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

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10

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

12

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23

24 **Abstract**

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

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

42

43

44 **Introduction**

45 The glycaemic index (GI) ranks food according to how they impact on blood glucose
46 concentration immediately after consumption, with high GI foods causing a sharp increase in
47 plasma glucose and low GI foods providing a more sustained glucose release[1]. Epidemiological
48 and clinical studies have reported that prolonged consumption of a high GI diet is associated
49 with insulin resistance and type 2 diabetes [2,3], while low GI diets improve insulin sensitivity
50 and reduce body weight [4,5]. Experimental animal studies have also demonstrated that rats fed
51 on low GI diets have a reduced body fat mass, improved glucose tolerance and reduced
52 expression of lipogenic genes in the liver compared with those maintained on high GI diets [6-
53 8].

54 Epidemiological and experimental animal studies have demonstrated that exposure to an
55 elevated glucose supply *in utero,* as a consequence of gestational diabetes or even mild
56 impairments to maternal glucose tolerance, significantly increases the risk of the offspring
57 developing obesity, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) in adult life
58 [14-16]. This has led to suggestions that interventions that reduce maternal glucose concentrations
59 and/or improve maternal glucose tolerance, including a low GI diet, may have beneficial effects
60 on the long term metabolic outcomes of the offspring. A small number of human studies have
61 investigated the effects of low GI diets during pregnancy and lactation on infant outcomes[9, 10].
62 However, while some studies have supported the potential benefits of a low GI diet in
63 pregnancy for maternal and pregnancy outcomes, including a reduced risk of delivering a large
64 for gestational age infant, no studies to date have evaluated the impact of this diet on the
65 metabolic health of the offspring beyond the immediate postnatal period [13]. In addition,
66 whether the long-term metabolic effects of exposure to a low GI diet during the fetal and
67 suckling periods are dependent on the GI of the diet consumed after weaning is also unknown.

68 Therefore, the aims of the present study were to use a rodent model to 1) compare the effects
69 of maternal consumption of a low GI vs high GI diet during pregnancy and lactation on fat
70 deposition, glucose tolerance, hepatic fat content and gene expression in the offspring at
71 weaning and in young adulthood, and 2) determine whether the effects of maternal low GI diet
72 consumption on young adult offspring differed according to whether the offspring were weaned
73 onto a low GI or high GI diet.

74

75 **Methods**

76 *Dams and feeding regime*

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

83 Following acclimatisation, rats were assigned to either the low GI (n=14) or high GI (n=14)
84 group. The diets each group received were identical in appearance, energy content, macro- and
85 micronutrient composition, the only difference being the carbohydrate type; in the low GI
86 group the diet included carbohydrate in the form of Gel Crisp starch (Diet SF10-084) while in
87 the high GI group the diet included carbohydrate in the form of dextrinised starch (Diet SF10-
88 081). Both diets were manufactured by Specialty Feeds (Glen Forrest, Western Australia). A
89 validated *in vitro* starch digestion assay was used as an indicator of the likely glycaemic
90 response to each of the diets [19]. At the 20 min time point, the amount of rapidly available
91 glucose (RAG) in the high GI feed was 56% higher than the low GI feed (P= 0.006). Similarly,

92 at 120 min of digestion, the amount of glucose released was 44% higher in the high GI feed
93 (*P*=0.0006). In addition, an *in vivo* pilot study was undertaken in which we measured blood
94 glucose concentrations in rats for two hours after the consumption of either the high or low GI
95 diet. The results obtained confirmed that the diets resulted in post-prandial glucose curves
96 which were different and consistent with the profile expected for low and high GI foods (data
97 not shown).
98
99 The diets were provided *ad libitum* and all rats had free access to water throughout the
100 experiment. Female rats were fed their respective diets for a minimum of 4 weeks before mating
101 and throughout pregnancy and lactation. Body weight was determined weekly during this time.
102 Fresh food was provided every second day, and on each of these occasions, the remaining food
103 was weighed and the weight subtracted from the amount provided at the start of the 2 day
104 period to calculate food intake.

105 After 4 weeks, vaginal smears were performed daily to determine the stages of the estrous
106 cycle. On the night of diestrous/proestrous the female rat was placed with a male (fed *ad libitum*
107 on standard rodent chow) overnight. The presence of sperm in vaginal smears conducted the
108 following morning was considered as confirmation of successful mating and designated as
109 gestation day 0. A total of 4 males were used for mating and the same males were used for
110 mating females in both the low GI and high GI groups in order to minimise the influence of
111 paternal effects on offspring outcomes.
112
113 *Offspring*

114 Pups were born on day 21-22 of gestation. Within 24 hours of birth (postnatal day 1), pups
115 were culled to 8 per litter, with 4 males and 4 females where possible. Pups were weighed on
116 postnatal day 1 and every 2 days thereafter during the suckling period and were weaned on

117 postnatal day 21. At the time of weaning, tissue was collected from 1 male and 1 female pup
118 from each litter, remaining were group-housed with their same sex littermates (2 animals per
119 cage), and were provided with either the same diet as their mother or the alternate diet. This
120 gave rise to 4 groups (1) offspring of low GI dams weaned onto the same low GI diet (L-L,
121 n=14, 7 males and 7 females), (2) offspring of low GI dams weaned onto a high GI diet (L-H,
122 n=14, 7 males and 7 females), (3) offspring of high GI dams weaned onto a low GI diet (H-L,
123 n=14, 7 males and 7 females) and (4) offspring of high GI dams weaned onto a high GI diet
124 (H-H, n=14, 7 males and 7 females). Food intake was determined every 2 days in all offspring
125 and fresh food provided. Fresh water was available *ad libitum*. All offspring were weighed
126 once per week from weaning until 12 weeks of age.
127
128 *Intraperitoneal Glucose Tolerance Test (IPGTT)*
129 IPGTTs were performed after an overnight fast on dams at the end of lactation as well as on
130 the offspring at 3wks and 12wks of age. Baseline blood samples were collected from the tail
131 vein and a glucose bolus (2g/kg of 50% dextrose in sterile 0.9% saline) was then injected
132 intraperitoneally. Blood samples were collected from the tail vein at 5, 10, 15, 30, 60 and 120
133 minutes following glucose delivery. Glucose concentrations were determined using a handheld
134 Accu-Chek Performa glucometer (Accu-Chek Performa©, Roche, Germany) at each time-
135 point.
136
137 *Post mortem and tissue collection*
138 Post mortem and tissue collection was conducted on dams after weaning on the day following
139 the IPGTT and on 1 male and 1 female offspring per litter (selected at random) at weaning and
140 1 male and 1 female offspring per litter at 12 weeks of age. Dams and offspring were killed
141 using an overdose of CO_2 in the non-fasted state. Immediately after euthanasia, blood samples

142 were collected via cardiac puncture into heparinised tubes and centrifuged at 3,500 g at 4ºC for
143 15 minutes. The plasma was collected and stored at -20ºC for subsequent analyses of hormone
144 and metabolite concentrations. Body weight, length (nose to tail) and abdominal circumference
145 were determined. All internal organs were weighed and all visible fat depots, including omental
146 fat, retroperitoneal fat, gonadal fat, subcutaneous fat and interscapular fat, were dissected to
147 determine the weights of the individual depots. The weights of omental, retroperitoneal and
148 gonadal fat were added together to determine visceral fat mass, and the weights of all individual
149 fat depots were added together to determine total body fat mass. The weights of all fat depots
150 were expressed relative to body weight. At both weaning and 12 weeks of age, a sample of
151 liver (from the same site in each animal) was snap frozen in liquid nitrogen and stored at -80ºC
152 for subsequent analysis of lipid content and gene expression.

153 *Hepatic lipid content, RNA extraction and gene expression analysis*

154 Total hepatic lipid content was determined gravimetrically following homogenisation and
155 extraction of 200mg of frozen tissue in chloroform-methanol (2:1, v/v) as previously described
156 [20, 21]. Total mRNA was extracted from the liver using Trizol reagent (Invitrogen Australia,
157 Mount Waverley, Vic, Australia), purified using an RNeasy Mini kit (Qiagen Australia,
158 Doncaster, Vic, Australia) and cDNA synthesized using Superscript III reverse transcriptase
159 (Invitrogen Australia) and random hexamers.

160

161 Quantitative Real Time PCR was performed using the SYBR green system on the Applied
162 Biosystems ViiA 7 Real Time PCR machine (Applied Biosystems, Foster City, CA, USA).
163 The target genes included key genes involved in hepatic lipid metabolism and insulin
164 signalling: acetyl-CoA carboxylase (ACC), peroxisome proliferator activated receptor-α
165 (PPARα), sterol regulatory element binding protein-1α (SREBP1α), fatty acid synthase (FAS),
166 the phosphatidylinositol 3-kinase regulatory p85 subunit (PI3K-p85) and phosphokinase C-ζ

167 (PKCζ), all of which have been implicated in non-alcoholic fatty liver disease (NAFLD) [22, 23].
168 The primers were designed using the Primer3 and NCBI websites, with all primers crossing
169 exon-exon boundaries to prevent annealing to genomic DNA. All primers were validated for
170 use in our laboratory by running the PCR product on a gel to confirm amplicon size as well as
171 sequencing to ensure the correct gene was amplified. Primer sequences are shown in **Table 1**.
172 The expression of target genes was quantified relative to the housekeeper genes β-actin and
173 HPRT, using the Applied Biosystems Data Assist software (Applied Biosystems, Foster City,
174 CA, USA). Two quality controls as well as a negative RT control were used on each 96-well
175 plate to ensure inter-plate consistency and melt curves were obtained at the end of each run to
176 confirm amplicon heterogeneity.
177
178 *Plasma hormone and metabolite assays*
179 Plasma glucose, alanine amino transferase (ALT), uric acid, total cholesterol, HDL cholesterol
180 (Thermo Electron, Pittsburgh, PA), and NEFA (WAKO Pure Chemical Industries Ltd., Osaka,
181 Japan) were determined using a Konelab 20X (Thermoscientific, Vantaa, Finland). Plasma
182 leptin and insulin concentrations were measured using commercially available immunoassay
183 kits (Crystal Chem Inc, Downers Grove, IL, USA and ALPCO Diagnostics, Salem, NH, USA).
184 All assays were conducted in accordance with the manufacturer's instructions and intra- and
185 inter-assay coefficients of variation were always <10%.
186
187 *Statistical analyses*
188 Data are presented as mean ± SEM. The dam (litter) was used as the unit of analysis in all
189 statistical tests. A power analysis was conducted to determine sample size using changes in fat
190 mass as the primary outcome. The effect of the low or high GI diet in the dams and pre-weaning
191 offspring was determined using a Student's unpaired t-test. The area under the curve (AUC)

192 for glucose following the IPGTT was calculated for each animal using the incremental AUC
193 method. The relative effects of exposure to maternal low GI diet or high GI diet and exposure
194 to the diets after weaning were analysed using a 2-way ANOVA. When a significant interaction
195 between maternal diet and post-weaning diet was identified, all groups were analysed together
196 using a one-way ANOVA and Tukey's post hoc analysis. Differences in the effects of the low
197 GI and high GI diets over time were analysed using a repeated measures ANOVA. Male and
198 female offspring were analysed separately for all measures. Repeated measures ANOVAs were
199 performed using Stata 11 (StataCorp LP, Texas, USA). All other analyses were performed
200 using SPSS for Windows Version 19.0 (SPSS Inc., Chicago, IL, USA). A probability of $P<0.05$
201 was considered statistically significant.

202

203 RESULTS

204 Maternal outcomes

205 *Food intake and body weight*

206 Body weights were not different between dams assigned to the low GI and high GI diets at the
207 commencement of the experimental diets (high GI, 244.1 ± 5.4g; low GI, 257.9 ± 6.4g,
208 $P=0.11$). However, at the time of mating (i.e. ~4 weeks after commencement of the
209 experimental diets), dams in the low GI group were heavier than those in the high GI group
210 (**Fig 1A**, $P<0.05$). There was no difference, however, in the average body weight during
211 pregnancy or at the end of lactation (**Fig 1A**), and low GI dams gained less weight during
212 pregnancy than the high GI dams (high GI, 116.3 ± 5.1g; low GI, 90.9 ± 8.6g, $P<0.05$). There
213 was also no difference in maternal food intake between groups either before mating, during
214 pregnancy or during lactation (**Fig 1B).**

215

216 *Fat mass*

217 At the end of lactation, low GI dams had a higher abdominal circumference (high GI, 218 17.8±0.3cm; low GI 19.3±0.4cm, *P*<0.05) and higher gastrointestinal tract mass relative to 219 body weight compared to high GI dams (high GI 8.7±0.6%; low GI 11.6±0.7%, *P*<0.01). There 220 were no differences in the total percentage body fat or the weight of any of the individual fat 221 depots between the low GI and high GI groups (supplementary material-table 1). However, GI 222 dams had a lower amount of visceral fat as a proportion of their total fat mass compared to the 223 high GI dams (**Fig 2A**, *P*<0.05).

224

225 *Glucose tolerance and plasma measures*

226 There was no difference in fasting glucose levels between low GI and high GI dams before the 227 administration of the glucose bolus (high GI, 5.9 ± 0.3mmol/l; low GI, 5.9 ± 0.2mmol/l, 228 *P*=0.19). The low GI dams also had a lower peak glucose following intraperitoneal glucose 229 administration **(Fig 2B,** *P*<0.05) and a lower glucose AUC during the IPGTT compared to the 230 high GI group (**Fig 2C,** *P*<0.05). There were no differences in the plasma concentrations of 231 insulin, glucose, NEFA or leptin between low GI and high GI dams at the time of post-mortem 232 (supplementary material-table 1)**.**

233

234 Offspring outcomes birth to weaning

235 *Growth from birth to weaning*

236 There was no difference in birth weight between the low GI and high GI groups in either 237 females or males (females: high GI, 6.1 ± 0.1g; low GI, 6.1 ± 0.2g, *P*=0.93; males: high GI, 238 6.2 ± 0.3g; low GI, 6.5± 0.3g, *P*=0.43). Weight gain during the suckling period between groups 239 was also comparable (male F=1.74, *P*=0.26 and female F=1.09, *P*=0.31) and there was no

240 difference in body weight at weaning (3 weeks of age) (females: high GI, 42.6 ± 1.8 g; low GI,
241 43.3 ± 1.5g, *P*=0.99; males: high GI, 44.4 ± 1.9g; low GI, 45.3 ± 1.5g, *P*=0.70).

242

243 *Fat mass at 3 weeks of age*

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

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

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

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

259

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

261 Relative liver weights were not different between the low GI and high GI groups in either male
262 (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

263 3.91±0.09%, *P*=0.54) offspring. However, male offspring of low GI dams had a lower hepatic
264 fat content as a percentage of liver weight compared to high GI males (high GI 6.35± 0.50%;
265 low GI 4.07±0.40%, *P*<0.05). There was no difference in liver fat percentage in females (high
266 GI 6.13± 0.84%; low GI 6.67±1.06%, *P*=0.29).
267
268 Plasma concentrations of uric acid and alanine transaminase (ALT), both established
269 biomarkers of liver function, were not different between low and high GI groups in either males
270 or females. Hepatic expression of key genes involved in lipogenesis and insulin signalling
271 (ACCβ, PPARα, SREBP1α, FAS, PI3K-p85 and PKCζ) was also not different between the low
272 GI and high GI groups in either males or females (supplementary material- table 4).
273
274 **Offspring outcomes – post weaning**
275 *Food intake and growth*
276 There was no difference in food intake during the post-weaning period between offspring of
277 low GI and high GI dams (data not shown). In female offspring, the rate of weight gain from
278 weaning to 12 weeks of age was higher in offspring of low GI dams, independent of the post
279 weaning diet (F=5.14, *P*<0.01), and these offspring were heavier between 6 and 10 weeks of
280 age, although not at 12 weeks of age, compared to offspring of high GI dams **(Fig 4A)**. There
281 were no differences between groups in body weight in male offspring at any time after weaning
282 **(Fig 4B)**.
283
284 *Fat mass at 12 weeks of age*
285 In females, relative interscapular fat mass was significantly lower in offspring of low GI dams,
286 independent of their post-weaning diet (*P*<0.05**, Table 3),** however the relative mass of other
287 individual fat depots and the relative visceral and total fat mass were not different. In males,

288 there was no difference in total fat mass or the relative weight of any of the individual fat depots
289 between groups **(Table 3)**.
290
291 *Glucose tolerance and plasma hormone concentrations at 12 weeks of age*

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

300 *Hepatic lipid content and gene expression at 12 weeks of age*

301 There was no difference between groups in relative liver weight or liver fat content in either
302 male or female offspring at 12 weeks of age **(Table 4)**. In females, offspring of low GI dams
303 had increased plasma ALT concentrations in comparison with offspring of high GI dams,
304 independent of their post-weaning diet (low GI 130.75±53.15 IU/L vs high GI 15.75±5.02
305 IU/L, $P<0.05$).
306

307 In females, hepatic PI3K-p85 mRNA expression at 12 weeks of age was lower in offspring of
308 low GI mothers, independent of the post-weaning diet ($P<0.05$, **Table 4**). SREBP1α mRNA
309 expression at 12 weeks of age was higher in offspring of high GI dams who were weaned onto
310 the low GI diet compared to all other groups ($P<0.05$, **Table 4**). There was no effect of either

311 the maternal or post-weaning diet on hepatic mRNA expression of PI3K-p85 or SREBP1α in
312 males on in ACC, PPARα, PKCζ or FAS in either male or female offspring (**Table 4**).

313

314 DISCUSSION

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

326

327 *Maternal Outcomes*

328 The increase in bodyweight we identified in the dams consuming the low GI diet prior to mating
329 was unexpected given previous reports of low GI diets increasing satiety, lowering food
330 consumption and reducing weight gain[24-26]. We consider it likely, however, that our results
331 were biased by the fact that body weights were not recorded in the fasting state, since low GI
332 diets are known to increase the weight of the large bowel and caecum [26]. In line with this the
333 weight of the gastrointestinal tract at post-mortem was ~10 g heavier in the low GI compared
334 to high GI dams, and if this value was subtracted from the pre-pregnancy weights of the low

335 GI dams then the difference between groups was no longer significant. In support of this,
336 despite the increase in pre-mating bodyweight we found no difference in total fat mass between
337 the low GI and high GI dams at the end of lactation. Interestingly, however, the low GI diet
338 appeared to affect fat distribution, since the dams fed the low GI diet had a lower ratio of
339 visceral to total fat mass than their high GI counterparts. This is consistent with previous studies
340 in non-pregnant adults, which reported that low GI diets preferentially enhance the mobilisation
341 of visceral compared to subcutaneous fat[27, 28].
342

343 *Growth and metabolic outcomes in the offspring at weaning*

344 A key finding of the current study was that maternal consumption of a low GI diet reduced
345 visceral adiposity, lowered plasma glucose concentrations and increased glucose tolerance in
346 female, but not male offspring at weaning. While the mechanisms behind this remain unclear,
347 one possibility is that the exposure to lower glucose concentrations as result of higher glucose
348 tolerance during the development of adipose depots 'programmed' a reduced lipogenic
349 capacity in visceral adipocytes. This hypothesis is indirectly supported by a study in sheep
350 which demonstrated that exposure to elevated glucose concentrations *in utero* is associated
351 with a precocial up-regulation of lipogenic genes in the main visceral adipose depot of the fetus
352 [29]. However, further studies will be required to test this directly. While male offspring of low
353 GI dams did not exhibit any differences in body fat mass or glucose tolerance, they did have
354 reduced hepatic lipid content. The functional significance of this is not clear, however, since it
355 did not translate into alterations in hepatic gene expression or circulating of biomarkers liver
356 function, and was no longer present at 12 weeks of age, even when offspring were maintained
357 on the low GI diet after weaning
358

359 *Growth and metabolic outcomes in the offspring in young adulthood*

360 Interestingly, and contrary to expectations, female offspring of low GI dams exhibited a phase
361 of accelerated weight gain after weaning, independent of their post-weaning diet. This was
362 particularly unexpected given the reduced gestational weight gain in low GI dams, which is
363 generally associated with improved metabolic outcomes in the offspring[30, 31]. The period of
364 increased body in the current study coincided with the timing of puberty - a period associated
365 with a marked increase in secretion of gonadotrophin releasing-hormones and estrogen and
366 increased body fat accrual [32]. One possibility, therefore, is that this period of accelerated growth
367 may be the result of an interaction between the effects of sex hormones and programmed
368 changes in other insulin-responsive tissues, such as the skeletal muscle, induced by exposure
369 to a maternal low GI diet. Whilst it is possible that the higher body weight of the low GI dams
370 at mating may have contributed to this increased body weight, this appears unlikely given that
371 there were no differences in birth weight between the low and high GI pups, and that maternal
372 weight for the majority of pregnancy was not different between groups.
373
374 The higher SREBP1α mRNA expression in female offspring of high GI dams provided with a
375 low GI diet after weaning may be indicative of an increased propensity for excess hepatic lipid
376 storage, since SREBP1α activation is associated with the up-regulation of hepatic lipogenesis[33-
377 35]. The fact that SREBP1α expression was increased in offspring of high GI dams that were
378 switched to a low GI diet after weaning, but not in those who continued to consume the high
379 GI diet, suggests that this may have been driven by a 'mis-match' between the nutritional
380 environment experienced pre- and post- weaning. The concept of a mis-match between the
381 environment experienced in postnatal life compared to the environment 'predicted' by the
382 perinatal nutritional experience being associated with an increased risk of disease in postnatal
383 life, including metabolic disease, is well described, however this is the first time it has been
384 described in the context of switching from a high to low GI diet [36].

385

386 We also observed a reduced expression of PI3K-p85 mRNA in female offspring of low GI
387 dams at 12 weeks of age, independent of the post-weaning diet. PI3K plays a key role in the
388 response of the liver to insulin, as part of the PI3K/Akt axis, and activation of this kinase
389 suppresses gluconeogenesis and promotes glycogen/lipid synthesis and cell growth [37, 38, 39].
390 Consequently, the lower PI3K mRNA expression would be expected to reduce insulin-
391 stimulated hepatic lipogenesis, and therefore has the potential to inhibit hepatic fat storage in
392 response excess energy intake. In light of this finding, further studies focussed on the effect of
393 maternal low GI diets on the expression, protein abundance and activity of key components of
394 the insulin signalling pathway, and on the impact of obesogenic diets on hepatic lipid storage
395 are warranted, and will provide clearer insights into the potential longer-term benefits of
396 maternal low GI diets on hepatic function in the offspring [40]. Furthermore, the reduction in
397 PI3K is also difficult to reconcile with the elevated plasma ALT concentrations which were
398 also present in female offspring of low GI dams in young adulthood, since this is generally
399 considered to be a marker of poorer hepatic function. However, studies relating ALT levels to
400 hepatic function are generally restricted to adult humans, and the reliability as an indicator of
401 hepatic function in the perinatal period and/or in rodents is not clear.

402

403 *Perspectives and Significance*

404 The present study is the first to directly compare the effect of a maternal high vs low GI diet
405 on offspring metabolic outcomes beyond the immediate postnatal period. We demonstrated
406 that consumption of a low GI diet during pregnancy and lactation led to increased glucose
407 tolerance in the dam as well as reduced visceral adiposity and increased glucose tolerance in
408 the female offspring at weaning. The long term impact of the GI of the maternal diet on the
409 offspring was less clear; however the results did indicate a potential benefit of maternal low GI

410 diet consumption for reducing hepatic lipid synthetic capacity in female offspring, by reducing
411 the expression of PI3K in early adulthood. The increase in SREBP1α in the female offspring
412 of high-GI dams switched to a low GI diets, however suggests the existence of a complex
413 relationship between nutritional exposures pre- and post-weaning, which will need to be further
414 explored in future studies. Nevertheless, the results of the present study provide an important
415 foundation for future studies aimed at determining whether the changes in glucose tolerance,
416 fat deposition and hepatic gene expression associated with maternal low GI diet consumption
417 can translate into an improved capacity of the offspring of low GI dams to resist metabolic
418 challenges later in life.
419
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432 **Conflicts of Interest**

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

435 (www.glycemicindex.com) and is the co-author of lay books about the glycemic index of

436 foods. The other authors have no conflicts to declare.

437 **Ethical Standards**

438 The authors assert that all procedures contributing to this work comply with the ethical

439 standards of the relevant national guides on the care and use of Albino Wistar Rats and has

440 been approved by The University of Adelaide Animal Ethics Committee.

441 **Figure legends**

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

450

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

458

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

465 solid line, n=14) and high GI (open triangles, dashed line, n=12) dams at the end of lactation.
466 (D) No difference in glucose tolerance as indicated by AUC was observed between low GI and
467 high GI male offspring. Data presented as mean±SEM, statistical analysis done using a two-
468 way ANOVA within each sex.
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470
471 **Figure 4** Weight gain from weaning to 12 weeks of age in (A) female and (B) male offspring
472 of low GI (filled squares, solid line) and high GI (open triangles, dashed line) dams. * $P<0.05$
473 compared to the high GI group, n=14 for all groups. Data presented as mean±SEM, statistical
474 analysis done using a repeated measures ANOVA within each sex.
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477 **Table 1: Primer Sequences for Determination of Hepatic Gene Expression**

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Gene Accession number
PI3 Kinase p85	ACCAGTGTTGACCCTTCCTG	TGCTGGAGCTCTGTGTTCTG	NM_013005.1
ACCβ	CCATGCTTTTTCAGACAGGTGC	GGACACTGCGTTCCCATACT	NM_053922.1
SREBP-1α	GCGCCATGGAGGAGCTGCCCTT	GTCACTGTCTTGGTTGTTGATG	NM_ 001276707
PPARα	CCTGTGAACACGATCTGAAAG	ACAAAGGCGGATTGTTG	NM_031347.1
PKCζ	AAGTGGGTGGACAGTGAAGG	GGGAAAACGTGGATGATGAG	NM_022507.1
FAS	TGCTCCCAGCTGCAGGC	GCCCGGTAGCTCTGGGTGTA	NM_017332
HPRT	CTCATGGACTGATTATGGACAG	GCAGGTCAGCAAAGAACTTATA	NM_012583.2
β-actin	GCACCACACCTTCTACAATG	TGCTTGCTGATCCACATCTG	NM_017101.1

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479 **Table 2** Fat mass as % bodyweight in male and female offspring of high and low GI dams at 3 weeks of age

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

480 Data presented as mean±SEM, * indicates significantly different mean between groups within
481 each sex, P<0.05.

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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.

	Male				***Female***			
Parameter	**L-L**	**L-H**	**H-L**	**H-H**	**L-L**	**L-H**	**H-L**	**H-H**
Gonadal fat	1.13±0.12	1.17±0.15	1.34±0.17	1.18±0.16	1.95±0.19	2.17±0.21	2.42±0.35	2.40±0.23
Retroperitoneal fat	1.45±0.11	1.70±0.15	1.49±0.22	1.57±0.17	1.40±0.07	1.57±0.13	1.51±0.24	1.61±0.19
Omental fat	0.86±0.13	1.15±0.09	1.08±0.14	1.19±0.13	1.25±0.26	1.48±0.09	1.48±0.17	1.44±0.14
Visceral fat	3.44±0.28	3.97±0.34	3.92±0.51	3.95±0.37	4.60±0.49	5.11±0.36	5.42±0.75	5.44±0.33
Subcutaneous fat	3.65±0.19	4.15±0.23	3.78±0.58	3.91±0.22	3.35±0.63	3.98±0.41	4.22±0.40	4.03±0.87
Interscapular fat	0.21±0.01	0.26±0.02	0.26±0.03	0.23±0.03	0.25±0.02[a]	0.26±0.01[a]	0.30±0.03[b]	0.30±0.02[b]
Total fat	7.30±0.49	8.35±0.51	7.95±1.08	8.12±0.57	8.20±0.49	9.36±0.72	9.91±1.13	9.78±0.53

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.

	Male				***Female***			
Parameter	**L-L**	**L-H**	**H-L**	**H-H**	**L-L**	**L-H**	**H-L**	**H-H**
Relative liver weight (%)	4.32±0.08	4.16±0.11	4.13±0.09	4.22±0.08	4.18±0.09	3.98±0.11	4.00±0.10	3.93±0.11
% liver lipids	5.04±0.48	7.37±1.31	5.75±1.28	5.63±1.11	5.24±0.81	7.27±0.81	6.44±1.61	3.65±0.63
Hepatic Genes								
ACCβ	45.40±6.45	44.20±7.91	47.70±4.57	39.60±4.40	55.02±5.52	57.09±8.74	63.58±7.27	66.09±6.90
PPARα	10.40±1.93	16.70±3.28	17.40±3.04	15.70±1.32	11.47±2.44	14.24±2.97	15.45±2.27	16.67±0.08
SREBP1α	33.30±6.02	38.00±6.03	32.10±4.20	31.60±2.71	30.13±3.67[a]	26.13±3.31[a]	45.14±4.83[b]	30.05±3.64[a]
PI3K	27.45±4.10	37.19±10.35	55.10±9.99	38.35±9.08	33.29±4.16[a]	42.02±3.54[a]	50.09±4.80[b]	58.74±8.67[b]
G3PDH	381.94±54.09	301.41±70.21	381.30±53.71	357.65±44.14	334.68±40.07	296.78±36.27	358.22±24.41	389.77±44.82
FAS	287.93±38.55	521.25±19.59	358.66±96.05	426.68±99.98	508.01±88.17	407.36±125.01	378.02±69.38	396.31±61.04
PKCζ	0.67±0.13	0.45±0.11	0.51±0.04	0.43±0.04	0.53±0.07	0.94±0.14	0.84±0.09	0.82±0.04

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, $P<0.05$

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