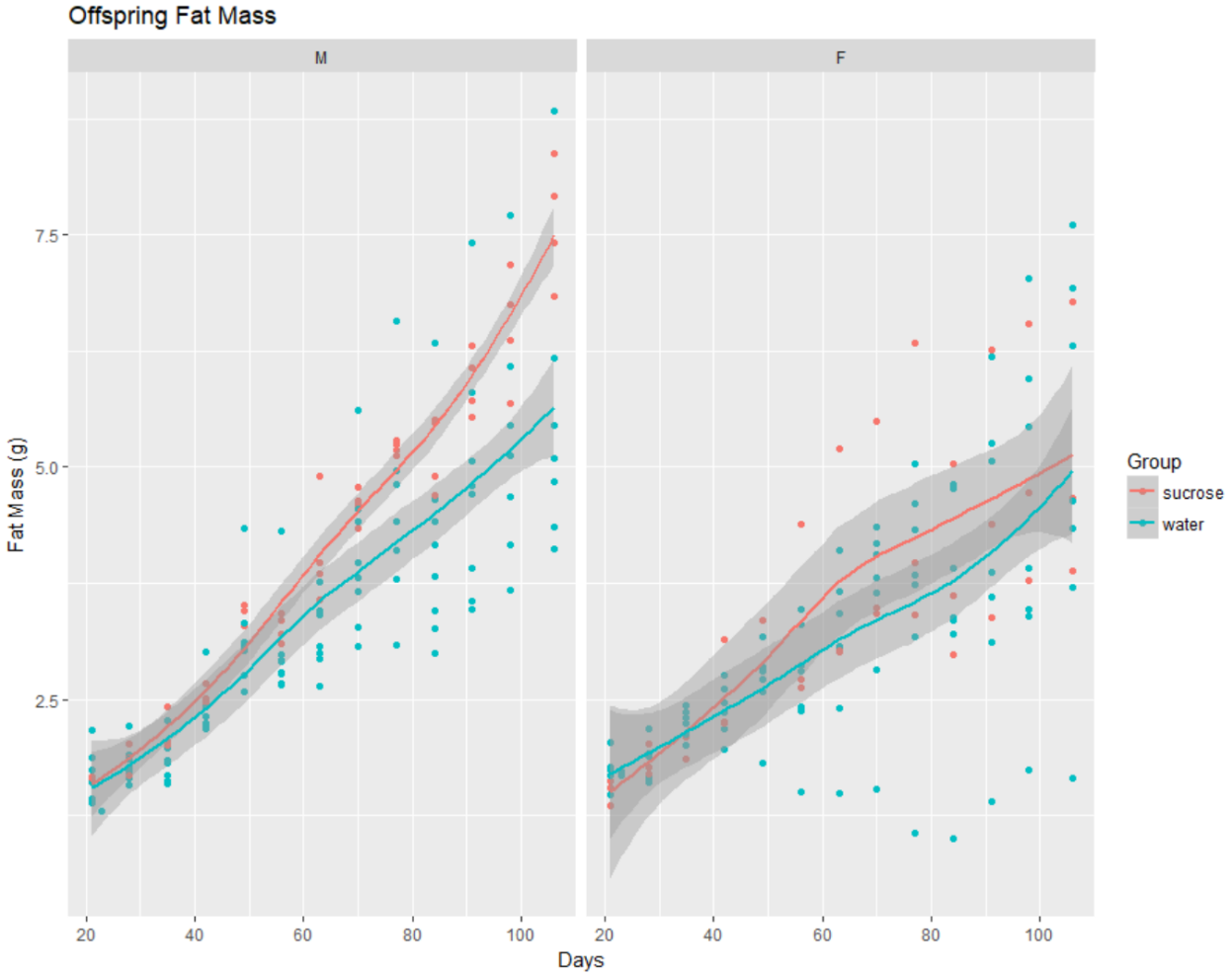
Offspring body weight in grams stratified by sex from weaning PND 21 to sacrifice PND 109. M denotes male sex and F denotes female sex.

Figure 5 B



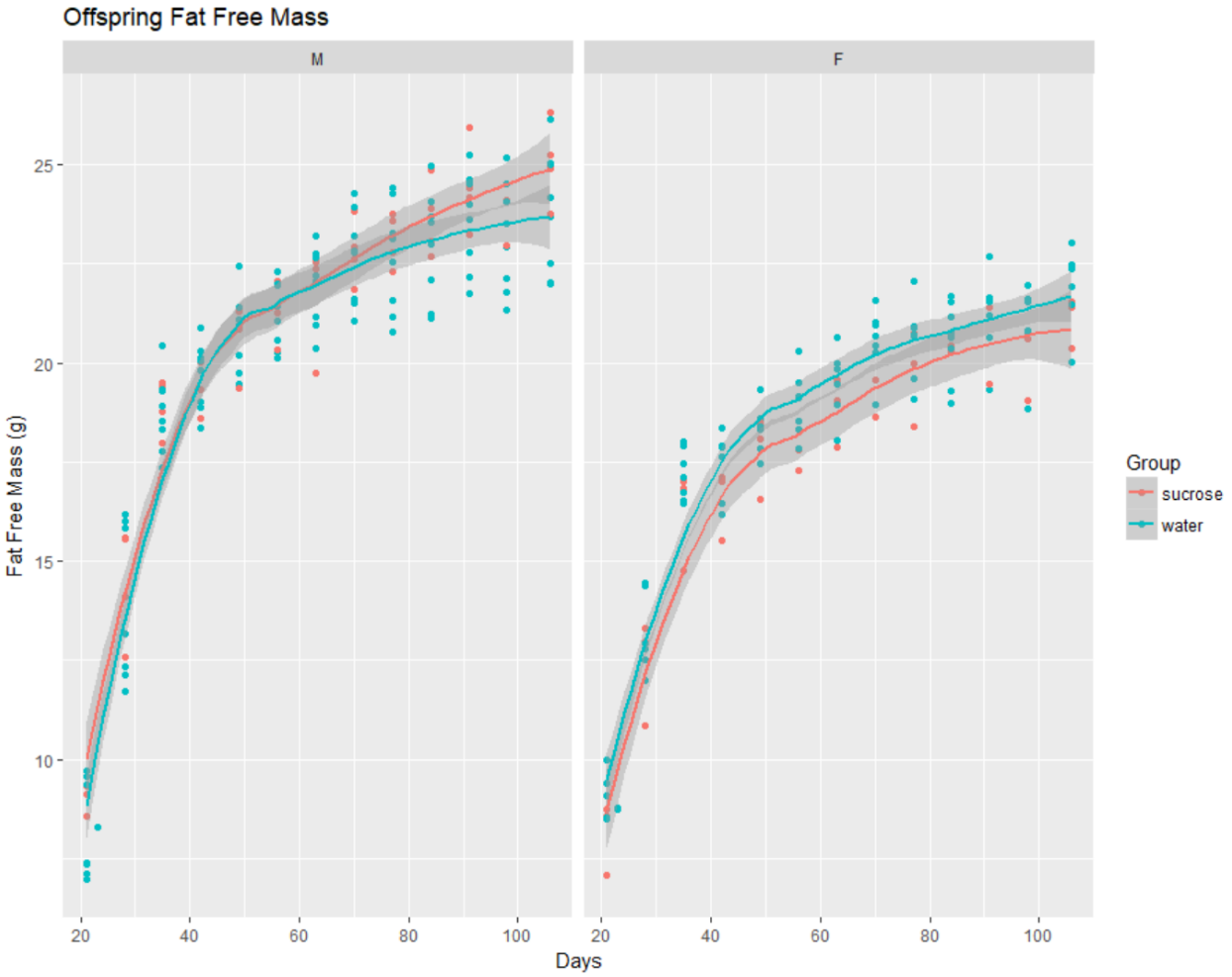
Offspring body weight at time of sacrifice PND 109, stratified by sex. Males exposed to sucrose had greater body weight than those exposed to water. Females had no differences in body weight by exposure group.

Figure 6



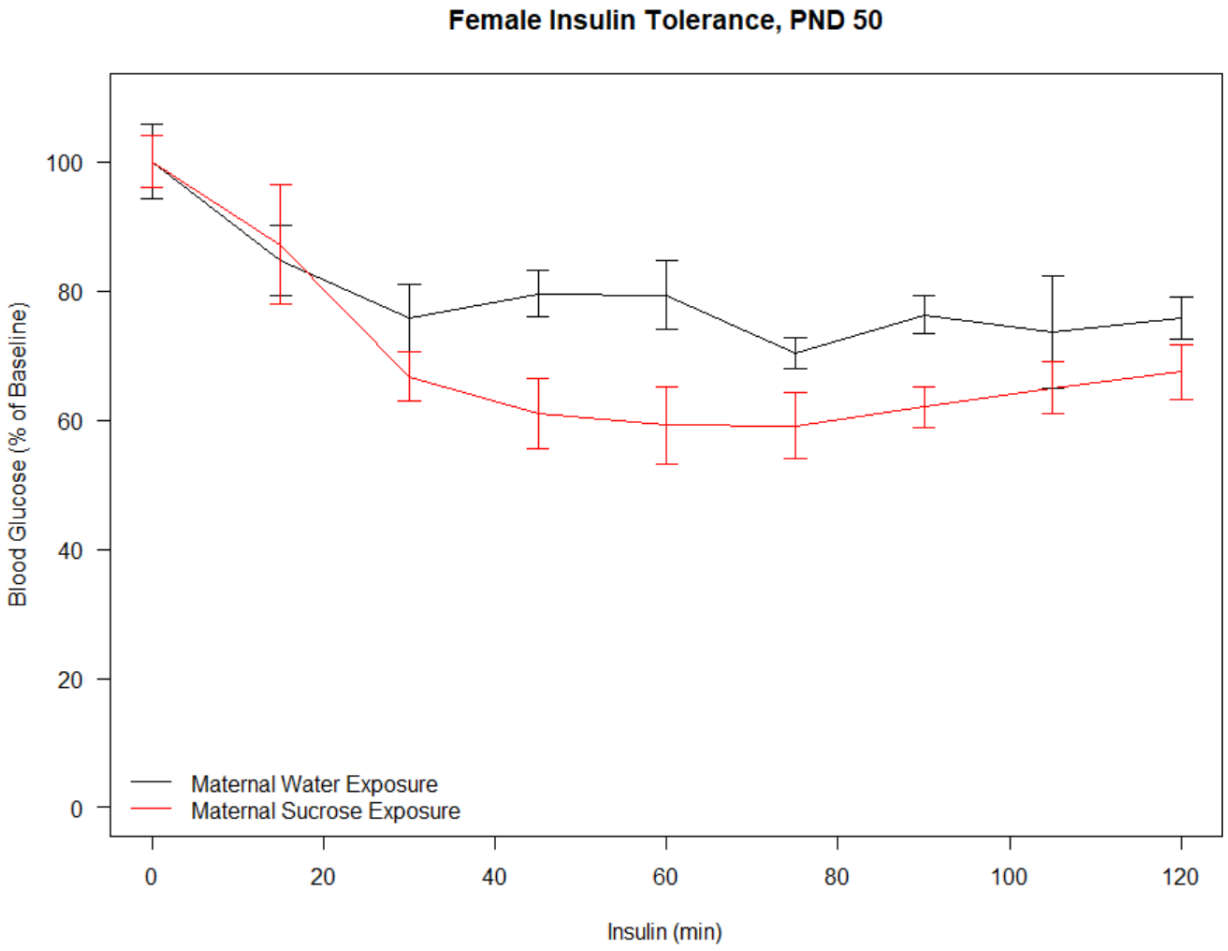
Offspring fat mass stratified by sex. M denotes male sex, and F denotes female sex.

Figure 7



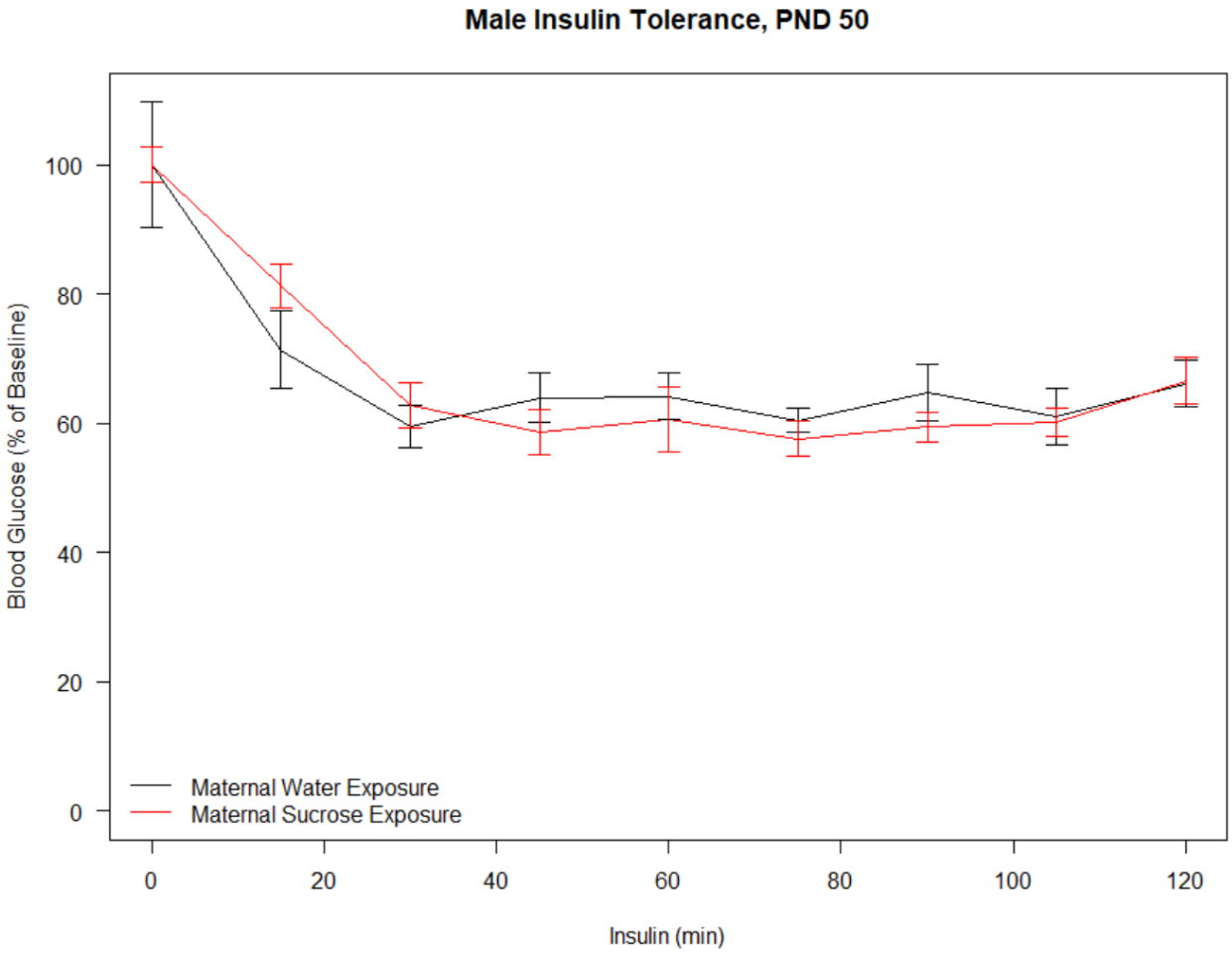
Offspring fat free mass stratified by sex. M denotes male sex and F denotes female sex.

Figure 8 A



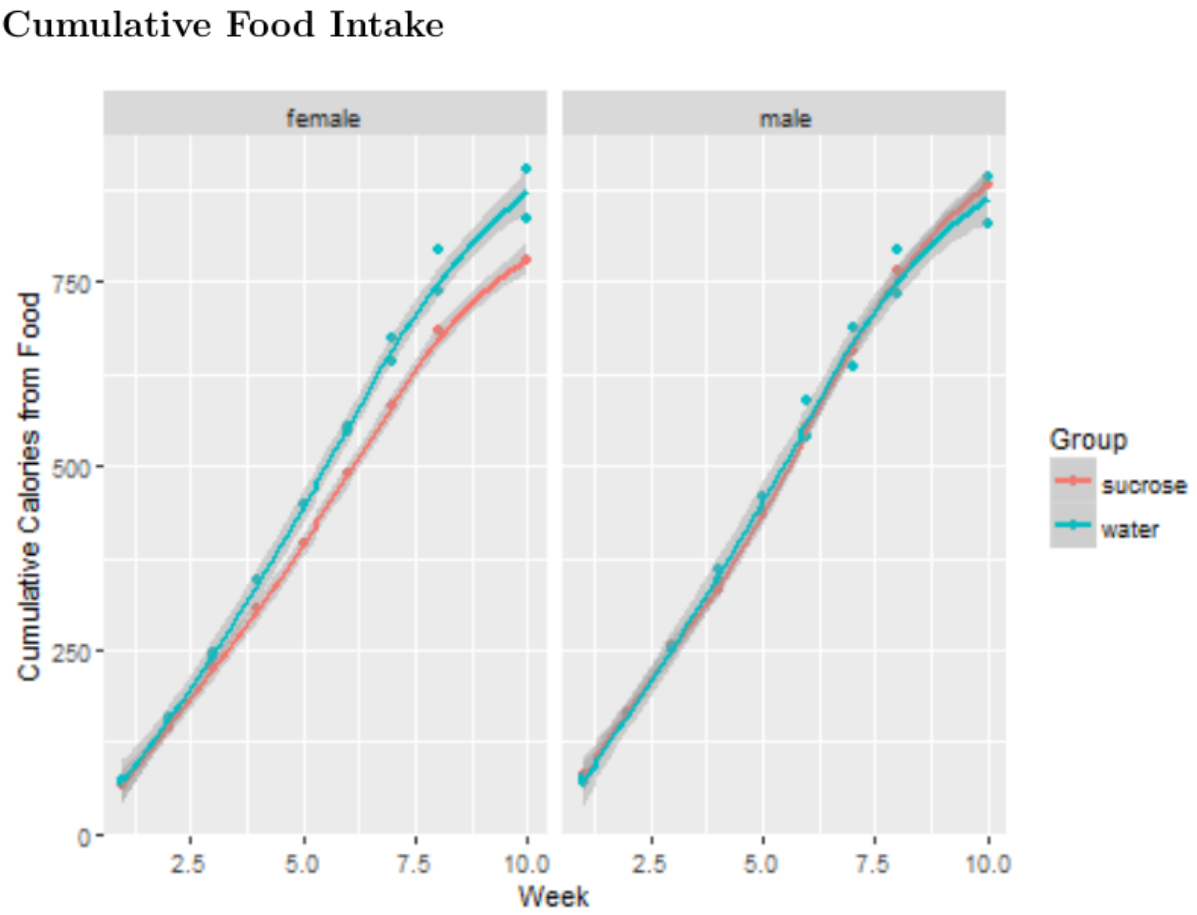
Insulin tolerance test (ITT) conducted after a 6 hour fast. An intraperitoneal injection of insulin was administered, then blood glucose was monitored every 15 minutes with glucometer. Results were normalized to blood glucose level at baseline. Females whose mothers were exposed to sucrose exhibit greater insulin tolerance beginning 45 minutes after administration of insulin.

Figure 8 B



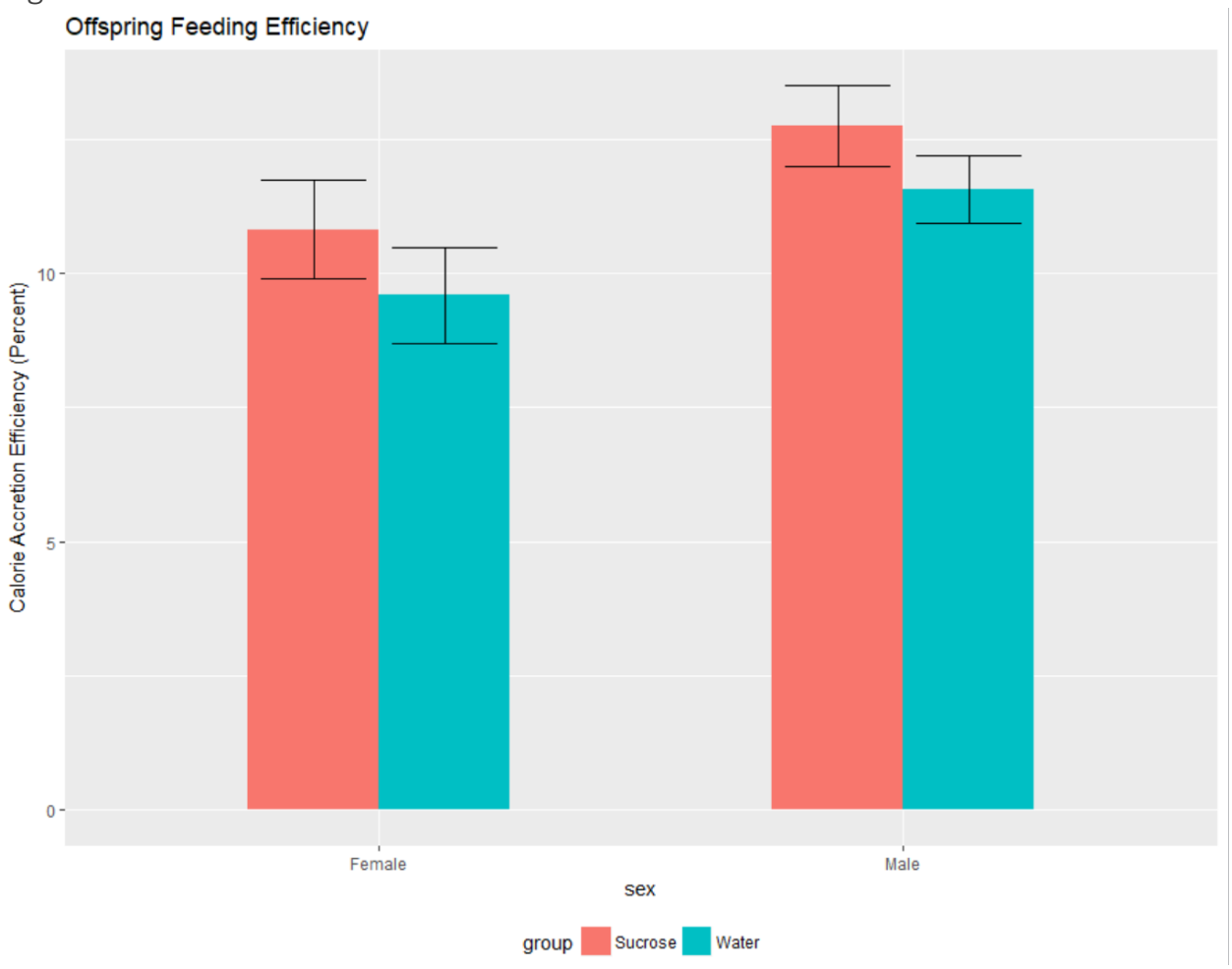
Insulin tolerance test (ITT) conducted after a 6 hour fast. An intraperitoneal injection of insulin was administered, then blood glucose was monitored every 15 minutes with glucometer. Results were normalized to blood glucose level at baseline. Males exhibit no differences in insulin tolerance between treatment groups.

Figure 9



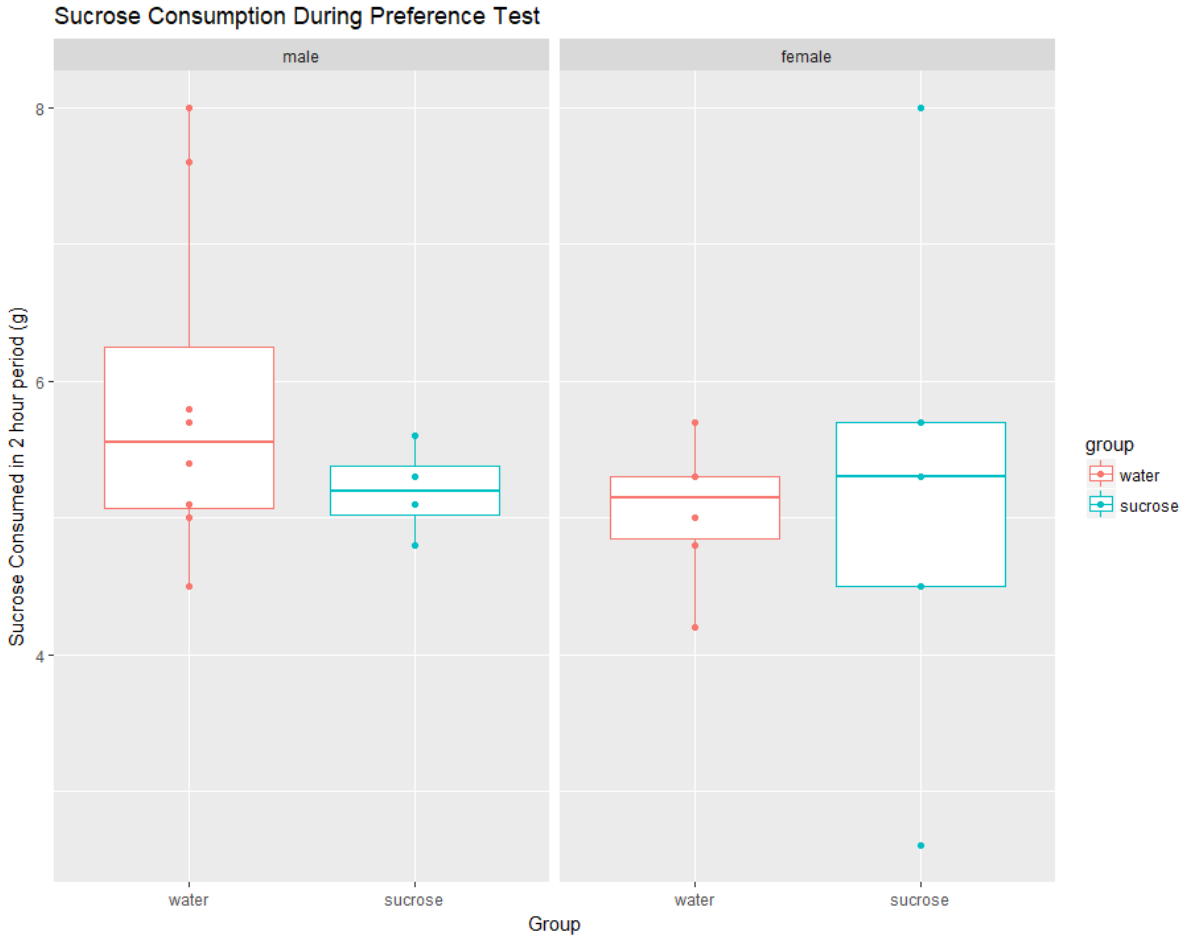
Cumulative food intake of offspring in kcals stratified by sex. There are exposure differences in food intake in males. Females exposed to sucrose consumed fewer calories than those exposed to water. Male food intake did not differ between treatment groups.

Figure 10



Feeding efficiency (FE), expressed as a percent, was calculated as FE= (Fat mass(g)*9kcal/g)+(lean mass(g)*4kcal/g)/(kcals consumed in study period)*100. Males exhibited greater FE than females. There were no differences in feeding efficiency by treatment group.

Figure 11



Animals were individually housed and deprived of water for the first 2 hours of the dark cycle. The next two hours of the dark cycle, animals were provided with two bottles; one containing water, another 10% sucrose. Results are grams of liquid consumed during 2-hour taste preference by maternal exposure group. There is no difference in sucrose taste preference between maternal exposure groups, or by sex.

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