

1 **Expression of cholesterol packaging and transport genes in human and rat placenta:**
2 **impact of obesity and a high-fat diet**

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16 Short Title: High-fat diet and placental CHO transport

17 **Abstract**

18 Evidence suggests that sub-optimal maternal nutrition has implications for the developing
19 offspring. We have previously shown that exposure to a low-protein diet during gestation was
20 associated with upregulation of genes associated with cholesterol transport and packaging
21 within the placenta. This study aimed to elucidate the effect of altering maternal dietary linoleic
22 acid (LA; omega-6) to alpha-linolenic acid (ALA; omega-3) ratios as well as total fat content
23 on placental expression of genes associated with cholesterol transport. The potential for
24 maternal BMI to be associated with expression of these genes in human placental samples was
25 also evaluated. Placentas were collected from 24 Wistar rats at 20d gestation (term=21-22d
26 gestation) that had been fed one of four diets containing varying fatty acid compositions during
27 pregnancy, and from 62 women at the time of delivery. Expression of 14 placental genes
28 associated with cholesterol packaging and transfer were assessed in rodent and human samples
29 by qRT-PCR. In rats, placental mRNA expression of *ApoA2*, *ApoC2*, *Cubn*, *Fgg*, *Mttp* and *Ttr*
30 was significantly elevated (3-30 fold) in animals fed a high LA (36% fat) diet, suggesting
31 increased cholesterol transport across the placenta in this group. In women, maternal BMI was
32 associated with fewer inconsistent alterations in gene expression. In summary, sub-optimal
33 maternal nutrition is associated with alterations in the expression of genes associated with
34 cholesterol transport in a rat model. This may contribute to altered fetal development and
35 potentially programme disease risk in later life. Further investigation of human placenta in
36 response to specific dietary interventions is required.

37 **Key Words:** Cholesterol, maternal nutrition, omega, placenta, pregnancy

38 Introduction

39 Maternal nutrition can have a profound impact on fetal development and future physiological
40 function and metabolic health ⁽¹⁾. A number of dietary perturbations, including maternal
41 undernutrition and low protein diets, have been associated with increased risk of obesity and
42 cardiovascular disease in the adult offspring ^(2, 3). In the context of the growing epidemic of
43 obesity, focus has shifted towards understanding the effects of nutritional excess and obesity
44 on offspring programming of disease. Studies have consistently demonstrated that these
45 exposures are associated with a substantial increase in the risk of poor metabolic health in the
46 offspring in both humans ⁽⁴⁾ and animal models ⁽⁵⁾. There is emerging evidence from animal
47 studies, however, that maternal high-fat diets also have the potential to program metabolic
48 outcomes in the offspring independent of the effects of maternal obesity. In addition, these
49 effects appear to depend not only on the amount of fat in the diet ⁽⁶⁾, but also on the fatty acid
50 composition ^(7, 8). The majority of studies to date that have investigated the effects of a
51 maternal high fat diet have utilised diets high in saturated fat. However, due to changes in
52 population level patterns in dietary consumption ^(9, 10), attention has now shifted toward the
53 roles of polyunsaturated fats within the diet.

54 The mechanisms underlying this early life programming of obesity and metabolic disease are
55 not completely understood. However, as the sole interface between the mother and the fetus,
56 structural and functional changes within the placenta have been implicated as playing a key
57 role ⁽¹¹⁾. Cholesterol is present in every cell of the human body and an adequate supply is
58 therefore critical for supporting normal fetal development. As the precursor for all steroid
59 hormone synthesis, cholesterol also plays an important role in placental function. During
60 pregnancy, the fetus obtains cholesterol via endogenous synthesis as well as transfer across
61 the placenta from the maternal circulation, disturbances to either of these processes have
62 negative impacts on fetal growth, cell proliferation, metabolism and the organisation of

63 tissues^(12, 13). The endogenous synthesis of cholesterol appears to be most critical for the
64 developing fetus, as defects in this pathway are known to be lethal⁽¹⁴⁾. Sub-optimal maternal
65 contribution of cholesterol across the placenta, however, has been associated with lower
66 birthweight^(15, 16) and microcephaly⁽¹⁶⁾ in humans, highlighting the importance of this
67 exogenous cholesterol supply.

68 Transport of cholesterol across the placenta is a complex process in both humans and rodents
69^(17, 18). Briefly, the majority of cholesterol circulates the body in the form of HDL, LDL and
70 VLDL cholesterol, which are associated with specific structural apolipoproteins (*ApoA2*,
71 *ApoB* and *ApoC2* respectively). The layer of trophoblast cells, located closest to maternal
72 circulation, take in LDL and VLDL through their respective receptors. HDL cholesterol can
73 be taken up via a specific receptor (scavenger receptor class B type 1; *SR-B1*) or by binding
74 to proteins such as megalin and cubilin (*Cubn*). Once within the cell, cholesterol is
75 hydrolysed into free cholesterol, bound to sterol carrier proteins and then transferred to the
76 basolateral membrane where it passes through the fetoplacental endothelium. The processes
77 governing cholesterol efflux from the endothelial layer are poorly understood, although it has
78 been shown that exogenous cholesterol is secreted into fetal circulation, through association
79 with various transporters⁽¹⁹⁾, where it is repackaged into fetal lipoproteins. This process is
80 facilitated by microsomal triglyceride transfer protein (*Mttp*). The finding that the placenta
81 expresses and secretes its own apolipoproteins such as *ApoB*⁽²⁰⁾ also raises the possibility that
82 cholesterol is repackaged into HDL, LDL and VLDL cholesterol within the placenta itself.

83

84 In addition to its critical role in fetal growth and development, there is emerging evidence
85 that alterations in placental cholesterol transfer capacity may also be a contributing factor to
86 metabolic programming. In a previous study⁽²¹⁾, we showed providing rats with a low protein

87 diet until day 13 of gestation, a dietary treatment previously associated with programming of
88 obesity, hypertension and glucose intolerance in adult offspring ^(22, 23), resulted in increased
89 placental expression of a number of genes associated with cholesterol and lipoprotein
90 transport and metabolism in the rat.

91 Given the similarity in the metabolic phenotype induced by maternal obesity/nutritional
92 excess and low protein diets, we hypothesised that programming of health and disease is
93 driven by perturbations of a small set of common ‘gatekeeper’ processes ^(24, 25) and changes in
94 placental cholesterol transfer and metabolism may be common mechanisms underlying
95 metabolic programming by different dietary exposures. Therefore, the aim of this current
96 study was to investigate the effect of a high maternal dietary omega-6:omega-3 fatty acid
97 ratio, associated with decreased placental weight ⁽²⁶⁾, against a lower ratio as well as total fat
98 intake, on the expression of genes associated with cholesterol and lipoprotein transport,
99 known to be affected by maternal diet, in the mature placenta of the rat. We also aimed to
100 investigate whether placental expression of these same genes differed according to maternal
101 body mass index (BMI) in a cohort of pregnant women.

102 **Materials and Methods**

103 *Animal Experiments and sample collection*

104 This paper reports data from the analysis of placentas that were collected as part of a previous
105 study ⁽²⁶⁾. Virgin female Wistar rats (n = 24; 75-100 g; Charles River, UK) were housed on
106 wood shavings in individually ventilated cages under a 12 h light/12 h dark cycle at a
107 temperature of 20–22 °C and had *ad libitum* access to food and water throughout the
108 experiment. Female rats were allowed to acclimatise to the unit for 1–2 weeks, during which
109 time they were fed on standard laboratory chow (2018 Teklad Global 18% Protein Rodent
110 Diet, Harlan Laboratories, UK). After acclimatisation, a tail vein blood sample was taken

111 from each animal for the determination of fatty acid status. The rats were then randomly
112 assigned to one of 4 dietary groups designed to provide either a high (9:1, high LA) or low
113 (1:1.5 low LA) ratio of linoleic acid (LA) to alpha-linolenic acid (ALA), achieved by altering
114 the amounts of flaxseed and sunflower oil included in the fat component of the feed. The
115 levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were
116 comparable in all diets, achieved by adjusting the amounts of coconut (SFA source) and
117 macadamia (MUFA source) oils in the diets. For each level of LA, diets containing either
118 18% or 36% fat by weight were developed. This resulted in four experimental diets; high LA
119 (18% fat), high LA (36% fat), low LA (18% fat) and low LA (36% fat) (n =5–7 per dietary
120 group). The list of ingredients and final fatty acid composition of the four experimental diets
121 have been published previously ⁽²⁶⁾.

122 All animal procedures were performed in accordance with the Animals (Scientific
123 Procedures) Act 1986 under Home Office licence and were approved by the Animal Ethics
124 Committee of the University of Nottingham, UK. Animals were pair housed from the start of
125 the experiment until mating, after confirmation of conception animals were individually
126 housed until completion of the experiment. Animals were maintained on their allocated diet
127 for a four week 'feed-in' period after which they were mated. Conception was confirmed by
128 the presence of a semen plug and this was recorded as day 0 of pregnancy. Female rats
129 remained on their respective diets until day 20 of gestation (full term = 22 days) at which
130 time rat dams were euthanised by CO₂ asphyxiation and cervical dislocation and fetuses by
131 cervical dislocation and exsanguination. All fetuses were weighed and sexed via
132 measurement of anogenital distance. Placentas from male fetuses were collected for analysis
133 and a tail sample from the fetus was collected for sex-genotyping by PCR for the SRY gene
134 ⁽²⁷⁾. Any samples found to be female or inconclusive (n = 5) were not included in placental

135 gene expression analysis. Full details of maternal weight gain, food intake and the effect of
136 the diets on fetal and placental weight are published elsewhere ⁽²⁶⁾.

137 *Human placental sample collection*

138 Ethical approval for the study was obtained from the Derbyshire Research Ethics Committee
139 (Ref: 09/H0401/90). Placental samples were obtained from patients attending the Department
140 of Obstetrics and Gynaecology, Royal Derby Hospital, Derby, UK. Patients provided
141 informed, written consent prior to undergoing elective caesarean section at term gestation
142 (>37 weeks), indications for which were maternal request, previous elective section or breech
143 presentation while cases with diabetes, hypertension, pre-eclampsia, were excluded.
144 Placentae, once checked by the midwife and with the cord clamped, were transported to the
145 lab within 20 minutes of delivery, where placental villous samples were taken midway
146 between the cord insertion site and placental periphery and frozen at -80°C prior to extraction
147 of RNA. Participants were stratified based on a BMI measurement taken during an antenatal
148 clinic appointment, resulting in 3 groups of women; BMI <25 kg/m² (n=20), BMI 25-35
149 kg/m² (n=21) and BMI >35 kg/m² (n=21).

150 *Sample Preparation and PCR*

151 RNA was isolated from 20-25mg of crushed snap-frozen human or rat placental tissue using
152 the Roche High Pure Tissue kit (Roche Diagnostics Ltd., UK) according to manufacturer's
153 instructions. RNA concentration was determined using a Nanodrop 2000 (Thermo Scientific)
154 and RNA quality was evaluated by agarose gel electrophoresis. cDNA was synthesised using
155 a RevertAid™ reverse transcriptase kit (Thermo Fisher Scientific, UK) with random hexamer
156 primers.

157 Gene targets were chosen based on our previous data ⁽²¹⁾ where RNASeq analysis of day 13
158 rat placentas revealed differential expression of 91 genes in response to maternal protein

159 restriction. Ingenuity pathway analysis identified 8 pathways that were significantly affected
160 ($P < 0.001$), 6 of which were closely related functionally with a strong emphasis of cholesterol
161 uptake and efflux across the placenta. Genes were selected based upon the ingenuity analysis
162 (*ApoA2*, *ApoB*, *ApoC2*, *Ttr*, *Fgg*, *serpin G1* and *Rbp4*). Additional genes were chosen that
163 were shown to be differentially expressed in the protein restricted condition (*Vill*, *Gpc3*,
164 *Prfl*, *Cubn*, *Mttp*) but not associated with pathways identified by Ingenuity analysis. *Tagln*
165 and *Tbp* gene targets were also chosen as preliminary RNASeq analysis suggested that they
166 may be sensitive to maternal dietary factors, resulting in 14 target genes for analysis. Real-
167 time PCR primers were designed using Primer Express software (version 1.5; Applied
168 Biosystems) from the RNA sequence, checked using BLAST (National Centre for
169 Biotechnology Information) and were purchased from Sigma (UK). The primer sequences
170 can be found in supplementary table S1. Real-time PCR was performed on a Lightcycler 480
171 (Roche, Burgess Hill, UK) using the 384 well format. Each reaction contained 5 μ l of cDNA
172 with the following reagents: 7.5 μ l SYBR green master mix (Roche), 0.45 μ l forward and
173 reverse primers (final concentration 0.3 μ M each) and 1.6 μ l RNase-free H₂O. Samples were
174 pre-incubated at 95°C for 5 min followed by 45 PCR amplification cycles (denaturation,
175 95°C for 10 seconds; annealing, 60°C for 15 seconds; elongation, 72°C for 15 seconds).
176 Transcript abundance was determined using a standard curve generated from serial dilutions
177 of a pool of cDNA made from all samples. Expression was normalised against the expression
178 of cyclophilin A, which was not significantly different between experimental groups.

179 *Statistical Analysis*

180 Data are presented as mean \pm SEM. Data were analysed using the Statistical Package for
181 Social Sciences (Version 24, SPSS Inc.). For animal data the effect of maternal dietary fatty
182 acid ratio and maternal dietary fat content on placental expression of target genes was
183 assessed using a two-way ANOVA, with dietary fat level and LA:ALA ratio as factors.

184 Human data were analysed using a one-way ANOVA with maternal BMI as a factor. Possible
185 co-variates were identified and corrected for within the analysis. A value of $P < 0.05$ was
186 considered statistically significant.

187 **Results**

188 *Rat placenta*

189 The results of the gene expression analyses in the rodent placentas is shown in Table 1.

190 Expression was measured for 13 genes (*ApoB* excluded), however, *Tbp* expression was not
191 detectable in any of the samples (data not shown) and so could not be included in the
192 analysis.

193 Placental *Mttp* mRNA expression was significantly increased (3 fold; $P < 0.01$) in dams
194 consuming a high LA compared to a low LA diet, independent of total dietary fat content
195 (Table 1). There was a significant interaction between the effects of maternal total dietary fat
196 and LA content in relation to mRNA expression of *ApoA2*, *ApoC2*, *Cubn*, *Fgg*, *Rbp4* and *Ttr*
197 ($P < 0.05$), such that expression of these genes was significantly increased in rats consuming
198 the high LA diets, but only when dietary fat content was also high (36% fat w/w). The
199 magnitude of this effect was greatest for *Cubn* (30-fold increase in the high LA (36% fat)
200 group when compared to the low LA (36% fat) group). A similar pattern was observed for
201 other key cholesterol-transport and metabolism genes in the placenta (*Gpc3*, $P = 0.072$; *Vill*,
202 $P = 0.054$), with 1.5-7 fold higher expression in placentas of dams exposed to a high LA diet,
203 but only when dietary fat content was also high. Placental expression of *Prfl*, *SerpinG1* and
204 *Tagln* was not affected by either maternal dietary fatty acid ratio or fat content.

205 *Human placenta*

206 The sociodemographic and clinical characteristics of the women who provided placental
207 samples for this study are summarised in Table 2. The average age, parity and gestation

208 length of women within this study was similar between women in the three BMI groups,
209 however the birth weights of infants born to women with a BMI >25 kg/m² were significantly
210 greater than those of women in the normal BMI range.

211 The results of the gene expression analyses in the human placentas is shown in Table 3. All
212 14 genes were measured and detected in these samples. Women with a BMI in the 25-35
213 kg/m² range (overweight to obese) exhibited a 2-6 fold higher mRNA expression of *ApoB* in
214 their placental samples compared to women with a BMI either <25 or >35 kg/m². The
215 expression of *Rbp4* was significantly lower in placentas obtained from women with a BMI
216 >25 kg/m² compared to those with a BMI <25 kg/m² (85% down-regulation, $P=0.001$). A
217 similar pattern was also observed for *Ttr*, with 71-88% down-regulation of expression in
218 women with a BMI >25 kg/m², although this did was not statistically significant ($P=0.053$).
219 The mRNA of other placental genes was not different between BMI groups.

220 **Discussion**

221 This experiment aimed to test the hypothesis that maternal diet, specifically, fatty acid
222 composition and quantity, and obesity would influence the expression of genes associated
223 with cholesterol uptake and transport in rat and human placenta. The results of the rat studies
224 suggested clear effects of maternal dietary fat content and composition, such that maternal
225 consumption of a higher-fat, high LA maternal diet was associated with increased expression
226 of key genes associated with these pathways, suggesting enhanced cholesterol transport to the
227 fetus in this group. In the human study, however, only two of these genes were differentially
228 expressed in placentas from women in different BMI categories, suggesting that maternal
229 obesity had a limited impact on placental cholesterol transport at the level of gene expression.

230 Within this study we have shown that exposure to a high LA, high-fat diet resulted in
231 increased expression of genes involved in the formation of apolipoproteins, cholesterol

232 uptake and cholesterol repackaging. These differences were not observed when either the fat
233 content and/or the fatty acid ratio was altered, suggesting a strong interaction between these
234 variables. Importantly, the effects observed on the placental gene expression profiles show
235 striking resemblances to our previous findings ⁽²¹⁾ where upregulation of these genes was
236 observed in the placentas of dams exposed to a low protein diet. These data suggest that, not
237 only do these differences persist to a late stage placenta (day 20), but, despite different
238 dietary interventions, the similarities in results suggest a common mechanism of action. The
239 upregulation of key genes in the rat placenta observed in this study suggests a state of
240 increased cholesterol uptake and efflux. While the potential impacts of this in the current
241 study are not clear, it has been demonstrated in previous studies that, exposure to excess
242 cholesterol during fetal development can be associated with adverse outcomes. In rodent
243 models, maternal hypercholesterolemia has been associated with growth restriction ⁽²⁸⁾,
244 altered liver development ⁽²⁹⁾ and atherosclerosis ^(30, 31). In humans, maternal
245 hypercholesterolaemia has been associated with the development of fatty streaks in fetal
246 arteries and cholestasis during pregnancy is associated with programming of an overweight,
247 insulin resistant phenotype in the child ^(32, 33). It will therefore be important in future studies
248 to determine the longer term consequence of the changes in placental gene expression for the
249 postnatal offspring.

250 Based on the substantial impact of maternal high-fat high LA feeding, a dietary pattern
251 commonly observed in the modern Western diet, we extended our study to determine if there
252 was any evidence to suggest an effect of maternal obesity on cholesterol transfer in the
253 human. There were, however, relatively few differences in the expression of key genes
254 associated with different BMI categories in human placental samples, although there were
255 some subtle differences in the expression of 3 genes (*ApoB*, *Rbp4* and *Ttr*) between BMI
256 categories. *Ttr* is a protein that binds to and transports *Rbp4*. In the bound state, *Rbp4* is

257 protected from glomerular filtration and so levels of these two proteins are often correlated.
258 As such, the similar patterns of expression across these two genes observed in this study was
259 anticipated. What was surprising, however, was our finding that women with a BMI above
260 the normal range ($>25 \text{ kg/m}^2$, overweight or obese) exhibited decreased placental expression
261 of these genes since elevated levels of circulating *Rbp4* have been associated with many of
262 the co-morbidities linked to obesity including hypertension ⁽³⁴⁾, insulin resistance and type 2
263 diabetes ^(35, 36). It is important to note, however, that these observations have all been
264 associated with circulating levels of *Rbp4*, whereas we measured gene expression in the
265 placenta. There is limited literature evaluating the role of *Rbp4* within the placenta during
266 pregnancy, particularly in association with maternal obesity. It may be, however, that in
267 obese mothers, placental expression is reduced to compensate for the increased maternal
268 circulating levels and therefore avoiding fetal exposure to high quantities of *Rbp4*. Further
269 experimentation, is required to determine expression of *Rbp4* and *Ttr* in both the mother,
270 fetus and the placenta throughout pregnancy and their association with maternal obesity.

271 Placental *ApoB* expression was increased in women whose BMI was above the normal range
272 ($>25 \text{ kg/m}^2$). A study by Dubé and colleagues ⁽³⁷⁾, showed increased circulating *ApoB*
273 concentrations in new-born infants of obese mothers, compared to mothers of normal weight,
274 in the absence of any difference in maternal circulating concentrations. It is therefore possible
275 that the high *ApoB* concentrations in infants of obese mothers may have been the result of
276 increased placental *ApoB* expression. If this abnormal lipoprotein profile is present in the
277 offspring and persists through childhood it may contribute to increased risk of cardiovascular
278 disease in later life.

279 One key limitation of the current study is that direct measurements of cholesterol transport or
280 measurement of the genes of interest at the level of protein were not analysed, and so care
281 must be taken when extrapolating the findings to functional outcomes. Further to this, human

282 participants within this study were stratified based on BMI whereas the animal experiments
283 utilised specific dietary interventions. There are many factors that can affect BMI and,
284 although nutrition is a key element, there is still a huge variety of nutritional habits that can
285 lead to individuals resulting in similar BMIs. As such, assumptions cannot be made about
286 specific nutrient intakes of the women based on this data. Finally, it is important to note that,
287 whilst there are many similarities between placental physiology and function in the rat and
288 humans, there are some key differences. Of particular importance to this study is the
289 difference in circulating progesterone levels at the end of pregnancy. In rodents there is a
290 dramatic decrease in circulating progesterone ⁽³⁸⁾, whereas in humans, progesterone levels are
291 increased or at least maintained at the time of parturition ⁽³⁹⁾. Progesterone is a key steroid
292 produced from cholesterol within the placenta and has been shown to regulate the expression
293 of some genes including *Rbp4* in other tissues ⁽⁴⁰⁾. As such, careful consideration of the
294 differences in placental hormone production, particularly steroid hormones, between the two
295 species should be made when drawing comparisons.

296 In conclusion, this study aimed to elucidate whether differences in placental expression of
297 genes involved in cholesterol transport and efflux were associated with altered maternal
298 nutrition in a manner similar to our previous observations in the low-protein model. We
299 demonstrated that exposure to high levels of omega-6 as part of a high-fat diet elicited a
300 similar pattern of placental gene expression, suggesting an increase in cholesterol transport
301 across the placenta. This highlights the potential for a common mechanism by which sub-
302 optimal maternal nutrition during pregnancy alters placental function, and potentially fetal
303 development, resulting in increased risk of disease in later life. We then carried out a
304 preliminary study which aimed to establish if similar alterations were observed in human
305 placentas. Although BMI was associated with some changes in expression, these observations

306 were not consistent and further experimentation is required on placental samples where the
307 specific nutrient intake of the participants are known.

308

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317 Conflicts of interest

318 None

319 Ethical Standards

320 The authors assert that all procedures contributing to this work comply with the ethical
321 standards of the relevant national guidelines on human experimentation and with the Helsinki
322 Declaration of 1975, as revised in 2008, and has been approved by the Derbyshire Research
323 Ethics Committee (Ref. 09/H0401/90).

324 The authors assert that all animal procedures were performed in accordance with the Animals
325 (Scientific Procedures) Act 1986 under Home Office licence and were approved by the
326 Animal Ethics Committee of the University of Nottingham, UK.

327

328 **References**

- 329 1. Langley-Evans, S.C., Nutrition in early life and the programming of adult disease:
330 a review. *J Hum Nutr Diet*, 2015. 28 Suppl 1: p. 1-14.
- 331 2. Le Clair, C., T. Abbi, H. Sandhu, and P.S. Tappia, Impact of maternal
332 undernutrition on diabetes and cardiovascular disease risk in adult offspring. *Can*
333 *J Physiol Pharmacol*, 2009. 87(3): p. 161-79.
- 334 3. Langley-Evans, S.C., Fetal programming of CVD and renal disease: animal models
335 and mechanistic considerations. *Proc Nutr Soc*, 2013. 72(3): p. 317-25.
- 336 4. Godfrey, K.M., R.M. Reynolds, S.L. Prescott, et al., Influence of maternal obesity
337 on the long-term health of offspring. *The lancet. Diabetes & endocrinology*, 2017.
338 5(1): p. 53-64.
- 339 5. Ribaroff, G.A., E. Wastnedge, A.J. Drake, R.M. Sharpe, and T.J.G. Chambers,
340 Animal models of maternal high fat diet exposure and effects on metabolism in
341 offspring: a meta-regression analysis. *Obesity reviews : an official journal of the*
342 *International Association for the Study of Obesity*, 2017. 18(6): p. 673-686.
- 343 6. Ainge, H., C. Thompson, S.E. Ozanne, and K.B. Rooney, A systematic review on
344 animal models of maternal high fat feeding and offspring glycaemic control. *Int J*
345 *Obes (Lond)*, 2011. 35(3): p. 325-35.
- 346 7. Muhlhausler, B.S. and G.P. Ailhaud, Omega-6 polyunsaturated fatty acids and the
347 early origins of obesity. *Curr Opin Endocrinol Diabetes Obes*, 2013. 20(1): p. 56-
348 61.
- 349 8. Ramsden, C.E., K.R. Faurot, D. Zamora, et al., Targeted alteration of dietary n-3
350 and n-6 fatty acids for the treatment of chronic headaches: a randomized trial.
351 *Pain*, 2013. 154(11): p. 2441-51.
- 352 9. Blasbalg, T.L., J.R. Hibbeln, C.E. Ramsden, S.F. Majchrzak, and R.R. Rawlings,
353 Changes in consumption of omega-3 and omega-6 fatty acids in the United States
354 during the 20th century. *The American Journal of Clinical Nutrition*, 2011. 93(5):
355 p. 950-962.
- 356 10. Sioen, I., L. van Lieshout, A. Eilander, et al., Systematic Review on N-3 and N-6
357 Polyunsaturated Fatty Acid Intake in European Countries in Light of the Current
358 Recommendations - Focus on Specific Population Groups. *Ann Nutr Metab*, 2017.
359 70(1): p. 39-50.
- 360 11. Tarrade, A., P. Panchenko, C. Junien, and A. Gabory, Placental contribution to
361 nutritional programming of health and diseases: epigenetics and sexual
362 dimorphism. *J Exp Biol*, 2015. 218(Pt 1): p. 50-8.
- 363 12. Singh, P., R. Saxena, G. Srinivas, G. Pande, and A. Chattopadhyay, Cholesterol
364 biosynthesis and homeostasis in regulation of the cell cycle. *PLoS One*, 2013.
365 8(3): p. e58833.
- 366 13. Fernandez, C., M. Martin, D. Gomez-Coronado, and M.A. Lasuncion, Effects of
367 distal cholesterol biosynthesis inhibitors on cell proliferation and cell cycle
368 progression. *J Lipid Res*, 2005. 46(5): p. 920-9.
- 369 14. Porter, F.D., Malformation syndromes due to inborn errors of cholesterol
370 synthesis. *The Journal of clinical investigation*, 2002. 110(6): p. 715-724.
- 371 15. Maymunah, A.-O., O. Kehinde, G. Abidoye, and A. Oluwatosin,
372 Hypercholesterolaemia in pregnancy as a predictor of adverse pregnancy
373 outcome. *African health sciences*, 2014. 14(4): p. 967-973.
- 374 16. Edison, R.J., K. Berg, A. Remaley, et al., Adverse birth outcome among mothers
375 with low serum cholesterol. *Pediatrics*, 2007. 120(4): p. 723-33.
- 376 17. Baardman, M.E., W.S. Kerstjens-Frederikse, R.M. Berger, et al., The role of
377 maternal-fetal cholesterol transport in early fetal life: current insights. *Biol*
378 *Reprod*, 2013. 88(1): p. 24.
- 379 18. Woollett, L.A., Review: Transport of maternal cholesterol to the fetal circulation.
380 *Placenta*, 2011. 32 Suppl 2: p. S218-21.

- 381 19. Stefulj, J., U. Panzenboeck, T. Becker, et al., Human endothelial cells of the
382 placental barrier efficiently deliver cholesterol to the fetal circulation via ABCA1
383 and ABCG1. *Circ Res*, 2009. 104(5): p. 600-8.
- 384 20. Madsen, E.M., M.L. Lindegaard, C.B. Andersen, P. Damm, and L.B. Nielsen,
385 Human placenta secretes apolipoprotein B-100-containing lipoproteins. *J Biol*
386 *Chem*, 2004. 279(53): p. 55271-6.
- 387 21. Daniel, Z., A. Swali, R. Emes, and S.C. Langley-Evans, The effect of maternal
388 undernutrition on the rat placental transcriptome: protein restriction up-regulates
389 cholesterol transport. *Genes & Nutrition*, 2016. 11(1): p. 27.
- 390 22. Langley, S.C. and A.A. Jackson, Increased systolic blood pressure in adult rats
391 induced by fetal exposure to maternal low protein diets. *Clin Sci (Lond)*, 1994.
392 86(2): p. 217-22; discussion 121.
- 393 23. Erhuma, A., A.M. Salter, D.V. Sculley, S.C. Langley-Evans, and A.J. Bennett,
394 Prenatal exposure to a low-protein diet programs disordered regulation of lipid
395 metabolism in the aging rat. *Am J Physiol Endocrinol Metab*, 2007. 292(6): p.
396 E1702-14.
- 397 24. Swali, A., S. McMullen, H. Hayes, et al., Cell Cycle Regulation and Cytoskeletal
398 Remodelling Are Critical Processes in the Nutritional Programming of Embryonic
399 Development. *PLOS ONE*, 2011. 6(8): p. e23189.
- 400 25. McMullen, S., S.C. Langley-Evans, L. Gambling, et al., A common cause for a
401 common phenotype: the gatekeeper hypothesis in fetal programming. *Med*
402 *Hypotheses*, 2012. 78(1): p. 88-94.
- 403 26. Draycott, S.A.V., G. Liu, Z.C. Daniel, et al., Maternal dietary ratio of linoleic acid
404 to alpha-linolenic acid during pregnancy has sex-specific effects on placental and
405 fetal weights in the rat. *Nutrition & Metabolism*, 2019. 16(1): p. 1.
- 406 27. McClive, P.J. and A.H. Sinclair, Rapid DNA extraction and PCR-sexing of mouse
407 embryos. *Mol Reprod Dev*, 2001. 60(2): p. 225-6.
- 408 28. Bhasin, K.K., A. van Nas, L.J. Martin, et al., Maternal low-protein diet or
409 hypercholesterolemia reduces circulating essential amino acids and leads to
410 intrauterine growth restriction. *Diabetes*, 2009. 58(3): p. 559-66.
- 411 29. El-Sayyad, H.I.H., M.M.S. Al-Haggag, H.A. El-Ghawet, and I.H.M. Bakr, Effect of
412 maternal diabetes and hypercholesterolemia on fetal liver of albino Wistar rats.
413 *Nutrition*, 2014. 30(3): p. 326-336.
- 414 30. Napoli, C., F. de Nigris, S. Welch John, et al., Maternal Hypercholesterolemia
415 During Pregnancy Promotes Early Atherogenesis in LDL Receptor-Deficient Mice
416 and Alters Aortic Gene Expression Determined by Microarray. *Circulation*, 2002.
417 105(11): p. 1360-1367.
- 418 31. Goharkhay, N., E. Sbrana, P.K. Gamble, et al., Characterization of a murine
419 model of fetal programming of atherosclerosis. *Am J Obstet Gynecol*, 2007.
420 197(4): p. 416.e1-5.
- 421 32. Papacleovoulou, G., S. Abu-Hayyeh, E. Nikolopoulou, et al., Maternal cholestasis
422 during pregnancy programs metabolic disease in offspring. *The Journal of Clinical*
423 *Investigation*, 2013. 123(7): p. 3172-3181.
- 424 33. Napoli, C., F.P. D'Armiento, F.P. Mancini, et al., Fatty streak formation occurs in
425 human fetal aortas and is greatly enhanced by maternal hypercholesterolemia.
426 Intimal accumulation of low density lipoprotein and its oxidation precede
427 monocyte recruitment into early atherosclerotic lesions. *J Clin Invest*, 1997.
428 100(11): p. 2680-90.
- 429 34. Solini, A., E. Santini, S. Madec, C. Rossi, and E. Muscelli, Retinol-binding protein-
430 4 in women with untreated essential hypertension. *Am J Hypertens*, 2009. 22(9):
431 p. 1001-6.
- 432 35. Yang, Q., T.E. Graham, N. Mody, et al., Serum retinol binding protein 4
433 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, 2005.
434 436(7049): p. 356-62.
- 435 36. Graham, T.E., Q. Yang, M. Blüher, et al., Retinol-Binding Protein 4 and Insulin
436 Resistance in Lean, Obese, and Diabetic Subjects. *New England Journal of*
437 *Medicine*, 2006. 354(24): p. 2552-2563.

- 438 37. Dube, E., A. Gravel, C. Martin, et al., Modulation of fatty acid transport and
439 metabolism by maternal obesity in the human full-term placenta. *Biol Reprod*,
440 2012. 87(1): p. 14, 1-11.
- 441 38. Barkley, M.S., Geschwind, II, and G.E. Bradford, The gestational pattern of
442 estradiol, testosterone and progesterone secretion in selected strains of mice. *Biol*
443 *Reprod*, 1979. 20(4): p. 733-8.
- 444 39. Tal, R., H.S. Taylor, R.O. Burney, S.B. Mooney, and L.C. Giudice, *Endocrinology of*
445 *Pregnancy*, in *Endotext*, K.R. Feingold, et al., Editors. 2000, MDText.com, Inc.:
446 South Dartmouth (MA).
- 447 40. Mullen, M.P., N. Forde, M.H. Parr, et al., Alterations in systemic concentrations of
448 progesterone during the early luteal phase affect RBP4 expression in the bovine
449 uterus. *Reprod Fertil Dev*, 2012. 24(5): p. 715-22.

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451

452 **Table 1** Rat placental gene expression at day 20 gestation

	High LA (18% Fat)	High LA (36% Fat)	Low LA (18% Fat)	Low LA (36% Fat)
<i>ApoA2</i> *	0.75 ± 0.24	3.24 ± 1.13	0.52 ± 0.08	0.33 ± 0.20
<i>ApoC2</i> *	0.72 ± 0.27	2.91 ± 1.00	0.56 ± 0.08	0.34 ± 0.21
<i>Cubn</i> *	0.81 ± 0.35	2.92 ± 1.15	0.35 ± 0.06	0.10 ± 0.04
<i>Fgg</i> *	0.76 ± 0.30	2.97 ± 1.09	0.36 ± 0.06	0.12 ± 0.05
<i>Gpc3</i>	0.96 ± 0.28	1.46 ± 0.43	0.56 ± 0.09	0.50 ± 0.09
<i>Mttp</i>	0.66 ± 0.25 ^a	2.12 ± 0.77 ^a	0.28 ± 0.05 ^b	0.22 ± 0.09 ^b
<i>Prfl</i>	1.65 ± 0.42	0.73 ± 0.14	1.17 ± 0.12	1.15 ± 0.31
<i>Rbp4</i> *	0.69 ± 0.29	2.34 ± 1.17	0.48 ± 0.12	0.13 ± 0.05
<i>SerpinG1</i>	1.03 ± 0.26	1.00 ± 0.13	0.84 ± 0.21	1.20 ± 0.33
<i>Tagln</i>	0.95 ± 0.13	0.87 ± 0.06	0.67 ± 0.04	1.09 ± 0.17
<i>Ttr</i> *	0.65 ± 0.22	2.43 ± 0.81	0.52 ± 0.08	0.32 ± 0.17
<i>Vill</i>	1.23 ± 0.42	2.67 ± 0.97	0.51 ± 0.10	0.54 ± 0.22

453 * Indicates a significant interaction effect of maternal dietary LA:ALA ratio and total dietary

454 fat content on placental gene expression (P<0.05).

455 Different superscript indicate significant differences between groups (P<0.01).

456 **Table 2** Human participant characteristics

BMI (kg/m²)	<25	25-35	>35
Age (years)	34.29 ± 1.28	33.55 ± 1.07	31.25 ± 1.04
BMI	21.88 ± 0.36	29.70 ± 0.70	40.10 ± 1.07
Parity	1.05 ± 0.11	0.75 ± 0.14	1.20 ± 0.19
Gestation length (weeks)	38.63 ± 0.23	38.68 ± 0.18	38.65 ± 0.20
Birthweight (g)*	3236 ± 88	3537 ± 112	3565 ± 108
Sex (% male)	45	45	65
N	21	20	20

457 Data are shown as mean ± SEM for N observations per group. *ANOVA indicated that, with
 458 adjustment for gestational age, birthweight was influenced by maternal BMI (P=0.021)

459 **Table 3** Human placental gene expression

	BMI <25	BMI 25-35	BMI >35
<i>ApoA2</i>	1.04 ± 0.21	1.22 ± 0.27	0.53 ± 0.06
<i>ApoB**</i>	0.34 ± 0.06	2.19 ± 0.55	1.04 ± 0.25
<i>ApoC2</i>	0.90 ± 0.15	1.02 ± 0.25	0.76 ± 0.13
<i>Cubn</i>	1.22 ± 0.21	1.15 ± 0.15	0.86 ± 0.09
<i>Fgg</i>	0.48 ± 0.14	0.82 ± 0.41	0.65 ± 0.42
<i>Gpc3</i>	0.97 ± 0.20	1.30 ± 0.20	0.83 ± 0.19
<i>Mttp</i>	0.57 ± 0.08	1.23 ± 0.37	0.42 ± 0.08
<i>Prf1</i>	1.14 ± 0.19	0.85 ± 0.10	1.03 ± 0.17
<i>Rbp4**</i>	0.50 ± 0.13	0.07 ± 0.01	0.08 ± 0.01
<i>SerpinG1</i>	1.45 ± 0.24	0.90 ± 0.07	0.90 ± 0.11
<i>Tagln</i>	0.97 ± 0.11	1.04 ± 0.10	1.11 ± 0.10
<i>Tbp</i>	1.02 ± 0.11	1.16 ± 0.11	1.00 ± 0.07
<i>Ttr</i>	1.92 ± 0.90	0.23 ± 0.08	0.56 ± 0.20
<i>Vill</i>	1.14 ± 0.20	1.82 ± 0.33	1.01 ± 0.18

460 * Indicates a significant effect of maternal BMI on placental gene expression (**P<0.01).

461

462 **Supplementary Table S1** Primer sequences used for the determination of gene expression by

463 RT-qPCR

Target Genes		Rat	Human
<i>Cyclophilin A</i>	FWD:	TGATGGCGAGCCCTTGG	CCCACCGTGTCTTCGA
	REV:	TCTGCTGTCTTTGGAACCTTGTC	TGCTGTCTTTGGGACCTTGTC
<i>ApoA2</i>	FWD:	ACTGACTATGGCAAGGATTTGATG	GACCGTGACTGACTATGGCA
	REV:	CTCCTGTGCATTCTGAAAGTAAGC	CAAAGTAAGACTTGGCCTCGG
<i>ApoB</i>	FWD:	-	GGGCAGTGTGATCGCTTCA
	REV:	-	GCGGGTCATGCCTTTGAT
<i>ApoC2</i>	FWD:	GAGCACTTGTTTCAGTTACTGGAACTC	GACAGCCGCCAGAACCT
	REV:	TGCTGTACATGTCCCTCAGTTTCT	TTGCTGTACAAGTCCCTGAGTTTC
<i>Cubn</i>	FWD:	TGCATGTCACCTTCACGTTT	GGCGGATCACCTAATGTTTAA
	REV:	TGTAAGCCTCTCCACTCC	TTGAATACTATCACATGCTCATTGTTG
<i>Fgg</i>	FWD:	CTGGCTGGTGGATGAACAAGT	TCTGGTTGGTGGATGAACAAGT
	REV:	TGGAGTAAGTGCCACCTTGGT	TTTTGAGTAAGTGCCACCTTGGT
<i>Gpc3</i>	FWD:	CGGTTTTCCAAGAGGCCTTT	TCGTGGAGAGATACAGCCAAAA
	REV:	GTAGAGAGACACATCTGTGAAAAATTCA	GGCTCAGGGCCCTTCATT
<i>Mtp</i>	FWD:	TTTTCTCTGTTTCTTCTCCTCGTA	GAGTGGATCTTCTTCTGCCTACACT
	REV:	AGCTTGATAGCCCGCTATTATTTAAT	CCAGAACCCGAGTAGAGAATGTCT
<i>Prfl</i>	FWD:	GCTGGCTCCCATTCCAAGAT	CAACTTTGCAGCCAGAAAGAC
	REV:	GCCAGGCGAAAAGTGTACATG	TGTGTACCACATGGAAAAGTGTAGAAG
<i>Rbp4</i>	FWD:	GAGGAAACGATGACCACTGGAT	ACGAGACCGGCCAGATGA
	REV:	TGCAGGCGGCAGGAATA	CACACGTCCCAGTTATTCAAAAAGA
<i>SerpinG1</i>	FWD:	GACAGCCTGCCCTCTGACA	GCCCAGACCTGGCCATAAG
	REV:	TTTCTTCCACTTGGCACTCAAG	CCAAGTTGGCGTCACTGTTG
<i>Tagln</i>	FWD:	GGCGTGATTCTGAGCAAGTTG	GGCGTGATTCTGAGCAAGCT
	REV:	CATGGAGGGCGGGTTCTC	GACCATGGAGGGTGGGTTCT
<i>Ttr</i>	FWD:	CCGTTTGCCTCTGGGAAGA	CTTGCTGGACTGGTATTTGTGTCT
	REV:	CCCCTCCGTGAACTTCTCA	AGAACTTTGACCATCAGAGGACACT
<i>Vill</i>	FWD:	FWD: AACCAGGCTTTGAACTTCATCAA	CTGAGCGCCCAAGTCAAAG
	REV:	REV: CGGACTCAGCCCCATCATT	CATGGCCTCGATCCTCCATA

464 Forward (FWD) and reverse (REV) primer sequences designed using Primer Express

465 software (version 1.5; Applied Biosystems)