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The effect of maternal and post-weaning low and high glycaemic index diets on glucose tolerance, fat deposition and hepatic function in rat offspring


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The effect of maternal and post-weaning low and high glycaemic index diets on glucose tolerance, fat deposition and hepatic function in rat offspring

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Short title: Maternal low/high GI diets and offspring outcomes

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Clinical studies have reported beneficial effects of a maternal low glycaemic index (GI) diet on pregnancy and neonatal outcomes, but the impact of the diet on the offspring in later life, and the mechanisms underlying these effects, remain unclear. In this study, Albino Wistar rats were fed either a low GI (n=14) or high GI (n=14) diet during pregnancy and lactation and their offspring weaned onto either the low or high GI diet. Low GI dams had better glucose tolerance (AUC_{glucose}, 1322 ± 55 vs 1523 ± 72 mmol.min/l, P<0.05) and a lower proportion of visceral fat (19.0 ± 2.9 vs 21.7 ± 3.8% of total body fat, P<0.05) compared to high GI dams. Female offspring of low GI dams had lower visceral adiposity (0.45 ± 0.03 vs 0.53 ± 0.03% body weight, P<0.05) and higher glucose tolerance (AUC_{glucose}, 1243 ± 29 vs 1351 ± 39 mmol.min/l, P<0.05) at weaning, as well as lower hepatic PI3K-p85 mRNA at 12 weeks of age. No differences in glucose tolerance or hepatic gene expression were observed in male offspring, but the male low GI offspring did have reduced hepatic lipid content at weaning. These findings suggest that consuming a low GI diet during pregnancy and lactation can improve glucose tolerance and reduce visceral adiposity in the female offspring at weaning, and may potentially produce long-term reductions in the hepatic lipogenic capacity of these offspring.

**Key Words:** programming, insulin resistance, fat mass
Introduction

The glycaemic index (GI) ranks food according to how they impact on blood glucose concentration immediately after consumption, with high GI foods causing a sharp increase in plasma glucose and low GI foods providing a more sustained glucose release. Epidemiological and clinical studies have reported that prolonged consumption of a high GI diet is associated with insulin resistance and type 2 diabetes, while low GI diets improve insulin sensitivity and reduce body weight. Experimental animal studies have also demonstrated that rats fed on low GI diets have a reduced body fat mass, improved glucose tolerance and reduced expression of lipogenic genes in the liver compared with those maintained on high GI diets.

Epidemiological and experimental animal studies have demonstrated that exposure to an elevated glucose supply in utero, as a consequence of gestational diabetes or even mild impairments to maternal glucose tolerance, significantly increases the risk of the offspring developing obesity, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) in adult life. This has led to suggestions that interventions that reduce maternal glucose concentrations and/or improve maternal glucose tolerance, including a low GI diet, may have beneficial effects on the long term metabolic outcomes of the offspring. A small number of human studies have investigated the effects of low GI diets during pregnancy and lactation on infant outcomes. However, while some studies have supported the potential benefits of a low GI diet in pregnancy for maternal and pregnancy outcomes, including a reduced risk of delivering a large for gestational age infant, no studies to date have evaluated the impact of this diet on the metabolic health of the offspring beyond the immediate postnatal period. In addition, whether the long-term metabolic effects of exposure to a low GI diet during the fetal and suckling periods are dependent on the GI of the diet consumed after weaning is also unknown.
Therefore, the aims of the present study were to use a rodent model to 1) compare the effects of maternal consumption of a low GI vs high GI diet during pregnancy and lactation on fat deposition, glucose tolerance, hepatic fat content and gene expression in the offspring at weaning and in young adulthood, and 2) determine whether the effects of maternal low GI diet consumption on young adult offspring differed according to whether the offspring were weaned onto a low GI or high GI diet.

Methods

Dams and feeding regime

This study was approved by the University of Adelaide Animal Ethics Committee. Twenty-eight (28) female Albino Wistar rats (~200g) were brought into the animal facility and housed individually in a 12hr light/12hr dark cycle environment at a constant temperature of ~25ºC. Rats were acclimatised to the environment for at least 1 week prior to the commencement of the experiment. During this time, they had free access to standard rodent chow (AIN93M, Specialty Feeds, Glen Forrest, Western Australia) and tap water.

Following acclimatisation, rats were assigned to either the low GI (n=14) or high GI (n=14) group. The diets each group received were identical in appearance, energy content, macro- and micronutrient composition, the only difference being the carbohydrate type; in the low GI group the diet included carbohydrate in the form of Gel Crisp starch (Diet SF10-084) while in the high GI group the diet included carbohydrate in the form of dextrinised starch (Diet SF10-081). Both diets were manufactured by Specialty Feeds (Glen Forrest, Western Australia). A validated in vitro starch digestion assay was used as an indicator of the likely glycaemic response to each of the diets. At the 20 min time point, the amount of rapidly available glucose (RAG) in the high GI feed was 56% higher than the low GI feed (P= 0.006). Similarly,
at 120 min of digestion, the amount of glucose released was 44% higher in the high GI feed ($P=0.0006$). In addition, an *in vivo* pilot study was undertaken in which we measured blood glucose concentrations in rats for two hours after the consumption of either the high or low GI diet. The results obtained confirmed that the diets resulted in post-prandial glucose curves which were different and consistent with the profile expected for low and high GI foods (data not shown).

The diets were provided *ad libitum* and all rats had free access to water throughout the experiment. Female rats were fed their respective diets for a minimum of 4 weeks before mating and throughout pregnancy and lactation. Body weight was determined weekly during this time. Fresh food was provided every second day, and on each of these occasions, the remaining food was weighed and the weight subtracted from the amount provided at the start of the 2 day period to calculate food intake.

After 4 weeks, vaginal smears were performed daily to determine the stages of the estrous cycle. On the night of diestrous/proestrous the female rat was placed with a male (*fed ad libitum on standard rodent chow*) overnight. The presence of sperm in vaginal smears conducted the following morning was considered as confirmation of successful mating and designated as gestation day 0. A total of 4 males were used for mating and the same males were used for mating females in both the low GI and high GI groups in order to minimise the influence of paternal effects on offspring outcomes.

**Offspring**

Pups were born on day 21-22 of gestation. Within 24 hours of birth (postnatal day 1), pups were culled to 8 per litter, with 4 males and 4 females where possible. Pups were weighed on postnatal day 1 and every 2 days thereafter during the suckling period and were weaned on
postnatal day 21. At the time of weaning, tissue was collected from 1 male and 1 female pup from each litter, remaining were group-housed with their same sex littermates (2 animals per cage), and were provided with either the same diet as their mother or the alternate diet. This gave rise to 4 groups (1) offspring of low GI dams weaned onto the same low GI diet (L-L, n=14, 7 males and 7 females), (2) offspring of low GI dams weaned onto a high GI diet (L-H, n=14, 7 males and 7 females), (3) offspring of high GI dams weaned onto a low GI diet (H-L, n=14, 7 males and 7 females) and (4) offspring of high GI dams weaned onto a high GI diet (H-H, n=14, 7 males and 7 females). Food intake was determined every 2 days in all offspring and fresh food provided. Fresh water was available *ad libitum*. All offspring were weighed once per week from weaning until 12 weeks of age.

*Intraperitoneal Glucose Tolerance Test (IPGTT)*

IPGTTs were performed after an overnight fast on dams at the end of lactation as well as on the offspring at 3wks and 12wks of age. Baseline blood samples were collected from the tail vein and a glucose bolus (2g/kg of 50% dextrose in sterile 0.9% saline) was then injected intraperitoneally. Blood samples were collected from the tail vein at 5, 10, 15, 30, 60 and 120 minutes following glucose delivery. Glucose concentrations were determined using a handheld Accu-Chek Performa glucometer (Accu-Chek Performa©, Roche, Germany) at each time-point.

*Post mortem and tissue collection*

Post mortem and tissue collection was conducted on dams after weaning on the day following the IPGTT and on 1 male and 1 female offspring per litter (selected at random) at weaning and 1 male and 1 female offspring per litter at 12 weeks of age. Dams and offspring were killed using an overdose of CO₂ in the non-fasted state. Immediately after euthanasia, blood samples
were collected via cardiac puncture into heparinised tubes and centrifuged at 3,500 g at 4°C for 15 minutes. The plasma was collected and stored at -20°C for subsequent analyses of hormone and metabolite concentrations. Body weight, length (nose to tail) and abdominal circumference were determined. All internal organs were weighed and all visible fat depots, including omental fat, retroperitoneal fat, gonadal fat, subcutaneous fat and interscapular fat, were dissected to determine the weights of the individual depots. The weights of omental, retroperitoneal and gonadal fat were added together to determine visceral fat mass, and the weights of all individual fat depots were added together to determine total body fat mass. The weights of all fat depots were expressed relative to body weight. At both weaning and 12 weeks of age, a sample of liver (from the same site in each animal) was snap frozen in liquid nitrogen and stored at -80°C for subsequent analysis of lipid content and gene expression.

Hepatic lipid content, RNA extraction and gene expression analysis

Total hepatic lipid content was determined gravimetrically following homogenisation and extraction of 200mg of frozen tissue in chloroform-methanol (2:1, v/v) as previously described. Total mRNA was extracted from the liver using Trizol reagent (Invitrogen Australia, Mount Waverley, Vic, Australia), purified using an RNeasy Mini kit (Qiagen Australia, Doncaster, Vic, Australia) and cDNA synthesized using Superscript III reverse transcriptase (Invitrogen Australia) and random hexamers.

Quantitative Real Time PCR was performed using the SYBR green system on the Applied Biosystems ViiA 7 Real Time PCR machine (Applied Biosystems, Foster City, CA, USA). The target genes included key genes involved in hepatic lipid metabolism and insulin signalling: acetyl-CoA carboxylase (ACC), peroxisome proliferator activated receptor-α (PPARα), sterol regulatory element binding protein-1α (SREBP1α), fatty acid synthase (FAS), the phosphatidylinositol 3-kinase regulatory p85 subunit (PI3K-p85) and phosphokinase C-ζ.
Gugusheff et al. (2016) investigated the outcomes of maternal low/high GI diets and offspring. PKCζ, all of which have been implicated in non-alcoholic fatty liver disease (NAFLD) 22, 23. The primers were designed using the Primer3 and NCBI websites, with all primers crossing exon-exon boundaries to prevent annealing to genomic DNA. All primers were validated for use in our laboratory by running the PCR product on a gel to confirm amplicon size as well as sequencing to ensure the correct gene was amplified. Primer sequences are shown in Table 1. The expression of target genes was quantified relative to the housekeeper genes β-actin and HPRT, using the Applied Biosystems Data Assist software (Applied Biosystems, Foster City, CA, USA). Two quality controls as well as a negative RT control were used on each 96-well plate to ensure inter-plate consistency and melt curves were obtained at the end of each run to confirm amplicon heterogeneity.

Plasma hormone and metabolite assays
Plasma glucose, alanine amino transferase (ALT), uric acid, total cholesterol, HDL cholesterol (Thermo Electron, Pittsburgh, PA), and NEFA (WAKO Pure Chemical Industries Ltd., Osaka, Japan) were determined using a Konelab 20X (Thermoscientific, Vantaa, Finland). Plasma leptin and insulin concentrations were measured using commercially available immunoassay kits (Crystal Chem Inc, Downers Grove, IL, USA and ALPCO Diagnostics, Salem, NH, USA). All assays were conducted in accordance with the manufacturer's instructions and intra- and inter-assay coefficients of variation were always <10%.

Statistical analyses
Data are presented as mean ± SEM. The dam (litter) was used as the unit of analysis in all statistical tests. A power analysis was conducted to determine sample size using changes in fat mass as the primary outcome. The effect of the low or high GI diet in the dams and pre-weaning offspring was determined using a Student’s unpaired t-test. The area under the curve (AUC)
for glucose following the IPGTT was calculated for each animal using the incremental AUC method. The relative effects of exposure to maternal low GI diet or high GI diet and exposure to the diets after weaning were analysed using a 2-way ANOVA. When a significant interaction between maternal diet and post-weaning diet was identified, all groups were analysed together using a one-way ANOVA and Tukey’s post hoc analysis. Differences in the effects of the low GI and high GI diets over time were analysed using a repeated measures ANOVA. Male and female offspring were analysed separately for all measures. Repeated measures ANOVAs were performed using Stata 11 (StataCorp LP, Texas, USA). All other analyses were performed using SPSS for Windows Version 19.0 (SPSS Inc., Chicago, IL, USA). A probability of $P<0.05$ was considered statistically significant.

RESULTS

Maternal outcomes

*Food intake and body weight*

Body weights were not different between dams assigned to the low GI and high GI diets at the commencement of the experimental diets (high GI, $244.1 \pm 5.4$g; low GI, $257.9 \pm 6.4$g, $P=0.11$). However, at the time of mating (i.e. ~4 weeks after commencement of the experimental diets), dams in the low GI group were heavier than those in the high GI group (Fig 1A, $P<0.05$). There was no difference, however, in the average body weight during pregnancy or at the end of lactation (Fig 1A), and low GI dams gained less weight during pregnancy than the high GI dams (high GI, $116.3 \pm 5.1$g; low GI, $90.9 \pm 8.6$g, $P<0.05$). There was also no difference in maternal food intake between groups either before mating, during pregnancy or during lactation (Fig 1B).

*Fat mass*
At the end of lactation, low GI dams had a higher abdominal circumference (high GI, 17.8±0.3cm; low GI 19.3±0.4cm, P<0.05) and higher gastrointestinal tract mass relative to body weight compared to high GI dams (high GI 8.7±0.6%; low GI 11.6±0.7%, P<0.01). There were no differences in the total percentage body fat or the weight of any of the individual fat depots between the low GI and high GI groups (supplementary material-table 1). However, GI dams had a lower amount of visceral fat as a proportion of their total fat mass compared to the high GI dams (Fig 2A, P<0.05).

**Glucose tolerance and plasma measures**

There was no difference in fasting glucose levels between low GI and high GI dams before the administration of the glucose bolus (high GI, 5.9 ± 0.3mmol/l; low GI, 5.9 ± 0.2mmol/l, P=0.19). The low GI dams also had a lower peak glucose following intraperitoneal glucose administration (Fig 2B, P<0.05) and a lower glucose AUC during the IPGTT compared to the high GI group (Fig 2C, P<0.05). There were no differences in the plasma concentrations of insulin, glucose, NEFA or leptin between low GI and high GI dams at the time of post-mortem (supplementary material-table 1).

**Offspring outcomes birth to weaning**

**Growth from birth to weaning**

There was no difference in birth weight between the low GI and high GI groups in either females or males (females: high GI, 6.1 ± 0.1g; low GI, 6.1 ± 0.2g, P=0.93; males: high GI, 6.2 ± 0.3g; low GI, 6.5± 0.3g, P=0.43). Weight gain during the suckling period between groups was also comparable (male F=1.74, P=0.26 and female F=1.09, P=0.31) and there was no
difference in body weight at weaning (3 weeks of age) (females: high GI, 42.6 ± 1.8 g; low GI, 43.3 ± 1.5 g, \( P = 0.99 \); males: high GI, 44.4 ± 1.9 g; low GI, 45.3 ± 1.5 g, \( P = 0.70 \)).

*Fat mass at 3 weeks of age*

In female offspring relative omental fat mass (\( P < 0.05 \)) and the total relative mass of visceral fat (\( P < 0.05 \)) at 3 weeks of age were both significantly reduced in the low GI group compared to the high GI group (Table 2). Individual weights of other fat depots and total relative body fat mass were not different (Table 2). In male offspring, there were no differences between groups in either total or relative fat mass at this time (Table 2).

*Glucose tolerance and plasma measures at 3 weeks of age*

At 3 weeks of age, female offspring of low GI dams had a lower peak plasma glucose post intraperitoneal glucose administration (Fig 3A, \( P < 0.01 \)) and a lower glucose AUC during the glucose tolerance test compared to female high GI offspring (Fig 3B, \( P < 0.05 \)). There was no difference in peak glucose or the glucose AUC in males (Fig 3C, D, \( P = 0.59 \)).

In females, non-fasting glucose concentrations at 3 weeks of age were lower in the low GI compared to the high GI group (high GI 11.62 ± 0.45 mmol/L; low GI 9.90 ± 0.39 mmol/L, \( P < 0.05 \)). There were no differences in glucose concentrations between groups in male offspring or in plasma NEFA, cholesterol, insulin or leptin concentrations in either females or males (supplementary material-table 2).

*Hepatic lipid content and gene expression at 3 weeks of age*

Relative liver weights were not different between the low GI and high GI groups in either male (high GI 4.00 ± 0.10%; low GI 4.01 ± 0.06%, \( P = 0.97 \)) or female (high GI, 3.89 ± 0.07%; low GI
Maternal low/high GI diets and offspring outcomes

3.91±0.09%, *P*=0.54) offspring. However, male offspring of low GI dams had a lower hepatic fat content as a percentage of liver weight compared to high GI males (high GI 6.35± 0.50%; low GI 4.07±0.40%, *P*<0.05). There was no difference in liver fat percentage in females (high GI 6.13± 0.84%; low GI 6.67±1.06%, *P*=0.29).

Plasma concentrations of uric acid and alanine transaminase (ALT), both established biomarkers of liver function, were not different between low and high GI groups in either males or females. Hepatic expression of key genes involved in lipogenesis and insulin signalling (ACCβ, PPARα, SREBP1α, FAS, PI3K-p85 and PKCζ) was also not different between the low GI and high GI groups in either males or females (supplementary material- table 4).

**Offspring outcomes – post weaning**

**Food intake and growth**

There was no difference in food intake during the post-weaning period between offspring of low GI and high GI dams (data not shown). In female offspring, the rate of weight gain from weaning to 12 weeks of age was higher in offspring of low GI dams, independent of the post weaning diet (*F*=5.14, *P*<0.01), and these offspring were heavier between 6 and 10 weeks of age, although not at 12 weeks of age, compared to offspring of high GI dams (Fig 4A). There were no differences between groups in body weight in male offspring at any time after weaning (Fig 4B).

**Fat mass at 12 weeks of age**

In females, relative interscapular fat mass was significantly lower in offspring of low GI dams, independent of their post-weaning diet (*P*<0.05, Table 3), however the relative mass of other individual fat depots and the relative visceral and total fat mass were not different. In males,
there was no difference in total fat mass or the relative weight of any of the individual fat depots between groups (Table 3).

Glucose tolerance and plasma hormone concentrations at 12 weeks of age

In female offspring, there was an interaction between the effects of the maternal and post-weaning diets in relation to glucose tolerance at 12 weeks of age. Thus, offspring of high GI dams tended to have lower glucose tolerance if they were weaned onto a low GI diet compared to if they were weaned onto a high GI diet (AUC_{glucose}, H-L, 1797 ± 194 vs H-H 1346 ± 97 mmol.min/L, P<0.07). However, no statistical difference was observed when the interaction was explored by using a one-ANOVA with post-hoc analysis. There were no differences in plasma glucose, NEFA, leptin or total cholesterol concentrations at 12 weeks of age in either males or females (supplementary material table 3).

Hepatic lipid content and gene expression at 12 weeks of age

There was no difference between groups in relative liver weight or liver fat content in either male or female offspring at 12 weeks of age (Table 4). In females, offspring of low GI dams had increased plasma ALT concentrations in comparison with offspring of high GI dams, independent of their post-weaning diet (low GI 130.75±53.15 IU/L vs high GI 15.75±5.02 IU/L, P<0.05).

In females, hepatic PI3K-p85 mRNA expression at 12 weeks of age was lower in offspring of low GI mothers, independent of the post-weaning diet (P<0.05, Table 4). SREBP1α mRNA expression at 12 weeks of age was higher in offspring of high GI dams who were weaned onto the low GI diet compared to all other groups (P<0.05, Table 4). There was no effect of either
the maternal or post-weaning diet on hepatic mRNA expression of PI3K-p85 or SREBP1α in males on in ACC, PPARα, PKCζ or FAS in either male or female offspring (Table 4).

DISCUSSION

This study was the first to directly compare the effect of a maternal high vs low GI diet on offspring and maternal metabolic outcomes beyond the immediate postnatal period. We showed for the first time that consuming a low GI diet pre-pregnancy and throughout pregnancy and lactation reduces visceral adiposity and increases glucose tolerance in female offspring at 3 weeks of age, and lowers female hepatic PI3K expression at 12 weeks. We also identified significant interactions between the maternal and post-weaning diet such the female offspring of high GI dams switched to a low GI diet had higher hepatic SREBP1α expression as adults. By examining the effect of high and low GI diets on gene expression and metabolic outcomes in the mother and offspring, this study provides a solid foundation for continuing investigations on the mechanisms underlying the effects of reducing the GI of the maternal diet on the metabolic health of the offspring.

Maternal Outcomes

The increase in bodyweight we identified in the dams consuming the low GI diet prior to mating was unexpected given previous reports of low GI diets increasing satiety, lowering food consumption and reducing weight gain\textsuperscript{24-26}. We consider it likely, however, that our results were biased by the fact that body weights were not recorded in the fasting state, since low GI diets are known to increase the weight of the large bowel and caecum \textsuperscript{26}. In line with this the weight of the gastrointestinal tract at post-mortem was \textasciitilde10 g heavier in the low GI compared to high GI dams, and if this value was subtracted from the pre-pregnancy weights of the low
GI dams then the difference between groups was no longer significant. In support of this, despite the increase in pre-mating bodyweight we found no difference in total fat mass between the low GI and high GI dams at the end of lactation. Interestingly, however, the low GI diet appeared to affect fat distribution, since the dams fed the low GI diet had a lower ratio of visceral to total fat mass than their high GI counterparts. This is consistent with previous studies in non-pregnant adults, which reported that low GI diets preferentially enhance the mobilisation of visceral compared to subcutaneous fat\textsuperscript{27, 28}.

\textit{Growth and metabolic outcomes in the offspring at weaning}

A key finding of the current study was that maternal consumption of a low GI diet reduced visceral adiposity, lowered plasma glucose concentrations and increased glucose tolerance in female, but not male offspring at weaning. While the mechanisms behind this remain unclear, one possibility is that the exposure to lower glucose concentrations as result of higher glucose tolerance during the development of adipose depots ‘programmed’ a reduced lipogenic capacity in visceral adipocytes. This hypothesis is indirectly supported by a study in sheep which demonstrated that exposure to elevated glucose concentrations \textit{in utero} is associated with a precocial up-regulation of lipogenic genes in the main visceral adipose depot of the fetus\textsuperscript{29}. However, further studies will be required to test this directly. While male offspring of low GI dams did not exhibit any differences in body fat mass or glucose tolerance, they did have reduced hepatic lipid content. The functional significance of this is not clear, however, since it did not translate into alterations in hepatic gene expression or circulating of biomarkers liver function, and was no longer present at 12 weeks of age, even when offspring were maintained on the low GI diet after weaning.

\textit{Growth and metabolic outcomes in the offspring in young adulthood}
Interestingly, and contrary to expectations, female offspring of low GI dams exhibited a phase of accelerated weight gain after weaning, independent of their post-weaning diet. This was particularly unexpected given the reduced gestational weight gain in low GI dams, which is generally associated with improved metabolic outcomes in the offspring\textsuperscript{30, 31}. The period of increased body in the current study coincided with the timing of puberty - a period associated with a marked increase in secretion of gonadotrophin releasing-hormones and estrogen and increased body fat accrual\textsuperscript{32}. One possibility, therefore, is that this period of accelerated growth may be the result of an interaction between the effects of sex hormones and programmed changes in other insulin-responsive tissues, such as the skeletal muscle, induced by exposure to a maternal low GI diet. Whilst it is possible that the higher body weight of the low GI dams at mating may have contributed to this increased body weight, this appears unlikely given that there were no differences in birth weight between the low and high GI pups, and that maternal weight for the majority of pregnancy was not different between groups.

The higher SREBP1α mRNA expression in female offspring of high GI dams provided with a low GI diet after weaning may be indicative of an increased propensity for excess hepatic lipid storage, since SREBP1α activation is associated with the up-regulation of hepatic lipogenesis\textsuperscript{33, 34}. The fact that SREBP1α expression was increased in offspring of high GI dams that were switched to a low GI diet after weaning, but not in those who continued to consume the high GI diet, suggests that this may have been driven by a ‘mis-match’ between the nutritional environment experienced pre- and post- weaning. The concept of a mis-match between the environment experienced in postnatal life compared to the environment ‘predicted’ by the perinatal nutritional experience being associated with an increased risk of disease in postnatal life, including metabolic disease, is well described, however this is the first time it has been described in the context of switching from a high to low GI diet\textsuperscript{36}. 
We also observed a reduced expression of PI3K-p85 mRNA in female offspring of low GI dams at 12 weeks of age, independent of the post-weaning diet. PI3K plays a key role in the response of the liver to insulin, as part of the PI3K/Akt axis, and activation of this kinase suppresses gluconeogenesis and promotes glycogen/lipid synthesis and cell growth. Consequently, the lower PI3K mRNA expression would be expected to reduce insulin-stimulated hepatic lipogenesis, and therefore has the potential to inhibit hepatic fat storage in response excess energy intake. In light of this finding, further studies focussed on the effect of maternal low GI diets on the expression, protein abundance and activity of key components of the insulin signalling pathway, and on the impact of obesogenic diets on hepatic lipid storage are warranted, and will provide clearer insights into the potential longer-term benefits of maternal low GI diets on hepatic function in the offspring. Furthermore, the reduction in PI3K is also difficult to reconcile with the elevated plasma ALT concentrations which were also present in female offspring of low GI dams in young adulthood, since this is generally considered to be a marker of poorer hepatic function. However, studies relating ALT levels to hepatic function are generally restricted to adult humans, and the reliability as an indicator of hepatic function in the perinatal period and/or in rodents is not clear.

Perspectives and Significance

The present study is the first to directly compare the effect of a maternal high vs low GI diet on offspring metabolic outcomes beyond the immediate postnatal period. We demonstrated that consumption of a low GI diet during pregnancy and lactation led to increased glucose tolerance in the dam as well as reduced visceral adiposity and increased glucose tolerance in the female offspring at weaning. The long term impact of the GI of the maternal diet on the offspring was less clear; however the results did indicate a potential benefit of maternal low GI
diet consumption for reducing hepatic lipid synthetic capacity in female offspring, by reducing
the expression of PI3K in early adulthood. The increase in SREBP1α in the female offspring
of high-GI dams switched to a low GI diets, however suggests the existence of a complex
relationship between nutritional exposures pre- and post-weaning, which will need to be further
explored in future studies. Nevertheless, the results of the present study provide an important
foundation for future studies aimed at determining whether the changes in glucose tolerance,
fat deposition and hepatic gene expression associated with maternal low GI diet consumption
can translate into an improved capacity of the offspring of low GI dams to resist metabolic
challenges later in life.

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Conflicts of Interest

JBM is the President of the GI Foundation (www.gisymbol.com), a not-for-profit entity that
endorses healthy low GI foods. She manages a GI testing service at the University of Sydney

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(www.glycemicindex.com) and is the co-author of lay books about the glycemic index of foods. The other authors have no conflicts to declare.

**Ethical Standards**

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of Albino Wistar Rats and has been approved by The University of Adelaide Animal Ethics Committee.
**Figure legends**

**Figure 1** (A) Average maternal body during pre-pregnancy (4 weeks after the commencement of the diets), pregnancy and lactation in high GI (open bar, n=14) and low GI dams (closed bar, n=14), *P*<0.05. The average bodyweight is calculated based on the weekly bodyweights recorded within each time period. (B) Maternal food intake during pre-pregnancy, pregnancy and lactation in high GI (open bar, n=14) and low GI dams (closed bar, n=14). Food intake was measured every two days throughout the experiment and the data was normalised to bodyweight. Data presented as mean±SEM, statistical analysis done using a Student’s unpaired T-Test.

**Figure 2** (A) The relative proportion of visceral fat at the end of lactation in high GI (open bar, n=14) and low GI dams (closed bar, n=14), *P*<0.05 (B) Maternal glucose concentrations during an IPGTT in low GI (filled squares, solid line, n=14) and high GI (open triangles, dashed line, n=14) dams at the end of lactation. Low GI dams had a lower peak in glucose (*P*<0.05) (C) Glucose tolerance was better in low GI compared to high GI dams as indicated by lower AUC and lower peak plasma glucose concentrations during the IPGTT (*P*<0.05). Data presented as mean±SEM, statistical analysis done using a Student’s unpaired T-Test.

**Figure 3** (A) Blood glucose concentrations during an IPGTT in female offspring of low GI (filled squares, solid line, n=14) and high GI (open triangles, dashed line, n=12) dams at the end of lactation. Female low GI offspring had a significantly lower peak in glucose (*P*<0.05). (B) Glucose tolerance was better in low GI compared to high GI female offspring as indicated by lower AUC and lower peak plasma glucose concentrations during the IPGTT (*P*<0.05). C) Blood glucose concentrations during an IPGTT in male offspring of low GI (filled squares,
solid line, n=14) and high GI (open triangles, dashed line, n=12) dams at the end of lactation.

(D) No difference in glucose tolerance as indicated by AUC was observed between low GI and high GI male offspring. Data presented as mean±SEM, statistical analysis done using a two-way ANOVA within each sex.

Figure 4 Weight gain from weaning to 12 weeks of age in (A) female and (B) male offspring of low GI (filled squares, solid line) and high GI (open triangles, dashed line) dams. * P<0.05 compared to the high GI group, n=14 for all groups. Data presented as mean±SEM, statistical analysis done using a repeated measures ANOVA within each sex.
Table 1: Primer Sequences for Determination of Hepatic Gene Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
<th>Gene Accession number</th>
</tr>
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<tbody>
<tr>
<td>PI3 Kinase p85</td>
<td>ACCAGTGGTTGACCCTTCTCTG</td>
<td>TGCTGGAGCTCTGTGTGTTCTG</td>
<td>NM_013005.1</td>
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<tr>
<td>ACCβ</td>
<td>CCATGCTTTTTTCAGACAGGTGC</td>
<td>GGACACTGCGTTCCCATACT</td>
<td>NM_053922.1</td>
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<tr>
<td>SREBP-1α</td>
<td>GCGCCATGGAGGAGGCTGCCCCT</td>
<td>GTCACTGTTCTGTTGTTGATG</td>
<td>NM_001276707</td>
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<tr>
<td>PPARα</td>
<td>CCTGTGAACACGATCTGAAAG</td>
<td>ACAAAGGGGGATGTTG</td>
<td>NM_031347.1</td>
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<tr>
<td>PKCζ</td>
<td>AAGTGGGTGGACAGTGAAAG</td>
<td>GGGAAAACGTGGATGATGAG</td>
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</tr>
<tr>
<td>FAS</td>
<td>TGCTCCCAGCTGCAGGC</td>
<td>GCCCCGTagCTGCTGGTGA</td>
<td>NM_017332</td>
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<tr>
<td>HPRT</td>
<td>CTCATGGACTGATTATGGACAG</td>
<td>GCAGGTCAGCAAAGAACTTATA</td>
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<td>β-actin</td>
<td>GCACCACACCTTCTACAATG</td>
<td>TGCTTGTGATCCACATCTG</td>
<td>NM_017101.1</td>
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</table>
Table 2  Fat mass as % bodyweight in male and female offspring of high and low GI dams at 3 weeks of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High GI (n=14)</td>
<td>Low GI (n=14)</td>
<td>High GI (n=14)</td>
<td>Low GI (n=14)</td>
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<td></td>
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<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Gonadal fat</td>
<td>0.22</td>
<td>0.03</td>
<td>0.37</td>
<td>0.03</td>
<td>0.26</td>
<td>0.01</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Retroperitoneal fat</td>
<td>0.35</td>
<td>0.03</td>
<td>0.37</td>
<td>0.03</td>
<td>0.36</td>
<td>0.02</td>
<td>0.38</td>
<td>0.03</td>
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<tr>
<td>Omental fat</td>
<td>0.49</td>
<td>0.04</td>
<td>0.45</td>
<td>0.04</td>
<td>0.53</td>
<td>0.03</td>
<td>0.45*</td>
<td>0.03</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>1.06</td>
<td>0.07</td>
<td>1.17</td>
<td>0.15</td>
<td>1.23</td>
<td>0.13</td>
<td>1.02*</td>
<td>0.05</td>
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<tr>
<td>Subcutaneous fat</td>
<td>2.89</td>
<td>0.30</td>
<td>3.29</td>
<td>0.31</td>
<td>3.71</td>
<td>0.40</td>
<td>3.73</td>
<td>0.03</td>
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<tr>
<td>Interscapular fat</td>
<td>0.59</td>
<td>0.05</td>
<td>0.59</td>
<td>0.04</td>
<td>0.58</td>
<td>0.03</td>
<td>0.60</td>
<td>0.02</td>
</tr>
<tr>
<td>Total fat</td>
<td>4.55</td>
<td>0.04</td>
<td>5.04</td>
<td>0.41</td>
<td>5.44</td>
<td>0.48</td>
<td>5.34</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM, * indicates significantly different mean between groups within each sex, $P<0.05$. 
Table 3. Weights of individual fat depots and visceral and total fat mass as a percentage of bodyweight in the male and female offspring of High GI and Low GI dams fed a low or high GI diet at 12 weeks of age.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadal fat</td>
<td>1.13±0.12</td>
<td>1.17±0.15</td>
</tr>
<tr>
<td>Retroperitoneal fat</td>
<td>1.45±0.11</td>
<td>1.70±0.15</td>
</tr>
<tr>
<td>Omental fat</td>
<td>0.86±0.13</td>
<td>1.15±0.09</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>3.44±0.28</td>
<td>3.97±0.34</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>3.65±0.19</td>
<td>4.15±0.23</td>
</tr>
<tr>
<td>Interscapular fat</td>
<td>0.21±0.01</td>
<td>0.26±0.02</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Total fat</td>
<td>7.30±0.49</td>
<td>8.35±0.51</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. n=7 per sex for all groups Different letters denote significantly different means within each sex, \( P<0.05 \)
Table 4  Relative liver weight (as a % of body weight), % liver lipids and mean normalised expression of hepatic genes in male and female offspring of High GI and Low GI dams fed a low or high diet at 12 weeks of age.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative liver weight (%)</td>
<td>4.32±0.08</td>
<td>4.16±0.11</td>
</tr>
<tr>
<td>% liver lipids</td>
<td>5.04±0.48</td>
<td>7.37±1.31</td>
</tr>
<tr>
<td>Hepatic Genes</td>
<td></td>
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</tr>
<tr>
<td>ACCβ</td>
<td>45.40±6.45</td>
<td>44.20±7.91</td>
</tr>
<tr>
<td>PPARα</td>
<td>10.40±1.93</td>
<td>16.70±3.28</td>
</tr>
<tr>
<td>SREBP1α</td>
<td>33.30±6.02</td>
<td>38.00±6.03</td>
</tr>
<tr>
<td>PI3K</td>
<td>27.45±4.10</td>
<td>37.19±10.35</td>
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<tr>
<td>G3PDH</td>
<td>381.94±54.09</td>
<td>301.41±70.21</td>
</tr>
<tr>
<td>FAS</td>
<td>287.93±38.55</td>
<td>521.25±19.59</td>
</tr>
<tr>
<td>PKCζ</td>
<td>0.67±0.13</td>
<td>0.45±0.11</td>
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</tbody>
</table>

Data are presented as mean ± SEM. n=7 per sex for all groups. Values for gene expression data have been multiplied by one thousand for ease of presentation. Different letters denote significantly different means within each sex, P<0.05.
REFERENCES


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