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1   **Impact of Maternal Obesity on Offspring Adipose Tissue: Lessons for the Clinic**

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Short title: Maternal Obesity and Offspring Fat

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6 **Abstract/Summary**

7 Maternal obesity is a major risk factor for the subsequent development of obesity and type 2

8 diabetes in the child. This relationship appears to be driven largely by the exposure of the

9 fetus to an increased nutrient supply during critical periods of development, which results in

10 persistent changes in the structure and function of key systems involved in the regulation of

11 energy balance, appetite and fat deposition. One of the key targets is the fat cell, or adipocyte,

12 in which prenatal overnutrition programs a heightened capacity for fat storage. The

13 increasing prevalence of maternal obesity has led to an urgent need for strategies to break the

14 resulting intergenerational cycle of obesity and metabolic disease. This review will discuss

15 the relationship between maternal obesity and poor metabolic health of the offspring, with a

16 particular focus on the involvement of adipose tissue, recent clinical studies examining

17 potential strategies for intervention and priority areas for further research.

18

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22

Introduction

The world-wide increase in the prevalence of overweight and obesity has led to a corresponding increase in the number of women who are classified as overweight or obese when they enter pregnancy. Recent statistics from Australia and the US suggests that over 50% of women have a body mass index (BMI) >25kg/m^2 at the time of their first antenatal appointment, and there are predictions that this figure is likely to increase [1,2].

Maternal obesity has both short- and long-term adverse consequences for the mother and her infant. Obese women have a significantly increased risk of a number of pregnancy complications, including gestational diabetes (GDM) and pre-eclampsia, and their infants have a higher incidence of neonatal morbidity and mortality [3,4]. Infants born to overweight/obese women are more likely to be born large for gestational age (LGA) or macrososmic (>4000g), require resuscitation at birth and to suffer from jaundice and neonatal hypoglycaemia [4,5].

Most recently, attention has turned to the impact of maternal obesity on the longer term outcomes of the infants. Clinical studies from across the developed and developing world have provided compelling evidence that infants of overweight and obese mothers, in addition to being heavier at birth, have a significantly increased risk of obesity and associated metabolic disorders later in life [4,6]. This association between maternal obesity and the risk of obesity in the child has created an intergenerational cycle of obesity and metabolic disease, which threatens to continue to impact on the metabolic health of future generations.

This review will focus on current understanding of the mechanisms which underlie the increased risk of obesity in infants of overweight/obese mothers, with a specific focus on the effects on adipose tissue development. It will summarise the current state of the field in this

46 area of research, and provide insights into the key challenges and opportunities over the

47 coming 5 year period.

48

49 **Maternal Obesity: A New Obstetric Challenge**

50 The number of women entering pregnancy overweight or obese has risen dramatically in

51 recent years, in line with the rising prevalence of obesity in the general population. Data from

52 the US in the early to mid-2000s indicated that just over 30% of pregnant women were

53 classified as overweight or obese [2], however more recent studies suggest that this figure is

54 now likely to be closer to 50% [3]. This substantial rise in the number of overweight and

55 obese pregnant women represents a major challenge to clinicians, since this sector of

56 pregnant population is well-known to be at increased risk of a host of pregnancy

57 complications [3]. Furthermore, this risk increases with increasing maternal BMI and the

58 presence of other co-morbidities, including pre-pregnancy diabetes or hypertension. Women

59 who enter pregnancy overweight or obese are at higher risk of developing gestational

60 diabetes (GDM), pregnancy hypertension and pre-eclampsia. There is also a much greater

61 risk of infants being large for gestational age or macrosomic (a birth weight >4000g), which

62 creates difficulties during the delivery process, in particular shoulder dystosia, and the

63 majority of these infants are delivered by caesarean section [4,7]. Pregnancies complicated by

64 maternal obesity are also more likely to end in still birth or significant neonatal distress. The

65 infants born to overweight and obese women are also at higher risk of neonatal complications

66 than infants born to lean women, in particular post-natal hypoglycaemia, jaundice and

67 admission to neonatal intensive care [3].

68

69 While it is not unexpected that heavier mothers give birth to heavy infants, there is now

70 compelling evidence that the adverse effects of maternal obesity extend beyond the

71 immediate postnatal period, and predispose the infant to an increased risk of obesity and its

72 associated metabolic complications throughout child and adult life.

73 **Maternal Obesity and Nutrient Supply to the Developing Fetus**

74 The short and long term consequences of maternal overweight/obesity on the developing

75 infant are thought to result from the exposure of the fetus to an excess nutritional supply

76 during critical periods in its development [8-10]. This increased nutrient supply appears to be

77 due to the combined effects of the tendency of overweight/obese women to consume diets

78 which are poorer in quality than lean women [11] and the fact that these women also tend to

79 be more insulin resistant than their lean counterparts [2] **(Figure 1)**. While a reduction in

80 insulin sensitivity in the mother is a normal adaptation to pregnancy, designed to maximise

81 transfer of nutrients from mother to fetus rather than them being utilised by maternal tissues,

82 a large proportion of women in the overweight/obese population already have compromised

83 insulin sensitivity (or indeed are already borderline diabetic) when they fall pregnant. In these

84 cases, the normal adaptation to pregnancy can often progress to GDM, which is characterised

85 by maternal hyperglycemia during both post-prandial and fasting periods.

86

87 In addition to hyperglycemia in the mother, GDM is associated with elevated glucose

88 concentrations in the developing fetus. This fetal hyperglycemia stimulates the fetal pancreas

89 to secrete more insulin, resulting in fetal hyperinsulinemia which stimulates anabolic

90 processes in the fetus and results in increased fetal growth and fat deposition [12]. This 'fuel

91 mediated teratogenesis' was first described by Freinkel in the 1980s, is associated with the

92 increased birth weight and risk of fetal macrosomia which are characteristic of infants of

93 diabetic mothers. In addition to being heavier and fatter at birth, there is also compelling

94 evidence from both clinical and epidemiological studies that infants born to diabetic mothers

95   are at higher risk of obesity and its associated metabolic disorders throughout childhood and

96   adulthood [13,14] **(Figure 1)**.

97   **The Underlying Mechanisms: The Role of Adipose Tissue**

98   There have been a large number of studies in both small and large experimental animals

99   which have begun to shed light on the biological mechanisms which underlie the increased

100   risk of obesity in infants born to overweight/obese mothers. In small animal studies, maternal

101   obesity has most commonly been modelled by feeding rat/mouse dams on cafeteria or semi-

102   synthetic high-fat diets prior to pregnancy and during pregnancy and lactation [15,16]. We

103   and others have consistently demonstrated that offspring born to dams fed on these types of

104   diets have a significantly elevated body fat mass at weaning and remain heavier and fatter

105   through the life course than offspring of dams fed on control diets [17-19]. Importantly,

106   maternal high-fat and/or high-sugar diets have been associated with altered gene expression

107   within both the adipose tissue and hypothalamic appetite-regulating networks of the

108   offspring, which result in higher fat deposition and increased appetite drive (hyperphagia) in

109   the offspring into adult life [18-20].

110

111   Studies in large mammals, such as sheep and pigs, have the advantage over studies in smaller

112   animals that the ontogeny of fat development is much more similar to that in the human; i.e.

113   fat cell development begins before birth and extends into early postnatal life. This differs

114   from altical rodent species, in which fat development is virtually absent *in utero* and

115   appreciable fat deposits only start to appear during the suckling period. Some years ago, we

116   developed a model of maternal overnutrition in the sheep in which pregnant ewes were fed

117   ~55% above their maintenance energy requirements (as specified by the Ministry of

118   Agriculture, Fisheries and Food, UK [21]) in the later third of pregnancy, in order to mimic

119   exposure of the fetus to a moderate increase in nutrient supply during the major period of

120   intrauterine fat development [22]. This maternal overnutrition was associated with significant

121   increases in maternal glucose concentrations, and fetal glucose and insulin concentrations

122   were also significantly increased in fetuses of over-fed ewes compared to fetuses of ewes fed

123   at maintenance energy requirements [22].

124

125   Using this model, we studied the effect of fetal hyperglycemia/hyperinsulinemia on fetal fat

126   cells, and fat deposition in the lamb in the early postnatal period. We demonstrated that

127   maternal overnutrition was associated with a significant increase in the expression of the key

128   adipogeneic/lipogenic transcription factor, Peroxisome Proliferator Activated Receptor

129   gamma (PPARγ), in the fetal perirenal adipose depot (the main fat depot in fetal life), in

130   conjunction with an increase in the mRNA expression of key lipogeneic genes, lipoprotein

131   lipase (LPL) and glycerol-3-phospahte dehydrogenase (G3PDH) [23]. Importantly, this

132   upregulation of lipogeneic genes was associated with an increased accumulation of fat in the

133   early postnatal period, and lambs of over-fed ewes had a significantly higher mass of

134   subcutaneous fat relative to body weight by the end of the first month of life [24,25]. These

135   results suggested, therefore, that prenatal hyperglycemia led to a precoical upregulation of

136   lipogenic genes in adipose tissue, which resulted in increased fat deposition after birth. The

137   importance of the increased availability of nutrients in driving these effects is highlighted by

138   the presence of a direct relationship between fetal glucose concentrations and the level of

139   PPARγ mRNA expression in the perirenal adipose tissue in late gestation – consistent with

140   clinical evidence that intrauterine glucose concentrations provide a sensitive marker of fetal

141   adiposity [26]. In other studies, we and others have also reported that infusing glucose

142   directly into the fetal sheep in late gestation is associated with a significant increase in the

143   size of the lipid droplets in fetal fat depots, fetal fat mass and in leptin mRNA expression in

144 fetal fat depots [27,28], providing further evidence of the central role of glucose supply in

145 driving fat deposition *in utero*.

146

147 In addition to effects of maternal overnutrition on the adipose tissue and fat mass, there were

148 also significant effects on the regulation of appetite and feeding behaviour. Thus, lambs of

149 over-fed ewes had a significantly higher milk intake over the first month of postnatal life, and

150 did not appropriately upregulate the expression of the appetite-inhibiting neuropeptide,

151 Cocaine and Amphetamine Regulated Transcript (CART) in response to a positive energy

152 balance [25]. This appeared to be a result of a downregulation of the expression of the leptin

153 receptor in the central appetite regulating centre of these lambs as their fat mass increased,

154 consistent with the development of central resistance to the actions of leptin's appetite-

155 suppressing actions [25]. These findings, summarised in **Figure 2**, led us to hypothesise that

156 the primary event in the pathway linking an increased nutrient supply in utero to increased

157 propensity to obesity in postnatal life was the programming of an increased capacity for

158 lipogenesis in fetal fat depots [9].

159

160 These sheep studies are supported by the finding from studies in pigs, largely conducted by

161 Hausman and colleagues, which examined the effect of maternal diabetes and obesity on the

162 structural and functional development of fetal adipose tissue in late gestation. In these

163 studies, diabetes in the sow was associated with increased adipose tissue mass in fetuses at

164 112 days of gestation, without a change in body weight [29]. Importantly, the activity of key

165 lipogenic genes, in particular lipoprotein lipase (LPL), was significantly upregulated in the fat

166 depots of fetuses of diabetic sows, and the number and size of the lipid droplets within the

167 fetal fat depots were significantly increased [29,30]. These observations supported the

168 concept that fetal adipose *de novo* fatty acid synthesis was stimulated in diabetic pregnancies,

169 and is likely to represent the primary mechanism by which increased lipid accumulates in the

170 offspring. In a separate series of studies, Hausman and colleagues studied the development of

171 fat depots in fetal pigs from sows who were genetically obese, compared to lean controls. As

172 in the fetuses of diabetic sows, the adipocytes of fetuses of obese sows were large, more

173 abundant and had higher LPL activity in late gestation, and these changes preceded the onset

174 of obesity in this genetically obese breed [31,32].

175

176 Taken together, the results of large animal studies suggest that fetal overnutition, induced as a

177 result of either maternal obesity and/or maternal hyperglycemia, is associated with a

178 precocial upregulation of lipogeneic genes in fetal adipose depots which persists after birth

179 and is associated with an increased capacity for lipid storage in postnatal life, and consequent

180 propensity to obesity.

181

182 **Maternal Obesity before Conception: Another Important Window**

183 While the majority of studies to date have focussed on the consequences of maternal obesity

184 and/or overnutrition during pregnancy, there is mounting evidence that poor metabolic health

185 in the mother prior to and immediately after conception may also have negative effects on the

186 long-term metabolic health of the offspring. Both clinical and experimental studies have

187 shown that maternal obesity/overnutrition in the periconceptional period, independent of the

188 nutritional environment later in development, can result in altered development of the adipose

189 tissue and increased propensity to obesity in the offspring later in life. In humans, it is clearly

190 very difficult to separate the effects of these two periods in the vast majority of pregnancies,

191 however it has been reported that maternal obesity is associated with poorer developmental

192 competence and poorer quality oocytes – which has negative effects on subsequent embryo

193 development [33,34].

194   The negative impact of maternal obesity prior to conception on long-term offspring

195   development has also been demonstrated in experimental animal models. McMillen and

196   colleagues conducted an elegant study in the sheep in which embryos were transferred from a

197   donor ewe who had been fed either on a control diet or on a high plane of nutrition (to induce

198   maternal weight gain) for the 4 months before conception to a lean recipient ewe at 6 d post-

199   conception, such that embryos of the 'over-fed' ewes were only exposed to the obesogenic

200   environment during the periconceptional period [35]. The lambs from the 'obese' and 'lean'

201   donor ewes were subsequently studied at 4 months of age. The study showed that female

202   offspring exposed to maternal obesity in the periconceptional period had a higher fat mass as

203   a percentage of body weight at 4 months of age compared to control lambs[35]. The study

204   also determined whether these effects could be reversed by restricting the energy intake of the

205   'obese' dams to induce weight loss in the period immediately prior to conception. While

206   maternal weight loss in the 'obese' donors prevented the subsequent increase in fat mass in

207   the lambs [35], periconceptional weight loss also resulted in heightened stress

208   responsiveness, suggesting that maternal energy restriction diets before or in the early part of

209   pregnancy may not be desirable [36,37].

210

211

212   **Implications for the Clinic**

213   In humans, as in sheep and pigs, the major period of fat development begins in late gestation

214   and extends into the first year of life. The rising incidence of maternal overweight and

215   obesity, coupled with the increased consumption of energy-dense, nutrient poor 'junk' foods,

216   by pregnant and lactating women has led to growing concerns about the long-term

217   consequences of this obesogenic environment on future generations. As a result, more recent

218 studies, in both humans and experimental animal models, have become increasingly focussed

219 on identifying potential strategies for intervention. This is particularly important in light of

220 the evidence from animal studies suggesting that the structural and functional changes

221 induced in adipose (and other) tissues as a result of prenatal exposure to an increased nutrient

222 supply in utero are very difficult, if not impossible, to reverse through nutritional

223 interventions applied later in development [15,38,39]. In humans, three key windows of

224 opportunity have been identified in relation to introducing nutritional interventions to

225 improve long-term health outcomes in the offspring, namely prior to pregnancy, during

226 pregnancy and in early infancy.

227 **Interventions Prior to Pregnancy**

228 As discussed above, the evidence showing that exposure to maternal obesity/overnutrition

229 during the periconceptional period alone can result in an increased propensity for fat

230 accumulation in the offspring suggests that, ideally, women who are overweight/obese should

231 consider undertaking diet/lifestyle interventions to normalise body weight and improve

232 metabolic health prior to conceiving. In addition, the fact that significant weight loss

233 immediately before conception or in the early stages of pregnancy has the potential to

234 negatively impact on the stress axis of the offspring [36], implies that any weight reduction

235 program should be undertaken some time before the woman plans to conceive. To date, there

236 have been no clinical trials in this area, and there remains an urgent need for research to

237 enable evidence-based guidelines for the nutritional management of overweight/obese

238 women in the lead up to pregnancy to be developed.

239

240

241

242 **Interventions During Pregnancy**

243 While weight reduction/improved nutrition prior to pregnancy may be ideal, this is not

244 always possible or practical, and pregnancies are not always planned and many clinicians do

245 not see women until they are well into their pregnancy. As a result, the majority of clinical

246 studies which have focussed on diet/lifestyle interventions introduced during pregnancy

247 and/or lactation. A summary of some of the potential nutritional interventions which have

248 been suggested/tested to date is presented in **Figure 4**, and the proposed interventions

249 discussed in more detail in the following paragraphs.

250

251 Early studies in this area focussed on the potential for interventions aimed at improving

252 maternal glucose control to improve pregnancy/neonatal outcomes. The findings from two

253 large-scale clinical studies, the Australian Carbohydrate Intolerance Study in Pregnant

254 Women (ACHOIS) and the Maternal-Fetal Medicine Unit (MFMU) Network study, provided

255 encouraging data which suggested that aggressive treatment of mild gestational diabetes,

256 compared to routine care, resulted in reduced risks of pre-eclampsia, perinatal morbidity and

257 fetal overgrowth (large for gestational age deliveries and fetal macrosomia) [40,41]. While

258 the long-term consequences of these interventions on the fat mass/metabolic health of the

259 children is not known, these studies nevertheless suggest that improving maternal glucose

260 control, and thereby reducing maternal and fetal glycaemia, has the potential to improve the

261 long term metabolic health outcomes of the child.

262 The absorption of digested carbohydrate from foods in the form of glucose is the major

263 dietary factor affecting postprandial blood glucose concentrations and insulin secretion, and

264 as a result the quality and quantity of carbohydrates in the diet are key determinants of

265 postprandial glucose concentrations[42]. The glycemic index (GI) describes the effects of

266 different carbohydrate foods on blood glucose levels; carbohydrates that break down quickly

267   during digestion and release glucose rapidly into the bloodstream have a high GI whereas

268   carbohydrates that break down more slowly, releasing glucose more gradually into the

269   bloodstream, have a low GI. Thus, consumption of a low GI diet is associated with lower

270   fasting and postprandial glucose concentrations than consumption of high GI diets [42]. Low

271   GI diets have received significant attention in adult nutrition in relation to their effects on

272   body weight and insulin action, and switching overweight and/or type 2 diabetic individuals

273   from typical western diets to low GI diets can improve insulin sensitivity and assist with

274   maintenance of weight loss [43-46].

275   The role of low GI diets in improving glucose control have led to suggestions that reducing

276   the GI of diets consumed by women during pregnancy may have the potential to improve the

277   metabolic health outcomes of the child by reducing maternal and fetal glucose

278   concentrations. There is some evidence that adopting a low GI diet during pregnancy may

279   offer benefits for maternal/child health, with a systematic review of human studies reporting

280   that four of the eight studies carried out to date showed a protective association between low

281   GI diets and pregnancy-related outcomes, and none showed negative effects [47]. In both

282   normal and diabetic women in these studies birth weight, birth weight z-score and ponderal

283   index of offspring were lower in women consuming the low GI diet compared to those

284   consuming a standard Western diet or low-fat diet, and there was a reduced risk of delivering

285   a large for gestational age or macrosomic infant [47,48]. Again, however, there are limited

286   data on the potential for this intervention to improve the metabolic health of the offspring in

287   the longer term. An exception is a recent study by Danielsen and colleagues, which reported a

288   direct relationship between the GI of the maternal diet at gestational week 30 and markers of

289   the metabolic syndrome, including fasting insulin, insulin sensitivity (assessed by HOMA-IR)

290   and leptin, in the children at 20 years of age [49]. While interesting, it is important to note

291   that this was an observational study, rather than a controlled trial, so it is not possible to

292 exclude the possibility of bias/confounding. Further follow-up of existing randomised trials

293 will help to determine whether low GI diets may offer true potential to improve the metabolic

294 health outcomes in offspring of diabetic mothers. Nevertheless, the available evidence

295 suggests that low GI diets can be followed safely during pregnancy, and are associated with

296 favourable effects in the mother, including reduced pregnancy weight gain, improved glucose

297 tolerance and lower fasting glucose/insulin concentrations [47].

298 More recently, two large-scale randomised controlled trials have specifically focussed on

299 nutritional/lifestyle interventions in overweight/obese pregnant women for improving

300 pregnancy/neonatal outcomes. The LIMIT study, led by Dodd and colleagues at the

301 University of Adelaide, included over 2200 overweight and obese women who were

302 randomised to receive either standard antenatal care or a comprehensive diet and lifestyle

303 interventions in the second half of pregnancy [50]. As the name suggests, one of the principal

304 aims of this study was to limit gestational weight gain in this population of pregnant women,

305 since previous studies had suggested that a high percentage of overweight/obese pregnant

306 women exceed the recommended weight gains for pregnancy, and that this is associated with

307 poor pregnancy/neonatal outcomes [51]. The first results of the LIMIT trial, published in the

308 British Medical Journal in early 2014, suggested that the nutritional and lifestyle intervention

309 was associated with significant improvements in the nutritional quality of the maternal diet,

310 but no differences in gestational weight gain in comparison with standard care [50]. Despite

311 this however, there was a significant reduction in the number of babies born >4000g in the

312 intervention arm, suggesting that the diet and lifestyle intervention had the potential to reduce

313 the incidence of fetal overgrowth [50]. Importantly, these results also imply that it may be

314 possible to achieve beneficial outcomes in the absence of reductions in gestational weight

315 gain. The UPBEAT trial, led by Poston in the UK and colleagues, is another large-scale RCT

316 which aims to test the ability of a complex diet and lifestyle intervention during pregnancy to

317 reduce the incidence of GDM and LGA deliveries (defined as a birth weight >4000g) [52].

318 Again, this study is specifically targeted towards overweight and obese women, and

319 researchers aim to recruit in excess of 1500 pregnancies in order to achieve appropriate

320 statistical power to test their primary hypotheses [52]. The results of these large scale RCTs,

321 and further follow-ups of the children in these trials in order to examine the longer term

322 metabolic outcomes will be important to guide policy and practice decisions in relation to the

323 management of overweight and obese pregnancies.

324 In addition to whole diet approaches to improving the metabolic health outcomes of children,

325 interventions with specific nutrients, or combinations of nutrients, have also received some

326 attention, particularly in relation to the level of omega-6 and omega-3 polyunsaturated fatty

327 acids (n-6 and n-3 PUFA) in the maternal diet during pregnancy and lactation. This interest

328 has stemmed largely from data derived from *in vitro* and adult rodent studies suggesting that

329 these two classes of fatty acids have contrasting roles in relation to fat cell differentiation and

330 lipid storage. These studies indicate that the n-3 PUFA, in particular the marine-derived long

331 chain n-3 PUFA (n-3 LCPUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid

332 (EPA), inhibit the proliferation and differentiation of pre-adipocytes [53,54] and inhibit the

333 expression of the key lipogenic genes in adult adipose tissue, resulting in a reduced

334 accumulation of lipid [55-58]. In contrast, the n-6 PUFA, LA and AA, have pro-adipogenic

335 actions and promote the hyperplastic and hypertrophic expansion of adipose depots [53,59].

336

337 These data have led to the suggestion that increasing the ratio of n-3 to n-6 PUFA in the

338 maternal diet during pregnancy and/or lactation may be a potential strategy for reducing fat

339 mass in the offspring [60]. To date there is little evidence from either animal or human

340 studies to support this hypothesis [61,62]. However, there have been no attempts to date to

341 determine whether specific sub-groups, for example women who consume poor quality diets

342 or are obese/overweight, could potentially benefit. The field awaits the outcomes of larger,

343 adequately powered clinical studies to resolve this question.

344

345 **Management of Infants of Obese Mothers**

346 In human infants, fat development is not complete at birth, but continues throughout the first

347 year of postnatal life [54]. Consequently, infant nutrition also plays an important role in

348 defining an individual's future risk of obesity. Importantly, data from animal studies provides

349 evidence of an interaction between the prenatal and early postnatal nutritional environment in

350 defining an individual's risk of obesity and insulin resistance in the longer term. Thus, fat

351 deposition and metabolic/cardiovascular deficits in offspring of rat dams fed on high-

352 fat/cafeteria diets during pregnancy and lactation are exacerbated when these offspring are

353 also fed on high-fat diets after weaning [18].

354

355 As discussed above, infants born to obese mothers are likely to be more susceptible to weight

356 gain and fat disposition after birth compared to infants from lean women. Thus, close

357 monitoring of the growth and nutritional intakes of these infants in the early postnatal period

358 has the potential to provide a means of limiting the negative impacts of the intrauterine

359 obesogenic environment. Research in this area is still in its infancy, and there are currently no

360 specific guidelines for the management of infants of obese mothers, and this remains a fertile

361 area for research. There have been some suggestions that encouraging 'catch down' growth,

362 that is limiting the rate of infant weight gain, of infants who are born heavy at birth may be

363 beneficial for reducing the subsequent risk of obesity, but studies in this area are lacking. It is

364 also important to note that weight gain in and of itself does not always provide an appropriate

365 measure of growth quality (i.e. lean vs fat mass), and assessment of body composition, and

366   the distribution of fat between subcutaneous and visceral fat compartments, is necessary to

367   gain an overall picture of the growth profile of these infants.

368

369   Perhaps the most important issue in relation to infant nutrition is the impact of breastfeeding

370   vs formula feeding on long-term health outcomes. A number of systematic reviews have

371   supported the suggestion that breastfeeding, particularly an extended duration of breast

372   feeding, reduces the risk of obesity by ~20% in comparison with formula feeding [63]. It is

373   important to note, however, that the biological effects are difficult to separate from social

374   factors and that many of these studies were conducted at a time when the range of infant

375   formulas available was much more restricted, such that the true effect size is difficult to

376   assess. In addition, no studies of breast vs formula feeding in relation to obesity risk have

377   specifically focussed on infants of obese mothers. It is also evident that the composition of

378   the breast milk, in particular the fat content, varies markedly between women and closely

379   related to the fatty acid composition of the maternal diet [64,65]. There is strong evidence

380   that the n-3 and n-6 PUFA content in human breast milk are directly related to the content of

381   these fatty acids in the maternal diet [54,66]. In addition, studies in rodents, including recent

382   work in our laboratory, indicate that higher maternal intakes of saturated and trans fatty acids

383   is directly related to increased content of these in the milk supply [67] (*Vithayathil, Gibson &*

384   *Muhlhausler, unpublished observations*). This implies that the composition of the maternal

385   diet during lactation, as well as during pregnancy, is likely to be important in determining the

386   long term metabolic health outcomes of the child.

387   If the mother is unable to or chooses not to breastfeed, then the selection of an appropriate

388   formula becomes important. Recent studies have implicated the higher protein content of

389   infant formulas in comparison to human breast milk in the higher infant growth rates and

390 heighted obesity risk in formula fed infants [68], and one randomised controlled trial has

391 suggested that feeding infants a formula with a lower protein content reduced their BMI and

392 obesity risk at 6 years of age [69]. There has also been a recent randomised trial specifically

393 focussed on infants of obese mothers, which reported that providing these infants with a

394 lower protein formula was associated with a lower rate of weight gain between 3 and 6

395 months of age [70]. Long-term follow up of the infants in this study to evaluate their long-

396 term outcomes in relation to fat deposition and metabolic health will provide critical insights

397 into the potential utility of low protein formulas in the management of infants born to

398 overweight/obese mothers.

399 **Expert Commentary**

400 The rising incidence of maternal obesity has led to an urgent need to identify appropriate and

401 effective interventions to control the resulting intergenerational cycle of obesity and poor

402 metabolic health. From early studies of development programming, largely focused on the

403 metabolic consequences of exposure to sub-optimal nutrition intake [71], the attention of the

404 developmental programming field has turned to the long-term consequences of periantal

405 exposure to maternal obesity. It is clear from these studies that infants born to obese women

406 are at increased risk of obesity and its related comorbidities as both children and adults, and

407 this has created an intergenerational cycle of poor metabolic health which is fuelling the

408 propagation of the obesity epidemic.

409

410 While the underlying mechanisms are still being explored, the data to date suggests that it is

411 exposure of the fetus/infant to an increased nutrient supply (in particular glucose and

412 potentially fat) during critical periods of development which plays a central role in the early

413 programming of obesity and metabolic disease. The work from our group and others suggests

414 that the developing fat cell is a particularly important target of this metabolic programming,

415 and that exposure to an increased nutrient supply *in utero* results in persistent alterations in

416 the structure/function of adipose cells which increases their capacity to store lipid in postnatal

417 life. While not the topic of this review, there is also evidence that other key organs/regulatory

418 systems, including the liver, skeletal muscle and central systems regulating appetite, reward

419 processing and glucose control, are also impacted by prenatal nutritional excess **(Figure 3)**.

420 As a consequence of these programmed alterations in the structure and function of these

421 systems, individuals exposed to an obesogenic environment in utero have an increased

422 propensity to accumulate fat deposits after birth, and are therefore at increased risk of obesity.

423

424 From this increased understanding of the biological mechanisms underpinning the

425 relationship between maternal and infant/child obesity has come the recognition that the

426 manifestations of prenatal overnutrition are largely permanent, and unlikely to be reversed by

427 interventions applied later in life. Thus, while it may be possible to prevent excess weight

428 gain by closely monitoring diet and physical activity, the heightened susceptibility to weight

429 gain and obesity remains. As a result, it is clear that interventions to improve the metabolic

430 health of infants of obese mothers need to be applied as early as possible in order to be

431 effective in improving long term outcomes.

432

433 More recently, research has turned toward potential interventions before and during

434 pregnancy, and after birth, which could potentially improve the long-term metabolic health of

435 the increasing number of infants whose mothers enter pregnancy overweight and obese. As

436 highlighted above, while improving metabolic health well before conception is likely to be

437 ideal, this is not always practical, and encouraging weight loss immediately prior to

438 conception or in the early stages of pregnancy also appears to carry short and long-term risks.

439 For this reason, the majority of intervention studies have focussed on diet and lifestyle

440 interventions applied during pregnancy.

441

442 There is evidence from animal studies that maternal exercise may offer some benefits for

443 offspring of mothers consuming high-fat/high-sugar junk food diets during pregnancy,

444 however human studies suggest that increasing the physical activity in pregnant women is

445 extremely challenging [50]. There is emerging evidence from large randomised controlled

446 trials that improving the quality of the maternal diet during pregnancy independent of the

447 level of physical activity and maternal weight gain, may reduce the incidence of LGA

448 deliveries [50]. Further well-powered and robustly designed clinical studies are needed to

449 determine whether specific nutritional interventions, for example increasing the supply of n-3

450 LCPUFA, limiting n-6 PUFA intake or reducing the GI of the diet in overweight/obese

451 women has the potential to reduce the subsequent risk of obesity/poor metabolic health in

452 their offspring. In addition, there is growing interest in identifying potential approaches for

453 management of infants of obese mothers to improve their long-term metabolic health;

454 research in this area is currently extremely limited research, and more studies are urgently

455 needed.

456

457

458 **Five-Year View**

459 As animal studies have provided new insights into the mechanisms which underlie

460 developmental programming of obesity by nutritional exposures during the perinatal period,

461 it has become increasingly clear that intervening early, preferably well before birth, to

462 improve the nutritional environmental experienced during development is critical for

463 improving long term metabolic health outcomes.

464   While there has been a move, both in experimental animal models and in clinical studies,

465   towards research focussed on potential strategies for optimising the metabolic outcomes of

466   infants from pregnancies complicated by maternal obesity, more such studies are desperately

467   needed. Over the coming 5 years, the results of current large-scale RCTs in this area and of

468   follow-up of the infants from these studies will start to emerge and will begin to develop a

469   basis for clear evidence-based guidelines regarding the nutritional management of

470   overweight/obese pregnant women.

471

472   In addition, the ability to more accurately measure body composition and body fat

473   distribution in infants will provide more insights into how different nutritional practices

474   influence growth quality (as well as quantity), and whether specific nutritional modifications,

475   such as increasing n-3 LCPUFA or reducing protein supply, in infants of obese mothers may

476   help to improve their long-term metabolic health. The ability to conduct genetic and

477   epigenetic studies in minute amounts of starting material, offers the potential to explore the

478   mechanistic pathways underlying the early origins of human obesity in more detail than ever

479   before, and the coming 5 years promises to see an explosion in the number of studies in this

480   area.

481

482   However, while technological advances will provide further insights into the underlying

483   biology, it will be critical to ensure that the design of clinical trials to test nutritional

484   interventions in pregnancy remains focussed on the core elements of the CONSORT

485   statement; a defined nutritional intervention, appropriate controls, a defined primary

486   outcome, adequate statistical power to address the primary question and, in the case of follow

487   up studies, low rates of attrition to preserve the integrity of the randomisation [72]. While

488 epidemiological and cohort studies are important for identifying potential links, it is only

489 through RCTs that we can confirm cause and effect relationships and establish definitely

490 whether specific interventions are (a) safe and (b) effective in improving the long-term

491 metabolic health outcomes in infants of overweight/obese mothers. Consequently, such

492 studies will be important in the effective translation of this research into clinical practice over

493 the coming 5 year time-frame.

494 **Acknowledgements**

498

499

500 **Key issues**

501   1. Maternal obesity is a major risk factor for obesity and associated metabolic disorders

502   in the child

503   2. The association between maternal obesity and obesity in the child is a result of an

504   increased nutrient supply to the fetus during critical periods of development

505   3. The fat cell is a key target of this developmental programming, and exposure to an

506   increased nutrient supply before birth prematurely 'switches on' key genes in adipose

507   tissue which are responsible for lipid storage and this results in an increased

508   propensity for fat accumulation after birth

509   4. The increase in fat storage places the individual at increased risk of obesity and

510   associated co-morbidities.

511   5. These effects are not easily reversible – therefore early intervention is essential.

6. In humans, the major period of fat cell development before birth to first year of life

7. Exposure to an increased nutrient supply during this period is an important determinant of fat cell size and number and the capacity for individuals for storing fat throughout the life course

8. Current research is focussed on potential nutritional interventions to improve outcomes in infants born to overweight/obese mothers, but few results have been published to date

9. There is an urgent need to accelerate this research, and to also focus on mechanistic studies in humans

10. The next 5 years is likely to see a move to the development of specific guidelines for the nutritional management of overweight/obese mothers and their infants.

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Figure Legends

Figure 1. Schematic representation of the association between maternal obesity/overnutrition, increased fetal growth and increased risk of obesity in later life.

Figure 2. Schematic of the proposed role of adipose tissue in the development of obesity and metabolic dysfunction after prenatal exposure to an excess energy supply. (1) Prenatal overnutrition results in increased expression PPARγ mRNA in visceral adipocytes before birth (2) After birth, signals from visceral adipocytes promote growth of the subcutaneous fat depot, leading to an increase in subcutaneous fat mass (3) Increased mass and leptin secretion from subcutaneous fat is associated with increased plasma leptin concentrations and development of central leptin resistance which leads to increased weight gain, obesity and ultimately metabolic dysfunction (from [9]).

Figure 3. Summary of potential mechanisms implicated with the association between exposure to maternal obesity before birth and increased risk of obesity in later life (adapted from

Figure 4. Nutritional interventions which may have potential to improve metabolic health of infants of obese mothers. Improving the overall quality of the maternal diet, increasing physical activity or reducing dietary GI during pregnancy could act either indirectly, through reducing pregnancy weight gain, or directly to improve maternal glucose tolerance and reduce fetal nutrient supply. Similarly, increased n-3 LPCUFA or decreasing n-6 PUFA intake may increase fetal n-3 LCPUFA supply and thereby reduce fetal fat deposition.