A Maternal Low Glycemic Index Diet for Improving Neonatal Metabolic Outcomes

By

Mohammed Al-Nussairawi

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Faculty of Sciences

School of Agriculture, Food & Wine

Waite Campus

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Declaration

I declare that this thesis is a record of original work and contains no material that has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text

Signature of student

Name of student

Mohammed Al-Nussairawi

Date

Signature of supervisor

Name of Supervisor

Beverly Muhlhausler

Date

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Preface

This research was conducted over 10 months as part of a Master of Biotechnology. In accordance with the requirements of the program, the research is presented in the format of a manuscript for submission to a peer-reviewed scientific journal. I have chosen to follow the format of the *Journal of Physiology*. My co-authors for the manuscript are Professor Robert Gibson, Dr Beverly Muhlhausler and Professor Jennie Brand-Miller. Dr Muhlhausler helped design the primers that I used for gene expression and assisted me with the interpretation of the results. Dr Beverly Muhlhausler developed the novel experimental design that I used in the experiment, carried out the spatial and temporal modelling and supervised me in other aspects of the data analysis. Dr Beverly Muhlhausler designed the project, supervised my research and reviewed drafts of the manuscript.

The manuscript in this thesis is intended as the first draft of a manuscript for future publication. The word count for the manuscript (excluding references and supplementary material) is 6731 words.

Research Manuscript

Abstract

Due to widespread anxiety about the obesity epidemic, type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD), and associated health care costs, the development of effective weight and dietary management strategies has become a public health priority. The aim of this study was to investigate the role of low glycaemic index (LGI) diets during pregnancy and lactation on metabolic outcomes of offspring in postnatal life. Rat model was used in this project. Dams were fed a LGI diet (n=8) or high GI (HGI) diet (n=8) during pregnancy and lactation. Body weight, body fat mass and glucose tolerance were determined in male and female offspring at three weeks of age. Livers were weighed and samples were collected to determine the hepatic mRNA expression of key genes involved in lipogenesis and the insulin-signalling pathway by qRT-PCR at three weeks of age. Maternal weight at the end of lactation was significantly higher in the LGI group compared to the HGI group (P<0.05), but there were no differences in glucose tolerance between dams in the LGI and HGI groups at the end of the dietary intervention. In addition, there were no differences between the weight of LGI and HGI offspring at birth, and no differences in body fat mass, body weight or liver weight between LGI and HGI offspring at three weeks of age. Glucose tolerance was improved in three-week-old female LGI offspring (P<0.05), but not in males. The hepatic expression of G3PDH was lower in LGI male offspring at three weeks of age, but there was no difference in the expression of any other genes between groups. These results provide evidence that a LGI diet does not affect body weight and liver function, but it improves glucose tolerance in female offspring at three weeks of age.

Abbreviations ACC, Aceyl-CoA carboxylase;(SREBP)-1,Sterol Regulatory Element-binding Protein; PI3K, phosphatidyl inositol 3-kinase; GAPDH, glyceraldehyde-phosphate-dehydrogenase; G3PDH, Glycerol 3-phosphate dehydrogenase; HPRT, Hypoxanthine phosphoribosyltransferase; PPARα, Peroxisome proliferator-activated receptor alpha; FAS, Fatty Acid Synthase; Ef, Amplification efficiency;CYPa,Cyclophilin A; PKC zeta,protein kinase C; (T2DM) type 2 diabetes mellitus; Hb A1c, Glycated haemoglobin A1c; NAFLD, non-alcoholic fatty liver disease;GAUC,glucose area under the curve; IAUC, insulin area under the curve; LGA,Large for gestational age**;**GI, Glycaemic Index; LGI, Low GI; HGI, High GI; CHOs, Carbohydrates; WHO, World Health Organization; Lo-Lo, Low-Low; Lo-Hi, Low-high; Hi-Lo, High-low; Hi-HI, High-high.

Introduction

Obesity represents one of the most important problems that affect people in modern society. Around the world, more than one billion adults are considered overweight, and more than 400 million are considered obese (van Dieren *et al.*, 2010). Additionally, the number of overweight children under five years of age is about 20 million worldwide (Ahmed *et al.*, 2009). Childhood obesity is an important risk factor for obesity later in life and is therefore expected to result in an increased number of obese adults in the future (Ahmed *et al.*, 2009). According to the WHO, by 2015, approximately 2.3 billion adults will be overweight (WHO, 2006). Obesity is closely associated with a number of widely recognised co-morbidities, including T2DM and the build up of fat in the liver (non-alcoholic fatty liver disease, NAFLD) (York *et al.*, 2009). The prevalence of these disorders (Bell *et al.*, 2005) is increasing in many countries around the world (Brand-Miller *et al.*, 2003).

There is increasing recognition of the importance of maternal nutrition for the long-term health of offspring. Research has shown that exposure to increased glucose levels before birth, as a result of maternal diabetes, maternal glucose intolerance or inappropriate maternal nutrient intake, promotes adipose tissue deposition in the foetus, increases the risk of pregnancy complications and is associated with an increased risk of obesity, T2DM and NAFLD in offspring later in life (Catalano, 2003; Muhlhausler *et al.*, 2007). Importantly, it has also been demonstrated that reducing maternal glucose concentrations during pregnancy results in improved pregnancy and infant outcomes (Moses *et al.,* 2006).

One of the main dietary factors determining postprandial glucose concentration is the quantity and quality of CHOs in the diet. The effect of different carbohydrates (CHOs) on glucose levels is determined by the glycaemic index (GI). This concept, first introduced by Jenkins and colleagues in the early 1980s, classifies CHOs

according to their resultant glycaemic responses (McGonigal & Kapustin, 2008). Foods can be classified into two groups: low GI (LGI) and high GI (HGI). LGI foods are metabolised slowly, whereas HGI foods are metabolised quickly. All foods are ranked on a GI scale from 0 to 100, where LGI foods have a GI of less than or equal to 55,whereas HGI foods have a GI of greater than or equal to 70 (McGonigal & Kapustin, 2008). The main difference between LGI and HGI foods is their effects on plasma glucose concentrations.

HGI diets cause a rapid increase in blood glucose levels, which results in a robust insulin response and increase in plasma insulin levels. The high levels of insulin result in rapid declines in plasma glucose; consequently, eating HGI foods is associated with a series of peaks and troughs in plasma glucose concentrations. Over time, these exaggerated post-prandial rises and falls in plasma glucose levels can predispose an individual to develop insulin resistance (Ludwig, 2002; Krog-Mikkelsen *et al.*, 2011). In contrast, LGI foods cause a slower and more sustained release of glucose and insulin and therefore avoid these peaks and troughs in plasma glucose (Krog-Mikkelsen *et al.*, 2011).

A number of studies show the benefits of an LGI diet for glucose control and weight loss in adult rats (Kabir *et al.*, 1998) and humans (De Rougemont *et al.*, 2007); however, less is known about their role in pregnancy. The early work carried out in humans has provided evidence that a LGI diet may have benefits for pregnant women and their children (Moses *et al.*, 2006). In a study by Moses and colleagues, which included 62 pregnant women who consumed a LGI diet (n=32) or HGI diet (n=30), one of the key findings was that the babies of mothers in the HGI group were heavier (3408±78 compared to 3644±90 g; P=0.005) and more likely to be classified as large for gestational age (LGA) compared to the LGI group (Moses *et al.*, 2006). A recent systematic review of eight studies by Louie

and colleagues (2010) concluded that an LGI diet could benefit the offspring by reducing postprandial glycaemia(Louie *et al.*, 2010).These studies have provided encouraging results: LGI diets have no negative effects, but may have some benefits for the offspring during pregnancy by reducing maternal and foetal glucose concentrations (Galgani *et al.*, 2006) and reducing the risk of excess foetal growth (Scholl *et al.*, 2004).

However, these studies have only examined the effects of LGI diets on women during pregnancy and their infants at the time of birth, and no studies have investigated the effects of LGI diets during pregnancy on metabolic outcomes in the offspring beyond the immediate postnatal period. Therefore, this thesis will use an animal model to investigate the effects of maternal intake of a LGI diet compared to a HGI diet during pregnancy and lactation on metabolic health outcomes of offspring in postnatal life. In particular, it will focus on the effects of a LGI diet during pregnancy on glucose tolerance, body fat mass and hepatic gene expression.

Materials and Methods

Animals and feeding regime

The Animal Ethics Committee of the University of Adelaide approved this study (ethics number is S_2011_084). Thirty-two male and female Albino Wistar rats (approx. 150 g) were individually housed under a 12-hour light–dark cycle at a room temperature of 25°C. Rats were allowed to acclimatise to the animal facility for one week before the commencement of the experimental procedure. At the end of the acclimatisation period, rats were weight-matched and assigned to LGI (n=16) and HGI (n=16) diets (Specialty Feeds, Glen Forrest, Western Australia). Detailed nutrition or composition information of the LGI and HGI diets is shown in Table1. Every two days, food was replenished and the food intake was determined

by subtracting the amount of each food type remaining at the end of the two-day period from the amount originally provided.

Table 1: Nutritional details of LGI and HGI diets. The nutritional parameters and the ingredients are the same for both diets, except for the starch.

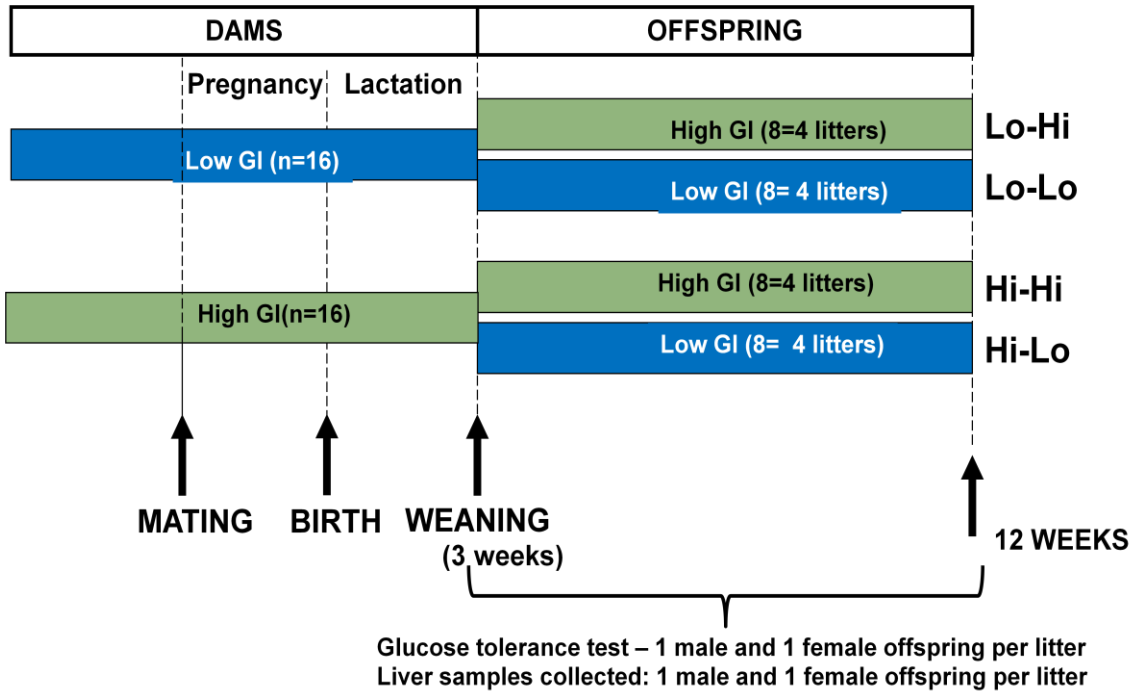
Nutritional Parameters			**Ingredients**	
Protein	19.4%		Casein(Acid)	200 g/Kg
Total fat	7.0%		Gel Crisp Starch (LGI) Dextrinised Starch (HGI)	636 g/Kg 636 g/Kg
Crude fibre	4.7%		Canola oil	70 g/Kg
AD fibre	4.7%		Cellulose	50 g/Kg
Digestible energy	16.3 MJ/Kg		DL Methionine	3.0 g/Kg
% Total calculated digestible energy from lipids	16.0%		Calcium Carbonate	13.1 g/Kg
			Sodium Chloride	2.6 g/Kg
% Total calculated digestible energy from protein	21.0%		AIN93 Trace Minerals	1.4 g/Kg
			Potassium Citrate	2.5 g/Kg
			Potassium Dihydrogen Phosphate	6.9 g/kg
			Potassium Sulphate	1.6 g/Kg
			Choline Chloride (75%)	2.5 g/Kg
			AIN93 Vitamins	10 g/Kg

Rat mating and pregnancy

Vaginal smears were conducted daily from two weeks before mating to determine the stages of the estrous cycle. After four weeks on their respective diets, on the evening of diestrous/proestrous, two female rats were placed in a cage with a proven male for 24 hours. Four mating males were used, and each male rat was mated with females from both LGI and HGI groups. Vaginal smears were performed the following morning to check for the presence of sperm to confirm successful mating, and this was designated as gestation day 0. Female rats were then removed from the males and housed individually. After mating, female rats were maintained on their respective diets throughout pregnancy and lactation. All dams were weighed weekly. Pups were born on days 21–22 of gestation. Within two days of birth, litter sizes were adjusted to eight pups, with four males and four

females where possible. Pups were weighed every two days until weaning and once per week after weaning, as shown in Figure 1. This thesis will include data for pregnancy, birth outcomes and pup outcomes up to weaning (three weeks of age).

Figure1: Experimental design



Intra-peritoneal glucose tolerance test (IPGTT)

At three weeks of age, IPGTTs were performed on two offspring (one male, one female) per litter. At baseline time t=0, tail vein blood samples were taken before administration of an intra-peritoneal injection of glucose (2mg/kg). Blood samples were taken from a rat's tail vein at six different times (5, 10, 15, 30, 60 and 120 minutes) post-injection. If the glucose values had not returned to baseline by 120 minutes, an additional sample was collected at 180 minutes post-injection. The Accu-Chek Performa glucometer was used to determine blood glucose concentrations at each time point.

Post-mortem and tissue collection

The dam and one male and one female pup from each litter were killed at weaning (three weeks of age) in order to determine the mass of visceral and subcutaneous fat and the collection of tissues. All post-mortems were conducted during the light phase (9–12 am). All rats were weighed immediately before the post-mortem and were then killed with an overdose of carbon dioxide. Blood samples were collected into heparinised tubes and centrifuged at 3500 *g*, 4°C for 15 minutes. The clear layer of plasma was removed and stored at –20°C for subsequent hormone and metabolite assays. The liver was weighed and liver samples were collected and stored at –80°C for subsequent molecular analysis.

Housekeeping genes selection

Four housekeeper genes (HKGs)— β actin quantitect primer assay (Qiagen Australia, Doncaster, Victoria), GAPDH, HPRT and CYPa (GeneWorks, Adelaide, South Australia) were tested in four GI pups at three weeks of age, two from the LGI group and two from the HGI group. Three HKGs β actin, GAPDH and HPRT were chosen for normalization using NormFinder and BestKeeper (www.gene-quantification.info) software due to their expression stability among these animals, and the CYPa gene was excluded from the study because it was not stable between the samples. However, in the final analysis, we have used HPRT as a best HKG because it is expression was the most stable between the groups.

RNA extractions and cDNA Reverse Transcription

Total RNA (100 mg for each sample) was extracted from 32 liver samples using Trizol reagent and then purified through RNA columns (QiagenRNeasy Mini Kit, Australia). RNA purity was confirmed by measuring the absorbance at 260 and 280nm, RNA integrity was confirmed by using agarose gel electrophoresis and

cDNA was synthesised (20 µL for each liver sample) using reverse transcriptase SuperScriptIII (Invitrogen, Australia) with random hexamers.

Primer design and validation

The primers were designed using the Primer3 and NCBI websites. Some of them were designed manually and others via NCBI. The primers were designed to cross exon-exon boundaries to ensure they did not anneal to genomic DNA, and they were validated in rat liver samples. The size of the amplicon was confirmed by running an agarose gel. All PCR products were sequenced before the experiment to ensure the amplification of the correct gene, and a qRT–PCR melt–curve analysis was performed at the end of each PCR run to confirm amplicon homogeneity.

Real-time PCR and gene expression

Gene expression was determined by Real-time PCR using the ViiA7 platform (Applied Biosystems, University of Adelaide, South Australia).

The amplification efficiencies were determined for all sets of genes, and the amplification efficiency was between 99 and 100 percent for all primers. A constant amount of cDNA (3.3 µL) was used for each qRT–PCR measurement, and three replicates were performed for each gene. The expression of all genes was normalised to three housekeeper genes β actin, GAPDH and HPRT—using BestKeeper and NormFinder software**.** Each plate included no template control (NTC) and β actin quality control (QC) to verify inter-plate reliability. Each qRT–PCR reaction (10 µL total volumes in each well) was included:6.6 µL Molecular grade water,3.3 µL cDNA, 3.3 µLForward primer, 3.3 µL Reverse primer and 16.5 µL Sybr Green (Bio-Rad Australia).

Table 2: Primer sequences for PI3 Kinase, ACCbeta, SREBP-1a, PPARalpha, PKCzeta, FAS, GAPDH, HPRT, G3PDH and CYPa

Gene	Sequence	Accession number	Working concentration nM
PI3 Kinase p85	F 5' ACCAGTGTTGACCCTTCCTG '3 R 5' TGCTGGAGCTCTGTGTTCTG '3	NM_013005.1	900
ACC β	F 5' CCATGCTTTTTCAGACAGGTGC'3 R 5' GGACACTGCGTTCCCATACT '3	NM_053922.1	900
SREBP-1a	F 5' GCGCCATGGAGGAGCTGCCCTTCG '3 R 5' GTCACTGTCTTGGTTGTTGATG'3	NM_001033694.1	900
PPARalpha	F 5' CCTGTGAACACGATCTGAAAG'3 R 5' ACAAAGGCGGATTGTTG'3	NM_031347.1	900
PKCzeta	F 5' AAGTGGGTGGACAGTGAAGG '3 R 5' GGGAAAACGTGGATGATGAG '3	NM_022507.1	900
FAS	F 5' TGCTCCCAGCTGCAGGC'3 R 5' GCCCGGTAGCTCTGGGTGTA'3	NM_017332	300
GAPDH	F 5' AGGGCTGCCTTCTCTTGTGA '3 R 5' TGGGTAGAATCATACTGGAACATGTAG'3	NM_17008.4	300
HPRT	F 5' CTCATGGACTGATTATGGACAGGAC'3 R 5' GCAGGTCAGCAAAGAACTTATAGCC'3	NM_012583.2	900
G3PDH	F 5' GCTTCGGTGACAACACCA'3' R 5' AGCTGCTCAATGGACTTTCC'3	NM_022215.2	300
CYPa	F 5' TATCTGCACTGCCAAGACTGAGTG'3 R 5' CTTCTTGCTGGTCTTGCCATTCC'3	NM_017101.1	900

The abundance of each mRNA transcript was measured, and the expression relative to β actin, HPRT and GAPDH was calculated using Q-gene qRT–PCR analysis software. This software provides a quantitative measure of the relative abundance of a specific transcript in different tissues using the comparative threshold cycle (*Ct*) method, which takes into account any differences in the amplification efficiencies of the target and reference genes.

Statistical analysis

Data are expressed as mean ± SEM. Relative fat mass and relative organ weight are expressed as per cent of body weight. Statistical analysis was conducted using the Statistical Package for Social Sciences software (SPSS) and Prism version 5.

The effects of maternal diet on pregnancy, birth outcomes and offspring outcomes at three weeks were assessed using an unpaired Student's T-test (two-tailed, unpaired with equal variance).

The effects of maternal diet on maternal outcomes (food intake, weight gain, glucose tolerance, liver weight, fat mass) were also determined using the Student's unpaired T-test (two-tailed, unpaired with equal variance).

The effect of diet and sex on gene expression was determined using a two-way analysis of variance (ANOVA).

Area under the curve (AUC) for the IPGTTs was calculated for each animal using Prism version 5, and the mean AUC between the LGI and HGI groups for the mothers and offspring was determined using a student's T-test.

Results were considered statistically significant where $P<0.05$.

Results

Maternal outcomes

Maternal food intake and body weight

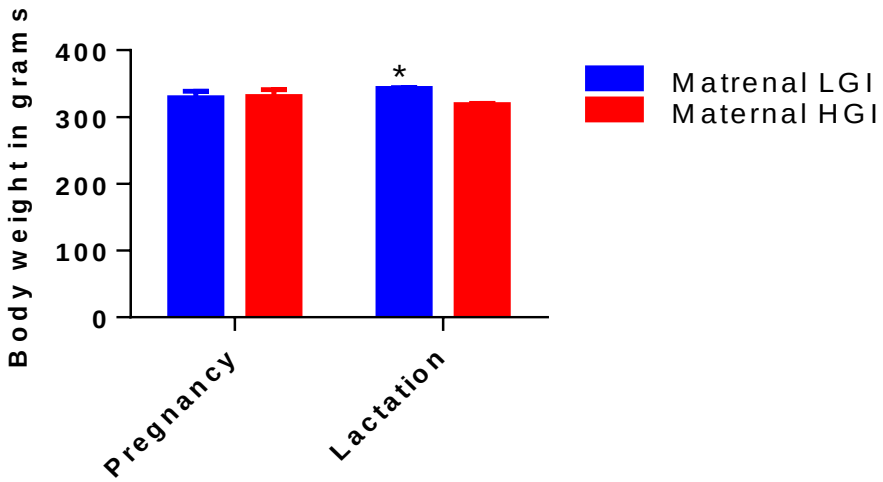
There were no differences in maternal food intake (grams/week/kg body weight) between the LGI and HGI groups either before pregnancy or during pregnancy and lactation (Table 3).

Table 3: Maternal food intake in grams during the dietary intervention. Data is expressed as Mean ± SEM.

Group	Before pregnancy (g)	Pregnancy (g)	Lactation (g)
LGI	362.05 ± 6.85	438.12 ± 13.32	829.55 ± 15.72
HGI	354.38 ± 3.09	401.49 ± 4.87	716.14 ± 20.58

There was also no difference in body weight between LGI and HGI mothers before (LGI=359.63 ± 13.86, HGI=364.13 ± 11.78)or at the end of pregnancy(Figure 2).Maternal weight at the end of lactation was significantly higher in LGI groups compared to HGI groups (*P*<0.05), as shown in Figure 2.

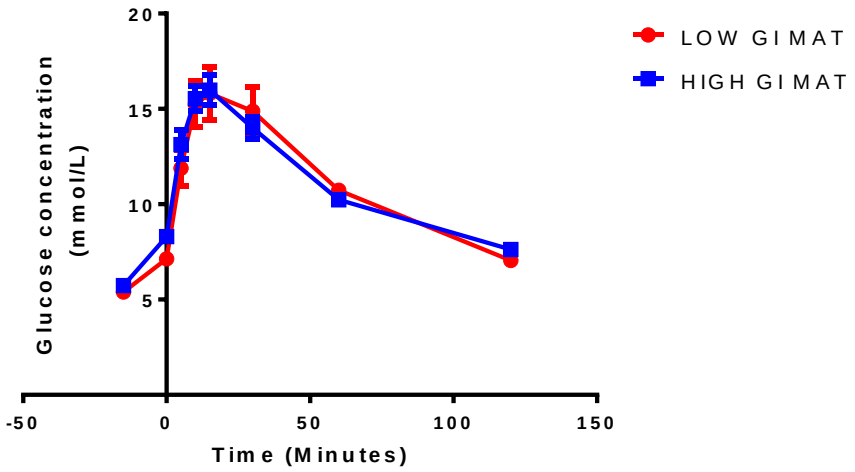
Figure 2: LGI and HGI maternal body weight during pregnancy and lactation. Data are expressed as Mean ± SEM.



Maternal glucose tolerance

There was no difference in glucose tolerance between LGI and HGI mothers at the end of the dietary intervention (Figure 3).

Figure 3: Comparison of glucose concentration values over time after intra-peritoneal glucose load, between maternal LGI and HGI mothers. Data are expressed as a mean ± SEM.



Liver weight and fat mass

There was no difference in the liver weight or fat mass between LGI and HGI mothers at the end of the dietary intervention (Table 4).

Table 4: Liver weight and fat mass for both LGI and HGI mothers. No significant difference in liver weight and fat mass in mothers during, and at the end of the dietary intervention. Values expressed as mean ± SEM.

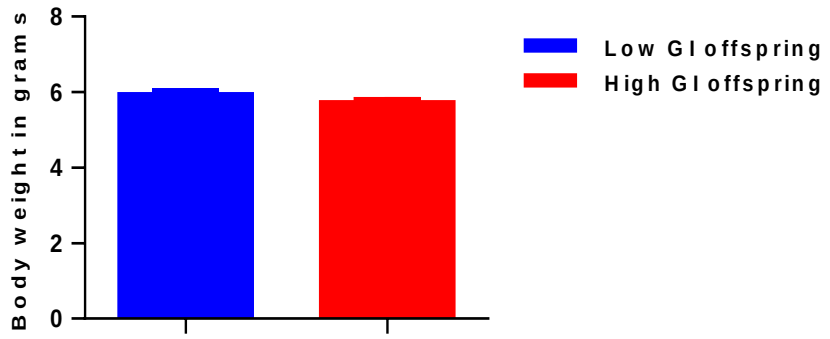
Group	Liver (g)	r Liver weight (g/g)	Total fat (g)	r Total fat (g/g)	Visceral fat (g)	r Visceral fat (g/g)
LGI	13.7 ± 0.6	0.1 ± 0.01	25.1 ± 3.0	0.1 ± 0.01	14.8 ± 1.6	0.2 ± 0.01
HGI	13.9 ± 1.1	0.2 ± 0.02	21.5 ± 2.7	0.3 ± 0.01	14.0 ± 1.3	0.3± 0.03

Offspring outcomes

Birth weight

There was no effect of maternal diet on the birth weight of either male or female offspring (Figure4).

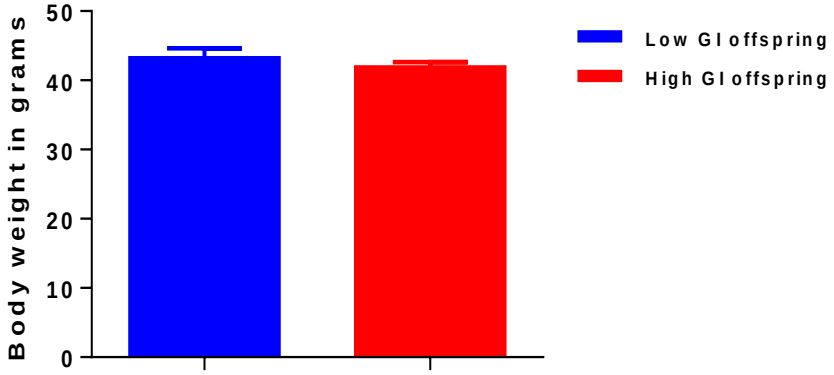
Figure 4: LGI and HGI offspring birth weight. Values expressed as a mean ± SEM



Body weight at three weeks of age

There was no difference in weight at three weeks of age between LGI and HGI groups for either males or females (Figure 5).

Figure 5: LGI and HGI offspring body weight at three weeks of age. Values expressed as a mean ± SEM. LGI=16; HGI 16



Offspring glucose tolerance at three weeks of age

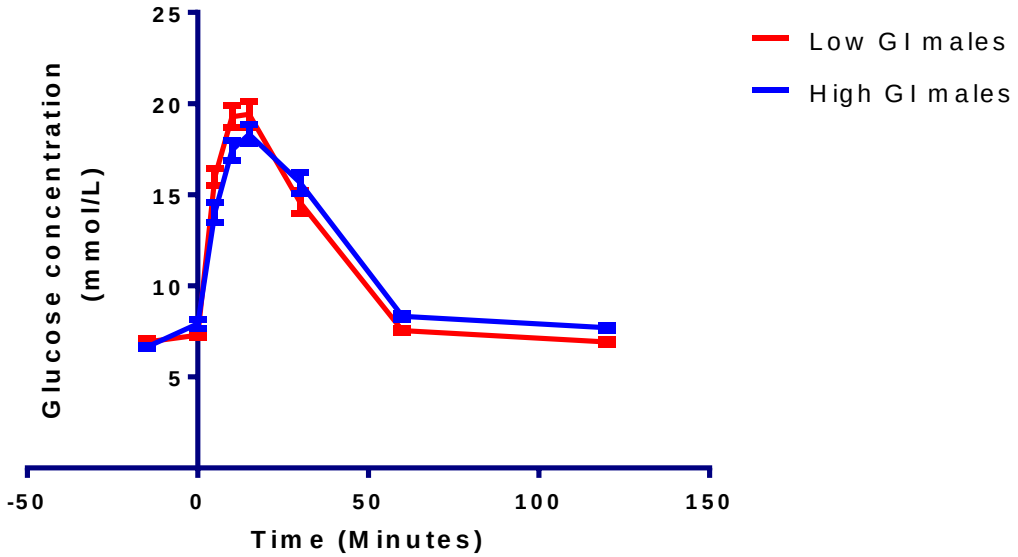
In males, there was no significant difference in glucose tolerance between LGI and HGI offspring at three weeks of age (Figure 6A). However, in females, glucose tolerance was significantly improved in the LGI group compared to the HGI group (P<0.05) (Figure 6B).

Table 5: Offspring AUC results. Values expressed as mean ± SEM.

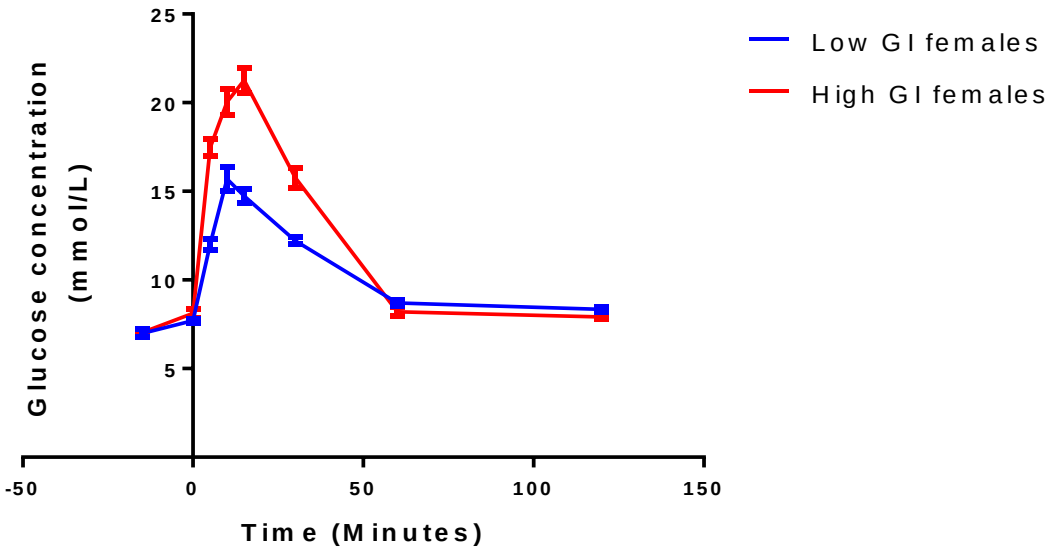
Group	Male three-week AUC (mmol/L) minutes	Female three-week AUC (mmol/L) minutes
LGI offspring	1255.81 ± 101.59	1331.75 ± 19.59
HGI offspring	1427.25 ± 62.33	1495.63 ± 62.10
T-test	0.172	0.024

Figure 6: Comparison of glucose concentration values over time after intra-peritoneal glucose load, between maternal LGI and HGI mothers. Values expressed as mean ± SEM. LGI mothers = 8; HGI mothers = 8

(A) LGI and HGI male offspring glucose tolerance



(B) LGI and HGI female offspring glucose tolerance



Offspring liver weight and fat mass at three weeks of age

There were no differences in the relative (r) liver weight and fat mass in the offspring at three weeks of age between LGI and HGI in either males or females (Table 6).

Table 6: Body weight and relative (r) body weight (g) of offspring at three weeks of age

Group	Liver weight (g)	r Liver weight (g/g)	Total fat mass (g)	r Total fat mass (g/g)	Visceral fat (g)	r Visceral fat (g/g)
LGI males	1.79 ± 0.076	0.039 ± 0.006	2.28 ± 0.28	0.06 ± 0.006	0.59 ± 0.098	0.013 ± 0.002
HGI males	1.69 ± 0.075	0.039 ± 0.002	2.08 ± 0.27	0.048 ± 0.005	0.52 ± 0.051	0.012 ± 0.001
LGI females	1.66 ± 0.088	0.039 ± 0.001	2.49 ± 0.24	0.058 ± 0.005	0.45 ± 0.042	0.013 ± 0007
HGI females	1.59 ± 0.100	0.039 ± 0.001	2.30 ± 0.34	0.055 ± 0.006	0.56 ± 0.080	0.014 ± 0.002

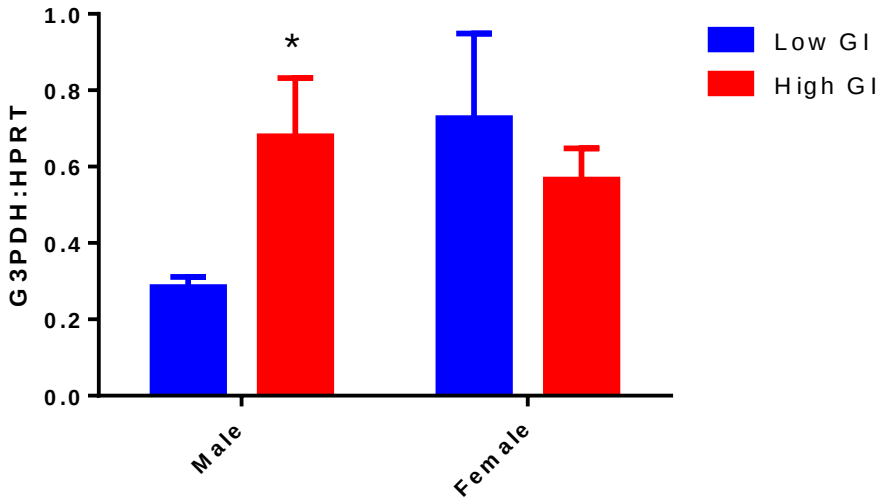
The effect of diets on gene expression of offspring at three weeks of age

In males, but not in females, G3PDH mRNA expression in the liver was lower in the LGI group compared to the HGI group (Figure 7). There were no significant differences between the LGI and HGI groups for the mRNA expression of PI3 Kinase and PKCzeta (insulin signalling) ACCbeta, and PPARalpha (fatty acid oxidation), SREBP-1a, FAS in (lipogenesis) the offspring at three weeks of age in either males or females (Table 7). There were significant differences between male and female offspring in the mRNA expression of PKCzeta, such that the expression of PKCzeta was higher in males compared to females, independent of maternal dietary group.

Table 7: mRNA expression of PI3Kinase, ACCbeta, SREBP-1a, PPARalpha,PKCeta and FAS relative to HPRT of the offspring at three weeks of age. Data are expressed as mean ± SEM. [#] indicates a significant difference between male and female offspring (P<0.05)

Gene	Male		Female	
	Low (n=8)	**High (n=8)**	**Low (n=8)**	**High (n=8)**
PI3Kinase	0.55 ± 0.14	0.63 ± 0.09	0.56 ± 0.14	0.56 ± 0.12
ACCbeta	0.19 ± 0.04	0.32 ± 0.14	0.33 ± 0.09	0.31 ± 0.08
SREBP-1a	0.47 ± 0.08	0.49 ± 0.09	0.38 ± 0.09	0.43 ± 0.09
PPARalpha	0.42 ± 0.13	0.49 ± 0.14	0.40 ± 0.10	0.35 ± 0.13
PKCzeta	0.068 ± 0.0012 [#]	0.065 ± 0.0015 [#]	0.013± 0.002	0.015 ± 0.003
FAS	0.52 ± 0.13	0.64 ± 0.11	0.53 ± 0.13	0.59 ± 0.12

Figure 7: G3PDH expression in the offspring relative to HPRT. G3PDHmRNAexpression was significantly lower in LGI offspring in males, but not in females (*P*<0.05).



Discussion

The current study is the first to show the effect of LGI and HGI diets during pregnancy and lactation on the metabolic outcomes of offspring at weaning. We found that female offspring of LGI dams had significantly improved glucose tolerance compared to the HGI group at three weeks of age. In males, the expression of the lipogenic gene G3PDH at three weeks of age was lower in the offspring of LGI dams compared to HGI dams. However, there were no significant differences between the LGI and HGI groups in terms of body weight or fat mass at weaning.

Maternal food intake, body weight and fat mass

We found that there were no differences in maternal food intake or body weight during pregnancy. The absence of effects on food intake is different from the results of a previous crossover study by Clapp and Beth (2007), which included seven adult women. This study indicated that there was high food intake (*P*<0.05) when participants consumed the LGI meal. Moreover, a significant weight loss in

response to the LGI diet was observed at the end of the study (P<0.05) (Clapp & Beth, 2007).

The lack of change in body weight during pregnancy is consistent with some studies in adult women, which have shown no change in body weight or fat mass after following an LGI diet (Aston *et al.*, 2008). A randomised crossover intervention of 19 women over a 12-week period showed that the LGI diet did not affect body weight and fat mass during the dietary intervention (Aston *et al.*, 2008). In another study, there was no difference in food intake, body weight and fat mass 10 weeks after consuming LGI or HGI diets in 45 overweight women (Sloth *et al.*, 2004).

However, other studies have shown significant weight loss over shorter periods after consuming LGI diets compared to standard GI diets (De Rougemont *et al.*, 2007). A five-week randomised intervention trial study by De Rougemont and colleagues (2007) showed that there were benefits of an LGI diet compared to a HGI diet in terms of weight loss. The study demonstrated that mean body weight was significantly different in the LGI group compared to the HGI group (De Rougemont *et al.*, 2007).

A study by Ebbeling and colleagues involved 14 subjects who were divided into two groups: experimental and conventional. It was demonstrated that the experimental group, who consumed a reduced-GI diet for 12 months, exhibited a reduction in body weight and fat mass (P<0.05), while subjects in the conventional group, who consumed a reduced-fat diet for the same period, did not exhibit any significant difference in weight loss or fat mass (Ebbeling *et al.*, 2003). In these studies, weight loss resulted from the decreasing level of body fat mass. Indeed, studies in rats showed that an LGI diet resulted in a decrease in lipogenesis in adipocytes after three weeks (Kabir *et al.*, 1998).

In the present study, we evaluated the effect of the LGI diet during pregnancy, which is a time when individuals usually accumulate body fat mass and lipogenesis is up-regulated. Therefore, this normal adaptation of pregnancy might alter mother's response to the LGI diet. In contrast to our study, Clapp (1997) was the first researcher to support the hypothesis that an LGI diet may safely reduce the epidemic of weight gain. Twelve pregnant women were fed a LGI diet before pregnancy until eight weeks gestation and then randomised to continue on LGI and HGI diets. Clapp's study showed that the participants who consumed the LGI diet gained less weight during pregnancy than the HGI subjects (LGI 11.8 ± 2.3 kg; HGI 19.7 ± 1.2 kg). Additionally, the infants of those who were fed the HGI diet had a higher birth weight and fat mass (P<0.01) compared to the infants of mothers who were fed the LGI diet (Clapp, 1997).

Lactation weight

Interestingly, while there was no effect of the LGI diet on weight gain in pregnancy in this study, we found that the body weight of the LGI dams was significantly higher than the HGI dams. Lactation is a time when the fat stores which are accumulated in pregnancy and mobilised, and this may lead to the hypothesis that this mobilisation of fat stores was inhibited in the LGI dams.

Maternal glucose tolerance

Our study showed that there was no difference in maternal glucose tolerance between the LGI and HGI groups at the end of lactation. This is unexpected because a number of studies have shown that consumption of a LGI diet is associated with improved glucose tolerance and insulin sensitivity compared to a HGI diet. A meta-analysis by Brand-Miller and colleagues (2003) of randomised controlled trails that compared the effect of LGI and HGI diets in the management

of type 2 diabetes showed that LGI diets enhanced the glycaemic control compared to HGI (Brand-Miller *et al.*, 2003). Stevenson and others (2009) also reported that in eight sedentary women, the blood glucose concentrations were lower at 30, 45, 135 and 150 minutes after consuming an LGI breakfast compared to those who consumed a HGI diet ($P<0.05$). Further, the AUC for both glucose and insulin following the glucose challenge were lower in women who consumed a LGI breakfast compared to women who had a HGI breakfast (Stevenson *et al.*, 2009).

In studies of adult diabetics, a LGI diet showed benefits in terms of glucose tolerance. In one study, Rizkalla and others showed that after consuming a LGI diet, the postprandial plasma glucose, insulin profiles and glucose tolerance were significantly improved compared to a HGI diet (Rizkalla *et al.*, 2004).

In addition, previous studies of pregnant women showed benefits of LGI diets for maternal glucose tolerance in pregnancy. The findings of a randomised crossover design done by Clapp and others (2007) clearly showed that a LGI diet benefited seven healthy non-pregnant women in terms of glucose and insulin concentrations. Their study demonstrated that 16-hour GAUC and IAUC were significantly lower after consuming an LGI diet compared to a HGI diet. In contrast, the current study shows that the GAUC of the maternal groups were similar in both groups.

In healthy pregnant women, some studies showed conflicting results compared to the findings of the current study. These data indicates that the type of CHOs affects a healthy mother's blood glucose, alters placental growth and increases weight gain (Moses *et al.*, 2009). The effects of a LGI diet in non-healthy pregnant women, particularly those with T2DM, also showed a positive influence. The study by Moses and others (2009) showed that pregnant women in the HGI group who

had T2DM met the criteria to have high insulin levels compared to subjects in the LGI group (59 percent in HGI group *v*. 29 percent in LGI group) (Moses *et al.*, 2009).

A randomised crossover design was used to examine the effects of two isocaloric, high-carbohydrate diets on the whole-blood glucose and insulin responses to mixed caloric intake and exercise in healthy non-pregnant women(n=14) and pregnant (n=12) women. In non-pregnant women, the blood glucose response to LGI CHOs was 50 percent less than HGI CHOs, and the effect of exercise on blood glucose was more noticeable while eating the HGI CHOs (Clapp, 1998). During pregnancy, women on the LGI CHOs diet experienced no significant change in their glycaemic response to mixed caloric intake, whereas those who switched to the HGI CHOs showed a 190 percent increase in their response (Clapp, 1998). This result indicates that the type of CHOs plays an important role in the postprandial blood glucose profile response to exercise in healthy mothers. This is different to the current study, which showed that there is no difference in response to LGI or HGI diets in dams.

In crossover research, Lapp and Beth (2007) compared the effects of LGI and HGI diets on insulin sensitivity in seven adult women. In a three-week period, the subjects performed an exercise for 20 minutes, three times a week, and consumed LGI and HGI diets four times a day. Insulin sensitivity was 20 percent higher in the subjects after the consumption of the LGI diet, and glucose concentration in blood and insulinemia were lower (45 per cent) in the subjects after consuming the LGI diet (Clapp & Beth, 2007).

The differences between these findings and the findings in our study are possibly due to the differences in the metabolism of rats and humans or the differences in

the increase in whole-body glucose disposal in humans and how this is affected by CHO intake.

Offspring outcomes

Birth weight

In this study, there was no significant difference observed in offspring birth weight. Supporting these findings, in a randomised control trial, Rhodes and others (2010) investigated the effects of an LGI diet compared to a low-fat diet in 46 overweight pregnant women. Their study showed that there was no effect of a LGI diet on birth weight offspring of both groups measured by birth-weight z scores (Rhodes *et al.*, 2010). However, our finding was different from human clinical studies where LGI diets were associated with a relative decrease in birth weight. Moses and colleagues (2006) investigated the effects of a LGI diet compared to a HGI diet during pregnancy on obstetric outcomes in 70 healthy pregnant women. Their study demonstrated that the birth weight of offspring of women who consumed a HGI diet was higher compared to the offspring of women who had a LGI diet (Moses *et al.*, 2006).

Similarly, the study of Scholl and others (2004) on 1,082 healthy pregnant women also demonstrated that women who consumed a LGI diet had a considerably lower birth weight compared with the HGI diet (Scholl *et al.*, 2004). A randomised control trial of 800 non-pregnant women by Walsh and colleagues (2012), in which women were assigned to receive a LGI diet or a standard diet during pregnancy, reported that birth weight was lower in LGI groups compared to HGI groups (Walsh *et al.*, 2012).

The fact that we did not see a difference in birth weight in our study may be because rats are very small at birth, which would make subtle changes in birth

weight difficult to detect. The differences in birth weight between these studies may also be due to the different metabolisms and hormones in rats and humans.

Body weight and fat mass at weaning

Our study shows that there is no significant difference in body weight fat mass at weaning between the offspring of LGI and HGI mothers. This is in contrast with the results of a study by Moses and colleagues (2006) on human pregnant women, which showed that there was a significantly higher prevalence of LGA in the offspring of women who consumed a HGI diet compared to a LGI diet during pregnancy. Further, the fat mass, which is estimated by the ponderal index of the offspring at birth, was significantly lower compared to HGI offspring (Moses *et al.*, 2006).

Offspring glucose tolerance at three weeks of age

To the best of our knowledge, this is the first study to show that a LGI diet significantly improved glucose tolerance in female offspring at three weeks of age. To date, the mechanisms that underlie this improvement in the present study are unknown. However, the improvements in glucose tolerance in LGI female offspring in our study may be due to the changes in insulin sensitivity in peripheral tissues. Future studies are needed to discover the mechanisms that underlie the improvement in glucose tolerance at three weeks of age.

Effect of an LGI diet on genes underlying the liver function and insulin-signalling pathway in the liver

In the present study, the expression of G3PDH was significantly higher in HGI males three weeks after birth. G3PDH is a lipogenic gene that contributes to lipogenesis (Muhlhausler *et al.*, 2010) and promotes triglyceride synthesis (Al-

Hasani & Joost, 2005). The current study shows that there were no significant effects of LGI and HGI diets on the gene expression of the other genes involved in lipogenesis and insulin signalling in the liver, including ACC β, SREBP-1a, PPAR α, FAS and the insulin-signalling pathway (PI3 Kinase p85 α, PKCzeta) in the liver of offspring at three weeks of age. This increase in the expression of G3PDH in the liver at weaning in HGI offspring compared to LGI offspring could contribute to reduced-fat accumulation in LGI offspring compared to HGI offspring, and it could result in unwanted consequences for HGI offspring later in life, such an increased risk of NAFLD.

Summary

It is well known that the increasing incidence of obesity is associated with a number of obesity-related health complications, including T2DM and NAFLD. It is known that the maternal diet during pregnancy is an important determinant of health outcomes in the offspring. However, to date, there is a lack of understanding as to whether maternal LGI diets during pregnancy for insulin sensitivity, body fat mass and liver function in the offspring throughout the life course. The present study is the first to examine and compare the effects of maternal LGI and HGI diets on the metabolic outcomes of the offspring at three weeks of age.

The present study has shown that in females at three weeks of age, glucose tolerance significantly improved in the LGI group compared to the HGI group. No significant difference was noticed in maternal body weight before or at the end of pregnancy. Importantly, dams' body weight was significantly higher in the LGI group compared to the HGI group at the end of pregnancy. No difference was observed in weight at three weeks of age between LGI and HGI groups for either

males or females. In terms of the gene expression, the present study found no difference, not only in genes involved in the build up of fat and fat oxidation (FAS, SREBP and PPAR α) in the liver, but also in genes underlying the insulin-signalling pathway in the liver, which includes PI3K p85 α and PKCzeta.

However, the present study shows that G3PDH plays an important role in the lipogenesis (Muhlhausler *et al.*, 2010) and triglyceride synthesis (Al-Hasani & Joost, 2005), was expressed at higher levels in HGI males compared to the LGI group. This high expression of G3PDH may result in negative consequences later in life, such as NAFLD. The similarities in the offspring gene expression for both fat oxidation and the insulin-signalling pathway in the liver might be due to the age of the offspring. That is, when the offspring become older, the gene expression might change. To understand the mechanisms that underlie neonatal metabolic outcomes more clearly, further research is required to examine offspring later in life to see if phenotype emerges. In addition, future studies are needed to examine the liver fat mass and to confirm the results of these experiments on additional experimental animals.

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