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Altered materno-fetal transfer of 13C-polyunsaturated fatty acids in obese pregnant women

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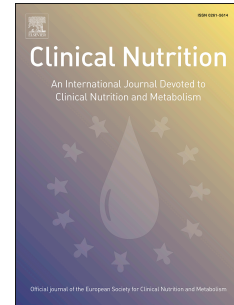
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3 **Altered materno-fetal transfer of ^{13}C-polyunsaturated fatty acids in obese**

4 **pregnant women**

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24 **Abbreviations:** AUC, area under the curve; BMI, body max index; CE, cholesterol

25 esters; DHA, docosahexaenoic acid; FA, fatty acid; GC-C-IRMS, gas chromatography

26 combustion isotope ratio mass spectrometry; GDM, gestational diabetes mellitus;

27 HOMA, homeostatic model assessment for insulin resistance, LA, linoleic acid; LC-

28 PUFA, long-chain polyunsaturated fatty acid; LD, lipid droplets; NEFA, non-esterified

29 fatty acids; OA, oleic acid; PL, phospholipids; TG, triglycerides.

30

Abstract

Background & Aims: Maternal obesity at conception is considered a major predictor of offspring obesity. This could by driven at least in part by an altered placental fat transfer. However, the pathophysiological mechanisms involved are not fully understood. We investigated the *in vivo* materno-fetal transfer of fatty acids (FAs) in obese pregnant women using stable isotopes.

Methods: Ten obese and ten normo-weight pregnant women (control) received orally a bolus of ^{13}C-labelled FAs 12h before elective caesarean section: oleic acid (^{13}C-OA), linoleic acid (^{13}C-LA) and docosahexaenoic acid (^{13}C-DHA). Maternal blood samples were collected at -12 (basal), -8, -4, -2, 0 hours relative to the time of cesarean section. At the time of birth, arterial and venous cord bloods as well as placental tissue were collected. FAs composition was determined by gas-liquid chromatography and isotopic enrichment by gas chromatography-combustion-isotope ratio mass spectrometry.

Results: Maternal plasma insulin and placental weight tended to higher values in obese pregnant women although they did not present serum hyperlipidemia. Higher concentrations of ^{13}C-LA and ^{13}C-DHA were found in non-esterified FAs fraction in maternal plasma of obese mothers. The ratio of placental uptake for ^{13}C-LA and ^{13}C-DHA was lower in obese women compared to normal weight pointing toward a limited capacity of FA placental transfer, especially of essential FAs. Maternal insulin was associated to this lower placenta/maternal plasma ratio for both ^{13}C-LA ($R= -0.563$, $P=0.012$) and ^{13}C-DHA ($R= -0.478$, $P=0.033$). In addition, the ratio cord/maternal plasma of ^{13}C-LA was significantly lower in obese women compared to controls.

3

54   **Conclusions:** In conclusion, obese mothers without hyperlipidemia showed a reduced

55   materno-fetal transfer of polyunsaturated FAs which could affect fetal development.

56   This affect dietary recommendation for obese pregnant women.

57

58   **Keywords:** placenta, labelled fatty acids, docosahexaenoic acid, linoleic acid, obesity.

59

60   **1. Introduction**

61

62   Obesity and overweight prevalence has reached epidemic proportions during the

63   last few decades, now affecting more than a third of populations in both developed and

64   developing countries (1-3). Accordingly, there is also an increasing number of obese

65   women becoming pregnant, along with a large number of women with high gestational

66   weight gain (4-5). Obesity with a body max index (BMI) >30 kg/m^2 at conception and

67   in the first trimester of pregnancy is considered one of the strongest early predictors of

68   childhood obesity (6-7). Ample evidence has linked nutrition and metabolic

69   environment during pregnancy with fetal development and long-term health outcomes

70   in the offspring, indicating that obesity programming starts already in utero (8-9).

71   Maternal obesity promotes a lipotoxic placental environment and altered fetal

72   lipid concentrations and modifies gene expression at term, e.g. with regards to

73   inflammatory signalling, oxidative stress and energy metabolism (10-12). Maternal

74   obesity is associated with increased child risks for disadvantaged neurodevelopment,

75   obesity, cardiovascular diseases and diabetes, and shortened life expectancy (13-14).

76   Expression of some genes involved in fatty acid (FA) transfer and lipid metabolism is

77   enhanced in placental tissue of obese women, e.g. FA transporter protein 1, perilipin 2,

78   and FAT/CD36, while expression of the FA transporter protein 4 gene was decreased

79   (12,15-18). It is important to determine whether placental changes may affect materno-

80   fetal FA transfer.

81   Previous studies with stable isotopes in gestational diabetes mellitus (GDM)

82   demonstrated an impaired materno-fetal transfer of docosahexaenoic acid (DHA) (19-

83   20), whereas the transfer of other non-long chain polyunsaturated FAs (LC-PUFA) was

84   not affected by GDM although tended to increase (21). GDM and obesity are often

5

85 linked, however little is known about the effects of maternal obesity without diabetes on

86 the placental function and transfer of FAs to the fetus (17). LC-PUFAs, especially

87 DHA, are of critical importance for the visual and neuronal development of infants (22).

88 In a recent cohort of 435 mother-infant pairs, the pre-pregnancy BMI affected the fetal

89 lipid profile with decreased cord n-6 LC-PUFAs and DHA (23). However, Segura et al.

90 reported an increase in placental LC-PUFAs in obese mothers, although the sample size

91 was small (18). In addition, there are discrepancies in placental total FA content in

92 obese placentas and several studies but not all reported higher fat content in this tissue

93 (18,24).

94   We studied for the first time the *in vivo* metabolism and materno-fetal transfer of

95 FAs labelled with stable isotopes in obese and normal weight pregnant women

96 administered 12h before elective caesarean section. Better knowledge on the materno-

97 fetal transfer of FAs in pregnancies with obesity may have potential implications on

98 effective dietary recommendations for obese pregnant women.

99

100   **2. Methods**

101

102   *2.1. Subjects*

103

104   10 obese pregnant women (pre-pregnancy BMI >30 kg/m^2) and 10 normal

105   weight women (pre-pregnancy BMI 20-25 kg/m^2) were recruited at term in the

106   Obstetrics and Gynecology Service of the Virgen de la Arrixaca Clinical Hospital

107   (Murcia, Spain). All participants fulfilled the following inclusion criteria: singleton

108   pregnancy, age 18-40 years, plan to undergo elective caesarean section at term,

109   omnivorous diet, no DHA supplements (last trimester), non-smoking and normal fetal

110   Doppler scan within the normal reference range (25) on the day before caesarean

111   section. Women with any health problem or pregnancy complications were excluded.

112   All women had a negative result in their O´Sullivan test in the second trimester, so

113   GDM patients were not included. All procedures followed were in accordance with the

114   Helsinki Declaration of 1975 as revised in 1983. Written informed consent was obtained

115   from all participating women. The protocol of this study was approved by the Ethics

116   Committee of the Virgen de la Arrixaca Clinical Hospital, Murcia, Spain.

117

118   *2.2. Stable isotope tracer administration*

119

120   Three free FAs uniformly labelled with ^{13}C stable isotope (ISOLIFE S.L.,

121   Wageningen, The Netherlands) were administered orally on a small piece of bread 12h

122   before elective caesarean section: ^{13}C-oleic acid (OA, 18:1n-9, 0.5 mg/kg body weight);

123   ^{13}C-linoleic acid (LA, 18:2n-6, 0.5 mg/kg) and ^{13}C-DHA (22:6 n-3, 0.1 mg/kg). The

124   labelled FAs were given at 9:00h in the morning and the caesarian section took place

7

125   12h later (21:00h). Subjects received a breakfast free of DHA and remained fasted until

126   the surgery. This schedule was set during the day in order not to disturb the mothers

127   night's sleep. In previous studies, 12h post-administration has been shown to be a good

128   model for the study of FA metabolism in pregnant women that allows the incorporation

129   of the tracers in the four plasma lipid fractions (26).

130

131   *2.3. Blood and placenta sampling*

132

133   Maternal blood samples were collected before tracer intake (basal time at -12h

134   respect to delivery) and every four hours (-8h and -4h), with an additional point at -2h

135   prior to the caesarean section (0h), using a venous line placed in the forearm of the

136   subject. We collected 10mL of maternal blood at each time point except for the sample -

137   2h before delivery when only 5mL blood were taken. Directly after birth, 2mL of each

138   cord venous and arterial blood were collected. All blood samples were collected into

139   EDTA-coated tubes and centrifuged for 3min at 1200g to separate plasma and red blood

140   cells. Samples were stored at -80ºC until analysis.

141   An aliquot of placental tissue was taken from the four imaginary quadrants of

142   the tissue, rinsed in cold 0.9% NaCl solution, immediately frozen in liquid nitrogen and

143   stored at -80°C until analysis.

144

145   *2.4. Plasma biochemical analysis*

146

147   Maternal plasma biochemical parameters at delivery and venous cord insulin

148   were analyzed by chemioluminescence (DIAsource INSIRMA, Nivelles, Belgium).

149   Glucose, serum total cholesterol, triglycerides (TG), LDL cholesterol and HDL

150 cholesterol were quantified by an automatic analyzer (Roche-Hitachi Modular PyD

151 Autoanalyzer, Mannheim, Germany).

152   The homeostatic model assessment (HOMA) index for insulin resistance was

153 calculated according to the following formula: (glucose mg/dL * insulin µU/mL) / 405.

154

155 *2.5. Placental lipid droplets isolation*

156

157   Lipid droplets (LD) were isolated from placental tissue as previously described (27).

158 Briefly, 5g of placenta were gently rinsed with cold 0.9% NaCl to eliminate blood. Samples

159 were transferred to 12mL buffer (25mM tricine, 250mM sucrose, 0.2mM phenylmethylsulfonyl

160 fluoride, pH 7.6) and homogenized using a T25 digital Ultra-Turrax® (IKA, Staufen, Germany)

161 disperser for 1min followed by homogenization in a tissue grinder Potter-Elvehjem (25 times).

162 Homogenates were centrifuged (3,000g, 10min, 4ºC). Supernatants were transferred to an Ultra-

163 Clear centrifuge tube (Beckman Coulter, CA, USA) and 2mL buffer (20mM HEPES, 100mM

164 KCl, 2mM $MgCl_2$, pH 7.4) were loaded on the top of the tissue homogenate. The supernatants

165 were again centrifuged (288,000g, 54min, 4ºC) in a Beckman Optima XL100 ultracentrifuge

166 equipped with a swinging bucket SW41Ti rotor (Beckman Coulter, CA, USA). After

167 centrifugation, the floating white band containing LD was collected.

168

169 *2.6. Analysis of fatty acid concentrations and enrichments*

170

171   FAs were analyzed in maternal plasma samples from all time points, placental

172 tissue and LD, venous and arterial cord plasma. Total lipids from 250µL of plasma and

173 0.8g of placental tissue were extracted into 3mL (plasma) or 6mL (placenta)

174 chloroform-methanol (2:1 v/v) according to the modified Folch method (28). Free

175 pentadecanoic acid, dipentadecanoyl phosphatidylcholine, tripentadecanoin and

176 cholesterylpentadecaoate (Sigma-Aldrich, Steinheim, Germany) were added as internal

177 standards. The lipid extract was dried down under nitrogen flow, redissolved in 400µL

178 chloroform-methanol (1:1 v/v) and applied to silica gel plates (Merck, Darmstadt,

179 Germany) for the isolation of lipid classes. Phospholipids (PL), non-esterified FAs

180 (NEFA), TG and cholesterol esters (CE) were isolated by development of the plates in

181 N-heptane/diisopropylether/glacial acetic acid (60:40:3, by vol). Synthesis of FAs

182 methyl esters was performed with 3N methanolic HCl (Supelco, PA, USA) at 90°C for

183 1h (29). A direct isolation and transesterification method was applied for NEFA

184 quantification in plasma samples (30). After drying under a gentle stream of nitrogen

185 the samples were redissolved in hexane containing butylated hydroxytoluene (2g/L) and

186 stored at -20°C until gas chromatography combustion isotope ratio mass spectrometry

187 analysis (GC-C-IRMS). ^{13}C enrichment of FAs was measured by GC-C-IRMS (Delta

188 XP, Thermo Electron, Bremen, Germany) equipped with a BPX70 (30m x 0.25mm x

189 0.25um) GC column (SGE, Milton Keynes, United Kingdom). The injection volume

190 varied amongst the samples, to achieve AUCs for all methylated FAs within the linear

191 dynamic range of the instrument. The procedure was slightly adapted from one

192 previously described (31).

193

194 *2.7. Expression of results*

195

196 From the ^{13}C/^{12}C ratio of the samples measured by GC-C-IRMS, the δ^{13}C

197 relative to the international Pee Dee Belemnite standard and the ^{13}C-FAs molar percent

198 excess was calculated (32), which represented the FA ^{13}C-enrichment. The

199 concentration of the labelled FAs (µmoL/L or nmoL/g ^{13}C-FA) was calculated by

200 multiplying the absolute concentrations of the FAs by their molar percent excess values.

201   For maternal plasma concentrations of ^{13}C-FAs, the area under the curve (AUC; µmol

202   ^{13}C-FA x h/L) was calculated by integrating the measured ^{13}C-FAs concentration until

203   delivery over time according to the trapezoidal rule.

204   The distribution ratio (%) of the tracer FAs between mother and fetus was

205   estimated by the calculation of the tracer concentration in venous cord blood relative to

206   AUC in maternal plasma. The distribution ratio of tracer FAs between placental tissue

207   and maternal plasma AUC was calculated as the percentage of the tracer concentration

208   in placenta relative to AUC in maternal plasma. Similarly, distribution ratios were also

209   calculated using enrichment values between venous cord blood/maternal plasma and

210   placenta/maternal plasma. More details on calculations of enrichments of maternal

211   plasma and placenta or cord blood can be found in Larqué et al. 2017 (33).

212

213   *2.8. Statistical analysis*

214

215   The results are expressed as means ± SEM. Obese and control groups were

216   compared using t-test. Pearson correlations were also performed. The significance level

217   was set at $P<0.05$. Statistical analysis was performed with SPSS software version 23.0

218   (SPSS, IL, USA).

219

220 **3. Results**

221   Maternal serum TG and cholesterol levels were similar between both groups,

222 which implies we enrolled "healthy obese" women without dyslipidaemia (**Table 1**).

223 Maternal BMI was significantly higher in obese than control pregnant women both

224 before pregnancy and at delivery (P<0.001) (Table 1). Placental weight tended to be

225 higher in the obese group ($P=0.051$), along with a trend towards higher insulin serum

226 concentrations ($P=0.090$) and HOMA index ($P=0.093$). The small sample size included

227 due to the demanding study procedures limited statistical power regarding describing

228 differences of anthropometrical measurements on the neonates (Table 1).

229   The maternal blood concentrations of labelled FAs were stable or slightly

230 decreasing by the time of delivery in both groups after 12h of administration (**Figure 1**).

231 No differences were found for ^{13}C-FA concentrations in total lipids of maternal plasma

232 (AUC) (**Table 2**). However, ^{13}C-DHA concentration in plasma NEFA was significantly

233 higher in the obese group compared to controls, following ^{13}C-LA the same trend

234 ($P=0.058$) (**Table 2**). These discrepancies were not produced by different dilution of the

235 tracers in the maternal unlabelled FA pools since both groups of pregnant women

236 presented similar NEFA concentrations in plasma (Table 1).

237   In placenta, the tracer concentrations per gram of tissue were similar between

238 groups in both total lipids and placental lipid fractions (Table 2 and **Figure 2A**).

239 Nevertheless, obese placentas tended to higher unlabelled fat content ($P=0.127$) (Table

240 1) while ^{13}C-FA concentration in placental LD followed the opposite sense, especially

241 for DHA (P=0.182, **Figure 2B**). Surprisingly, the placenta/maternal plasma ratio

242 showed lower FAs uptake in obese subjects, mainly for ^{13}C-LA and ^{13}C-DHA (**Figure**

243 **2C and 2D**). The differences were statistically significant when expressed as

244 enrichment ratios (Figure 2D). Maternal insulin was associated to this lower

245 placenta/maternal plasma ratio for both ^{13}C-LA (R= -0.563, *P*=0.012) and ^{13}C-DHA

246 (R= -0.478, *P*=0.033), indicating a likely role of this molecule in the uptake process of

247 FA from plasma by the placenta.

248   No differences were found for ^{13}C-FA concentrations in venous cord plasma,

249 although ^{13}C-DHA tended to a lower value in the obese subjects (*P*=0.069, **Figure 3A**).

250 Similar results were observed in artery cord plasma (**Figure 3B**). Arterio-venous

251 difference, which indicates an approximation of the tracer amount retained in the fetal

252 metabolism, was similar between obese and control subjects for all studied FAs ([13]C-

253 OA: Obese $0.005 \pm 0.002 \mu mol/L$ *vs.* Control: $0.004 \pm 0.005 \mu mol/L$, *P*=0.886 ; ^{13}C-LA:

254 Obese $0.010 \pm 0.003 \mu mol/L$ *vs.* Control: $0.007 \pm 0.007 \mu mol/L$, *P*=0.692 ; ^{13}C-DHA:

255 Obese $0.002 \pm 0.001 \mu mol/L$ *vs.* Control: $0.003 \pm 0.001 \mu mol/L$, *P*=0.620).

256   Materno-fetal transfer of ^{13}C-LA across the placenta, indicated by the ratio

257 between cord venous and maternal plasma AUC, was significantly lower in obese

258 mothers than in controls (**Figure 3C**), not only using concentration ratio but also

259 enrichment ratio (**Figure 3D**). A similar trend was observed for ^{13}C-DHA although no

260 statistical differences were found (Figure 3C and 3D).

261 **4. Discussion**

262

263   Studies on the mechanisms regulating the delivery of lipids to the human fetus

264 are critical to improve our understanding on fetal nutrition. This study evaluated the

265 incorporation and metabolism of stable isotope labelled-FAs (OA, LA and DHA) in

266 maternal, placenta and fetal compartments in "healthy" obese and normal weight

267 pregnant women. Obese mothers did not present hyperlipidaemia, and both groups

268 showed similar serum NEFA, cholesterol and TG levels. This should be taken in

269 consideration, since not all obese subjects develop hyperlipidemia and usually healthy

270 and unhealthy obese subjects are mixed. In fact, similar TG levels have been reported in

271 obese mothers and in normal weight mothers (34), while GDM mothers usually present

272 elevated TG levels (35-36).

273   In the present study there were no major differences between groups in the

274 incorporation of the administered tracer FAs in maternal plasma. Obese women

275 presented higher ^{13}C-DHA and ^{13}C-LA concentration in plasma NEFA compared to

276 controls. The second half of gestation is characterised by a catabolic state that induces

277 an increase of NEFA and glycerol levels in plasma (37). Nevertheless, the lack of

278 differences in total unlabelled plasma NEFA concentration between both groups could

279 be due to the relative "healthy" state of obese subjects in this study. The higher ^{13}C-

280 DHA and ^{13}C-LA concentration in maternal plasma NEFA could indicate an impaired

281 PUFA placental uptake in these subjects. In fact, maternal insulin was inversely

282 associated to their ratio of placental uptake, as occurs in GDM (38). The trend to higher

283 peripheral insulin resistance (HOMA) in obese women may enhance insulin anabolic

284 effect in the placenta, altering structure and FA transport as occurs in GDM (39).

14

285   Total ^{13}C-FA concentrations in placenta were similar in both groups of pregnant

286   women but obese placentas showed a trend towards higher concentration of unlabelled

287   FAs, which agrees with findings of higher fat accumulation in overweight and obese

288   placentas (10,24). An increase of inflammation molecules, PPAR-ɣ, (40-42) or higher

289   fat synthesis from high glucose supply (43) may promote heavier placentas with higher

290   fat content.

291   ^{13}C-LA and ^{13}C-DHA placental uptake ratio was lower in obese women than in

292   controls and they were negatively correlated to maternal insulin level, possibly as a

293   compensatory mechanism to limit the total materno-fetal FA transfer. LD are

294   intracellular structures that store neutral lipids (TG and CE), and they are implicated in

295   the regulation and hydrolysis of cell fat stores (44). There were not differences in ^{13}C-FA

296   concentrations in placental LD but since they pointed to lower values in obese placentas

297   (Figure 2B), an endogenous origin of placental fat depots from maternal glucose in

298   obese placentas might occur. Thus, placenta de novo lipogenesis will dilute the apparent

299   enrichment of ^{13}C-FA within placental LD pool. This might contribute to counteract

300   excessive maternal fat uptake by the placenta in these glucose-tolerant obese women.

301   Calabuig-Navarro et al. recently reported higher mRNA and protein expression of FA

302   esterification regulators along with a decreased FA oxidation capacity in placentas from

303   obese women (24). It is unknown whether women with more severe obesity,

304   hyperlipidemia and marked insulin resistance could present higher placental fat uptake

305   of non-LC-PUFAs as occurs in patients with GDM (21). Further studies are needed to

306   evaluate the impact of different degrees of obesity and insulin resistance in placenta

307   metabolism and function.

308   Brass and colleagues reported a sex effect on OA and arachidonic acid placental

309   uptake in obese women, while no sex differences were observed for DHA uptake (45).

15

310   Other authors also found different placental adaptations to maternal weight status (BMI

311   and gestational weight gain) depending on fetal sex (46). In our study, 6 neonates were

312   males in the obese group and only 3 in the control group (n=10/group). We cannot

313   discard an implication of fetal sex in the rate of FAs uptake by the placenta, but due to

314   the low numbers of subjects per group we cannot elucidate any possible sex related

315   effect on placental FA transfer.

316   We found significant lower materno-fetal transfer of ^{13}C-LA in normolipidemic

317   obese subjects compared to controls, with a similar trend for ^{13}C-DHA (P=0.148). In

318   fact, ^{13}C-DHA concentration in cord venous plasma of obese mothers was almost

319   significantly lower respect to normal weight women (P=0.069), while its concentration

320   in total lipids of maternal plasma or placenta were not different (Table 2). This may

321   support in part an impaired materno-fetal transfer of this FA. Since ^{13}C-DHA increased

322   significantly in plasma NEFA of obese subjects, an altered transfer of DHA in this lipid

323   fraction could affect obese pregnancies. These results are in agreement with decreased

324   cord blood DHA reported in obese mothers in a recent cohort study (23). Dubé and

325   colleagues also found lower LA transport in freshly isolated cytotrophoblast cells from

326   obese women relative to controls, although these differences were not observed using

327   syncytiotrophoblast cells (17). An impaired materno-fetal transfer of essential and LC-

328   PUFAs, especially DHA, during pregnancy could be considered as an important risk

329   factor for nervous, visual and immune development later in life (47). It has been

330   reported that children whose mothers were severely obese before pregnancy have

331   decreased cognition and language scores (48) as well as adverse psychosocial

332   development at 6 years of age (49). It is tempting to speculate that an increased

333   polyunsaturated FAs intake during pregnancy might overcome placental function

334   alterations in obese pregnant women and improve child outcomes, which should be

335 explored further. Arterio-venous ^{13}C-FA concentration differences, which reflect fetal

336 retention and metabolism, were similar in obese and normal weight women, although

337 with large standard deviations. Thus, we do not find indications for a change in fetal

338 metabolism of transferred ^{13}C-FAs despite altered placental metabolism and transfer of

339 FAs with maternal obesity.

340   One of the most important strengths of the present study is that timing of

341 caesarean sections were exactly scheduled which allowed us to collect representative

342 AUC maternal blood samples along the 12 hours of the study, as well as placental and

343 cord blood samples at a defined time interval after tracer administration. Limitations

344 include the small sample size as a consequence of the very demanding study protocol

345 with caesarean sections at night, and the inclusion of obese mothers without

346 hyperlipidaemia and marked insulin resistance, which could have influenced the results

347 obtained.

348

349   We conclude that placental metabolism is altered in normolipidemic obese

350 pregnant women reducing the materno-fetal transfer of LA and DHA, when compared

351 to normal weight women. Placental lipogenesis from glucose might reduce the placental

352 lipid uptake in these subjects to counteract excessive lipid transfer. It is uncertain

353 whether hyperlipidemic obese women placenta may still retain placental lipid uptake. It

354 appears prudent that obese pregnant women limit their intake of both sugars and

355 saturated fats while increasing polyunsaturated FAs consumption, which should be

356 further evaluated.

357

358

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Statement of Autorship

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performed research; A. Gázquez, D. van Harskamp, J.E. Oosterink and H. Schierbeek

analyzed data; A. Gázquez performed statistical analysis, A. Gázquez and E. Larqué

wrote the paper and had primary responsibility for final content.

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All authors read and approved the final manuscript. None of the authors reported any

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384

385   **6. References**

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541 **Legend of figures**

542 **Figure 1.** Time course ^{13}C-fatty acids concentrations in maternal plasma of obese and

543 normal weight pregnant women. A) ^{13}C-Oleic acid (^{13}C-OA). B) ^{13}C-Linoleic acid (^{13}C-

544 LA). C) ^{13}C-Docosahexaenoic acid (^{13}C-DHA). Time point 0 indicates the time of

545 caesarean section; tracers were administered 12h before surgery. Control group (open

546 squares), n=10 and obese group (black squares), n=10. Results are expressed as means ±

547 SEM. Common superscript letters indicate similar values between different time points.

548 Significant differences when P<0.05.

549

550 **Figure 2.** A: ^{13}C-fatty acid concentrations in placental non-esterified fatty acid fraction

551 (^{13}C-OA P=0.11, ^{13}C-LA P=0.13, ^{13}C-DHA P=0.19). B: ^{13}C-fatty acid concentrations in

552 placental lipid droplets (LD). C: Placental ratio between ^{13}C-fatty acid concentrations in

553 total lipids of placenta and maternal plasma. D: Placental ratio between ^{13}C-fatty acid

554 enrichments in total lipids of placenta and maternal plasma. Control group (open bars),

555 n=10 and obese group (black bars), n=10. Results are expressed as means ± SEM.

556 *Indicates statistically significant differences (P<0.05) between control and obese

557 group. OA, oleic acid; LA, linoleic acid; DHA, docosahexaenoic acid.

558

559 **Figure 3.** A: ^{13}C-fatty acid concentrations in cord venous plasma (^{13}C-DHA P=0.069);

560 B: ^{13}C-fatty acid concentrations in cord artery plasma. C: Placental ratio between ^{13}C-

561 fatty acid concentrations in cord venous and maternal plasma (^{13}C-DHA P=0.148). D:

562 Placental ratio between ^{13}C-fatty acid enrichments in cord venous and maternal plasma

563 (^{13}C-DHA P=0.169). Control group (open bars), n=10 and obese group (black bars),

564 n=10. Results are expressed as means ± SEM. *Indicates statistically significant

565 differences (P<0.05) between control and obese group. OA, oleic acid; LA, linoleic

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566   acid; DHA, docosahexaenoic acid.

567   Table 1. Characteristics of the mothers and neonates at delivery.

	Control (n=10)	Obese (n=10)	*P*
Maternal Age (years)	33.80 ± 1.87	34.50 ± 2.31	0.807
Gestational Age (weeks)	38.50 ± 0.28	38.40 ± 0.42	0.838
Maternal Pregestational BMI (kg/m^2)	22.51 ± 0.50	32.22 ± 0.92	**<0.001**
Maternal BMI at delivery (kg/m^2)	28.22 ± 0.57	35.63 ± 1.18	**<0.001**
Placental Weight (g)	612.86 ± 58.06	755.00 ± 42.82	0.051
Maternal Glucose (mg/dL)	59.80 ± 2.48	62.10 ± 6.271	0.377
Maternal Insulin (µU/mL)	9.47 ± 1.50	14.73 ± 2.68	0.090
HOMA index	1.39 ± 0.24	2.40 ± 0.55	0.093
Maternal TG (mmol/L)	1.94 ± 0.25	1.95 ± 0.17	0.973
Maternal plasma NEFA (mmol/L)	0.90 ± 0.11	0.91 ± 0.07	0.938
Maternal total cholesterol (mmol/L)	6.77 ± 0.40	6.31 ± 0.39	0.399
Maternal LDL cholesterol (mmol/L)	3.81 ± 0.31	3.37 ± 0.42	0.385
Maternal HDL cholesterol (mmol/L)	2.06 ± 0.24	2.00 ± 0.18	0.834
Maternal total FA (mmol/L)	14.54 ± 1.02	14.52 ± 0.98	0.986
Placental total FA (nmol/g)	32.57 ± 2.35	37.21 ± 1.99	0.127
Cord venous total FA (mmol/L)	3.27 ± 0.38	3.13 ± 0.36	0.776
Birth Weight (g)	3241 ± 134	3512 ± 169	0.203
Birth Length (cm)	49.80 ± 0.54	50.50 ± 0.71	0.418
Neonatal Cephalic Circumference (cm)	34.50 ± 0.34	34.65 ± 0.47	0.787
Neonatal Abdominal Circumference (cm)	33.75 ± 0.61	35.10 ± 0.74	0.170
Cord Glucose (mg/dL)	32.80 ± 5.19	46.63 ± 5.85	0.105
Cord Insulin (µU/mL)	6.16 ± 0.57	5.86 ± 0.42	0.676

568

569   Results are expressed as means ± SEM. Significant differences when *P* <0.05. BMI,

570   body mass index; FA, fatty acids; HDL, high density lipoproteins; HOMA, homeostatic

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571   model assessment; NEFA, non-esterified fatty acids; LDL, low density lipoproteins;

572   TG, triglycerides.

573   Table 2. ^{13}C-fatty acid concentration and enrichment in both plasma and placenta of obese and normal weight pregnant women.

	^{13}C-Oleic acid			^{13}C-Linoleic acid			^{13}C-Docosahexaenoic acid		
	Control	Obese	P	Control	Obese	P	Control	Obese	P
Plasma ^{13}C-FA AUC (µmol x h/L)									
Total lipids	20.92 ± 3.67	19.34 ± 1.70	0.687	30.94 ± 6.38	35.83 ± 4.04	0.503	3.61 ± 0.65	3.53 ± 0.38	0.906
Phospholipids	2.78 ± 0.89	2.02 ± 0.19	0.402	19.09 ± 5.36	21.25 ± 2.66	0.708	2.22 ± 0.38	1.85 ± 0.18	0.359
Triglycerides	16.18 ± 3.38	14.84 ± 1.48	0.694	8.48 ± 1.69	9.34 ± 1.44	0.684	1.46 ± 0.46	1.49 ± 0.20	0.947
Non-esterified FA	1.25 ± 0.19	1.29 ± 0.13	0.865	0.83 ± 0.08	1.19 ± 0.16	0.058	0.09 ± 0.01	0.18 ± 0.04	**0.046**
Cholesterol esters	1.42 ± 0.35	1.32 ± 0.26	0.804	3.29 ± 0.82	4.36 ± 0.73	0.313	0.02 ± 0.01	0.02 ± 0.01	0.608
Plasma ^{13}C-FA enrichment (MPE %)	0.04 ± 0.01	0.05 ± 0.01	0.649	0.06 ± 0.01	0.07 ± 0.01	0.356	0.05 ± 0.01	0.07 ± 0.01	0.100
Placenta ^{13}C-FA concentration (nmol/g)									
Total lipids	0.74 ± 0.04	0.69 ± 0.03	0.322	1.66 ± 0.17	1.69 ± 0.09	0.855	0.38 ± 0.04	0.35 ± 0.04	0.545
Phospholipids	0.66 0.03	0.60 0.02	0.168	1.52 0.13	1.53 0.07	0.990	0.30 0.03	0.26 0.08	0.375
Triglycerides	0.03 0.01	0.02 0.01	0.764	0.04 0.01	0.04 0.01	0.506	0.03 0.01	0.03 0.01	0.506
Cholesterol esters	0.01 0.01	0.01 0.01	0.663	0.02 0.01	0.02 0.01	0.731	0.01 0.01	0.01 0.01	0.267
Placenta ^{13}C-FA enrichment (MPE %)	0.03 0.01	0.03 0.01	0.144	0.07 0.01	0.07 0.01	0.788	0.03 0.01	0.03 0.01	0.673

574   Results are expressed as means ± SEM. Significant differences when $P < 0.05$. AUC, area under the curve; FA, fatty acid. AUC was calculated

575   using ^{13}C-FA concentrations from the four time points of plasma sampling (-8h, -4h, -2h, 0h). Plasma enrichment was calculated as mean of the

576   four ^{13}C-FA MPE measures. Control n=10, Obese n=10.