Fetal oxygen and glucose consumption in human pregnancy complicated by fetal growth restriction

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1 **Abstract**

2 In healthy pregnancy, glucose and oxygen availability are essential for fetal growth and wellbeing.

3 However, how substrate delivery and fetal uptake are affected in human pregnancy complicated

4 by fetal growth restriction (FGR) is still unknown. Here we show that the human FGR fetus has a

5 strikingly reduced umbilical uptake of both oxygen and glucose. In 30 healthy term and 32 FGR

6 human pregnancies umbilical volume flow (Qumb) and parallel umbilical vein (uv) and artery (ua)

7 blood samples were obtained at elective Caesarean section to calculate fetal glucose and oxygen

8 uptake as Qumb • Δ (uv-ua) differences. Umbilical blood flow was significantly lower in FGR

9 pregnancy (-63%, P<0.001) but not when normalized for fetal body weight. FGR pregnancy had

10 significantly lower umbilical oxygen delivery and uptake, both as absolute values (delivery: –78%;

11 uptake: –78%) and normalized (delivery: –50%; uptake: –48%) for fetal body weight (all P<0.001).

12 Umbilical glucose absolute delivery and uptake were significantly reduced (delivery: –68%;

13 uptake: –72%) but only glucose uptake was decreased when normalized for fetal body weight (–

14 30%, P<0.05). The glucose/oxygen quotient was significantly increased (+100%, P<0.05) while

15 glucose clearance was significantly decreased (71%, P<0.001) in FGR pregnancy (both P<0.05).

16 The human fetus in FGR pregnancy triggers compensatory mechanisms to reduce its metabolic

17 rate, matching the proportion of substrate consumption relative to oxygen delivery as a survival

18 strategy during complicated pregnancy.

19 **Key words:** fetus, oxygen consumption, glucose consumption, oxygen delivery, glucose delivery,

20 FGR

21 **Introduction**

Fetal growth restriction (FGR) is associated with poor placentation and incomplete remodeling of the uteroplacental spiral arteries (1). This implies reduction of utero-placental blood flow, thereby impairing oxygen and substrate delivery to the fetus and slowing its growth trajectory (2). Progressive severity of FGR based on increased utero-placental vascular resistance and fetal heart rate trace abnormalities is associated with increasing fetal hypoxia and potential fetal brain damage (3,4). Individuals who are born following FGR are known to be at increased risk of cardiovascular diseases (5). This is likely due to fetal metabolic adaptations essential to allow fetal survival within the adverse intrauterine environment, but also increases the risk of pathology in the offspring in later life. However, the nature of these fetal compensatory metabolic adaptations in FGR pregnancy remains very unclear, particularly for the human fetus.

Fetal conversion of energy into mass can most easily be determined by measurement of the rate of fetal oxygen uptake (6). Since there is no long-term storage of oxygen, its uptake and utilization are almost identical over short periods of time. Over the years, the chronically catheterized pregnant sheep model has permitted the estimation of fetal oxygen consumption by measuring the rate of umbilical blood flow together with the umbilical venous-arterial difference in oxygen content (7). Similarly, glucose uptakes have been measured in the pregnant sheep model (7) and recently in human pregnancy (8). Glucose represents the most important fetal nutrient and in human pregnancies the glucose/oxygen quotient has revealed that the utilization of glucose accounts for approximately 80% of oxygen uptake (9).

More recently, the technical improvement in the accuracy of measurement of umbilical venous blood flow in the human fetus from the second half of gestation (10; 11) has made it possible to estimate the fetal uptake of oxygen in human pregnancy (12). However, for human pregnancy, any information on fetal oxygen consumption in complicated pregnancy is still very limited compared

45 with measurements in normal pregnancies at term. Further, no information exists for the

46 consumption of glucose in the human fetus.

47 Therefore, the objective of this study was to determine changes in the umbilical uptake of both

48 oxygen and glucose in human fetuses of FGR compared to healthy term pregnancy. The study

49 tested the hypothesis that the human fetus in FGR pregnancy reduces its metabolic rate, changing

50 the proportion of substrate consumption relative to oxygen.

51 **Methods**

52 The study was performed at the Department of Mother and Child of the Luigi Sacco Hospital and

53 at the Department of Mother, Child and Neonate "L. Mangiagalli". The protocol of the study was

54 approved by the Institutional Review Board of the University of Milan. Informed consent was

55 obtained from all pregnant women prior to inclusion in the study.

56 The data that support the findings of this study are available from the corresponding author upon

57 reasonable request.

58 **Study population.** Thirty healthy control pregnancies (Controls) and thirty-two pregnancies

59 complicated by fetal FGR were studied at the time of elective Caesarean section. Gestational age

60 was calculated from the last menstrual period and confirmed by routine ultrasonography

61 performed between 11 and 13 weeks. Exclusion criteria were maternal chronic diseases,

62 gestational diabetes, alcohol abuse, drug addiction, any maternal therapy interfering with fetal

63 growth, labour, abnormal fetal karyotype, and fetal malformations or infections. All pregnancies

64 were singleton and none of the women smoked during pregnancy.

65 ***Controls:*** Normal healthy pregnancies were studied at term (37-41 weeks of gestation). All

66 patients had a normal pre gravid Body Mass Index (Table 1) and none had medical or obstetric

67 pathologies. Indications for Caesarean section were breech presentation (n = 9), maternal request

68 (n = 11) and repeat Caesarean section (n = 10). None of the babies showed signs of distress at

69 delivery. Neonatal weight was appropriate for gestational age according to Italian standards for

70 birth weight and gestational age (13).

71 *FGR*: Fetuses with FGR were identified by ultrasound through repeated longitudinal measurements

72 that demonstrated a reduction in fetal growth velocity. FGR was defined by measurements of

73 abdominal circumferences below the 10th percentile of reference values for fetuses of similar ages

74 (14), together with a shift in the growth curve by greater than 40 centiles (14). This definition is

75 now also included in a recent consensus document (15). None of the fetuses were affected with

76 abnormal karyotype, genetic syndromes, viral infection or major malformations. FGR was

77 confirmed at birth by neonatal weight below the 10th percentile according to Italian standards for

78 birth weight and gestational age (16).

79 FGR pregnancies were further classified into three groups, according to increasing severity, which

80 were defined by Doppler velocimetry of the umbilical artery and by fetal heart rate tracings (FHR),

81 as previously described (3). Type 1 FGR showed both normal pulsatility index (PI) and FHR (n =

82 14); Type 2 FGR showed abnormal PI and normal FHR (n = 7). Type 3 FGR showed both abnormal

83 PI and abnormal FHR (n = 11). All pregnancies complicated by FGR underwent a Caesarean section

84 in the interest of the mother and the fetus, according to our clinical protocol, and none were in

85 labour.

86 **Study protocol**. On the day of study, umbilical blood flow measurements were taken by ultrasound

87 before the induction of anaesthesia, as previously described (12). All patients underwent spinal

88 anaesthesia and none had any secondary effects, such as maternal hypotension. We previously

89 reported no significant differences in mean umbilical venous blood flow measured before and after

90   the induction of anaesthesia in control pregnancies (12). Umbilical arterial and venous blood

91   samples were withdrawn from a doubly clamped segment of the cord. Placentas were cleaned from

92   excess blood and weighed after removing fetal membranes and the umbilical cord.

93   **Umbilical blood flow measurement**. All ultrasound exams were performed using a 5 MHz convex

94   probe (Voluson 730 Expert-GE Medical Systems), as previously described (17). Briefly, cross-

95   sectional area (cm^2) of the umbilical vein was determined on a free-loop of the umbilical cord by

96   tracing the inner circumference of the vessel (17). The time-averaged peak velocity was measured

97   positioning the Doppler sample volume in the maximum velocity spot on a longitudinal vessel view.

98   Umbilical vein mean velocity was calculated as (time-averaged maximum velocity) • 0.5, assuming

99   a parabolic velocity profile (17). The average of three consecutive measurements of the above

100   variables was calculated. Umbilical venous blood flow (Q_{umb}) was calculated as: Q_{umb} ($ml.min^{-1}$) =

101   mean velocity ($cm.sec^{-1}$) • vessel area (cm^2) • 60 (17).

102   **Oxygenation and metabolic data**. Both umbilical venous (uv) and arterial (ua) blood were

103   immediately sampled from a doubly clamped segment of the cord. All samples were collected in

104   heparinized syringes that were sealed and stored on ice. Blood gases (pO_2 and pCO_2), pH,

105   haemoglobin concentration and O_2 saturation, lactate and glucose concentrations were measured

106   using a GEM Premier 3000 portable system (Instrumentation Laboratory). Oxygen (O_2) Content

107   was calculated as: Oxygen (O_2) Content (mmol/l) = Haemoglobin (g/l) • O_2 saturation (%) •

108   0.05982.

109   **Calculations**. Umbilical O_2 uptake was calculated according to the Fick principle:

110   Umbilical O_2 Uptake ($\mu mol \cdot min^{-1}$) = Q_{umb} ($ml \cdot min^{-1}$) • D (uv-ua) O_2 Content ($mmol \cdot l^{-1}$)

111   Q_{umb} adjusted to individual neonatal weights ($Q_{umb} \cdot Kg^{-1}$) was also calculated ($\mu mol \cdot min^{-1} \cdot Kg^{-1}$).

112   Fetal O_2 delivery and the coefficient of fetal O_2 extraction were respectively calculated as:

113   Fetal O_2 delivery ($mmol \cdot min^{-1}$) = Q_{umb} ($ml \cdot min^{-1}$) • uv O_2 Content ($mmol \cdot l^{-1}$)/1000

114   Fetal O_2 extraction (%) = [D (uv-ua)/uv] O_2 Content • 100

115   Glucose/O_2 metabolic quotient was calculated as:

116   [D (uv-ua) glucose concentration (mmol·l^{-1}) / D (uv-ua) O_2 Content (mmol·l^{-1})] • 6

117   Umbilical Glucose delivery and Uptake were calculated as for oxygen.

118   Fetal glucose clearance (ml·min^{-1}) = Umbilical Glucose Uptake (μmol·min^{-1}) / umbilical arterial

119   glucose concentration (mmol·l^{-1}).

120   **Statistical analysis**

121   Data are presented as mean ± standard error (SE). The coefficient of variation for mean umbilical

122   venous volume flow was 8.1%, as previously reported (17). Differences between groups were

123   assessed using the Student's *t* test for independent-samples, with applied correction when equality

124   of variances assumption was violated (Levene's test). Differences among variables, according to

125   severity of FGR, were compared by One-way ANOVA, with an appropriate *post-hoc* test.

126   Correlations describing the strength and direction of the relationships between two variables were

127   assessed using the Pearson Product-Moment correlation. Linear regression analyses were

128   performed by the least squares method. Umbilical blood flow, O_2 and glucose utilization were also

129   compared statistically using a General Linear Model (GLM) test with repeated measures when

130   appropriate. For all comparisons, a p value < 0.05 was considered significant. All tests were

131   performed using the statistical package SPSS (IBM SPSS Statistics, v. 21.00, Armonk, NY).

132

133   **Results**

134   Maternal and fetal characteristics for Control and FGR are presented in Table 1. Maternal age, BMI

135   and the fetal sex ratio were not significantly different between groups. Gestational age, neonatal

136   and placental weight and the feto/placental weight ratio were significantly lower in the FGR group.

137 When the weight of fetuses of Control pregnancy was estimated at the corresponding mean

138 gestational age at delivery for FGR fetuses (32.7 weeks) (Est FW [32.7wks]), fetuses of FGR pregnancy

139 remained significantly lighter (Est FW [32.7wks], Control: 2083.2 ± 69.8 g *vs.* FGR: 1313.3 ± 90.2 g,

140 p<0.001). When considering the severity of FGR, no significant differences were found for maternal

141 characteristics between FGR 1, FGR 2 and FGR 3 (data not shown).

142 Gestational age, and fetal and placental weight were significantly lower in FGR 2 and 3 compared

143 to FGR 1 (Table S1 in the online-only Data Supplement). However, no significant differences in fetal

144 characteristics were found between FGR 2 and FGR 3 (data not shown). Therefore, for further

145 analysis, severity groups for FGR 2 and 3 were combined.

146 **Umbilical oxygenation and blood lactate levels**. Umbilical venous and arterial values for pH, pO_2,

147 pCO_2, $SatO_2$, Hb, O_2 Content, glucose and lactate concentrations are presented in Table S2 in the

148 online-only Data Supplement. Compared to Controls, fetuses of human FGR pregnancy showed

149 lower values for umbilical vein and artery pH, pO_2, $SatO_2$, O_2 Content and uv glucose concentration,

150 as well as higher values for umbilical vein and artery pCO_2, lactate and ua Hb. Significant differences

151 were observed between FGR 1 and FGR 2+3 for umbilical artery pH and lactate, and umbilical vein

152 lactate concentration (Table S3).

153 **Umbilical blood flow measurements.** Both mean umbilical venous cross-sectional area and mean

154 umbilical blood flow velocity were significantly lower in fetuses of FGR compared to Control

155 pregnancy. Therefore, calculated mean umbilical blood flow was also significantly lower for fetuses

156 of FGR relative to Control pregnancy (Table 2) and with increasing FGR severity (Table S4).

157 However, when umbilical blood flow was normalized for fetal weight, there were no differences

158 between Control and FGR and no differences were found according to the severity of FGR (Table 2

159 and S4). Umbilical blood flow was positively related to gestational age, placental weight and fetal

160 weight in all groups, showing FGR 2+3 fetuses to the left of the relationship, FGR 1 in the middle

161 and Control fetuses to the right of the relationship (Figure 1 A-C).

162 **Umbilical O_2 delivery, uptake and extraction**. Fetal O_2 **delivery** resulted significantly lower in

163 FGR compared to Control pregnancy, also when normalized for fetal body weight (Table 2).

164 Since the mean values of umbilical veno-arterial O_2 Content decreased in FGR, **umbilical O_2 uptake**

165 was significantly lower in FGR compared to Control, either when expressed as absolute values or

166 when normalized for fetal body weight. Assuming constant umbilical oxygen consumption/kg, the

167 calculated umbilical O_2 uptake/kg at 32.7 weeks (FW $^{32.7wks}$) for fetuses of Control pregnancy was

168 398.8 ± 38.4 compared to 126.5 ± 18.3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ in the FGR group, reflecting a reduction of

169 ca. 68% in FGR pregnancy.

170 Values for **oxygen delivery and uptake** per kg body weight were also significantly lower when

171 FGR 1 and FGR 2+3 fetuses were separately compared to Controls, with no significant difference

172 between the two FGR groups (Figure 2 A-B).

173 The average coefficient of **fetal O_2 extraction** was similar in fetuses of Control and FGR pregnancy

174 (Table 2) and there was no significant relationship between fetal O_2 extraction and delivery in

175 fetuses of Control or FGR pregnancy (Figure 3A).

176 However, for both Control and FGR pregnancies, a significant negative correlation between fetal O_2

177 extraction and umbilical artery pO_2 was found, meaning fetuses with lower pO_2 values in the

178 umbilical artery extracted more O_2 (Figure 3B).

179 No significant difference was observed for any of the above parameters in relation to fetal sex.

180 **Umbilical glucose delivery, uptake, clearance and metabolic quotient.** Glucose concentration

181 in the umbilical vein, fetal **glucose delivery** and mean umbilical veno-arterial glucose

182 concentration difference were significantly lower in fetuses of FGR compared to Control pregnancy

183 (Table 2 and Table S2 in the on line-only Data Supplement). When FGR fetuses were evaluated

184 according to severity, significant differences persisted for glucose concentration in the umbilical

8

vein and for fetal glucose delivery (Table S3 and S4). Fetal glucose delivery per kg body weight was not significantly different in FGR compared to Controls (Table 2) nor when different severity groups of FGR were considered (Table S4). Umbilical **glucose uptake** was significantly lower in fetuses of FGR compared to Control pregnancy, either when expressed as absolute values or when normalized for fetal body weight (Table 2). When evaluated according to FGR severity, umbilical glucose uptake was significantly different among the 3 groups but this difference was not significant when normalized for fetal weight (Table S4). Fetal glucose clearance was also significantly decreased in fetuses of FGR pregnancy compared to Controls (Table 2). When evaluated according to severity, FGR 1 and FGR 2+3 had significantly lower fetal glucose clearance compared with Controls but the difference was not significant between the two groups of FGR (Table S4). The **glucose/O_2 metabolic quotient** was significantly increased in fetuses of FGR compared to Controls (Table 2). When analyzed according to FGR severity, one-way ANOVA comparisons revealed no significant difference between FGR groups (Table S4). Significant positive relationships occurred between umbilical glucose uptake and umbilical oxygen uptake normalized for fetal body weight (Figure 4A), and between umbilical glucose uptake and umbilical oxygen delivery (Figure 4B) in all fetuses, independent of Control or FGR pregnancy, with no significant differences between the two groups.

No significant difference was observed for any of the above parameters in relation to fetal sex.

Discussion

In the present study, we found significantly lower umbilical venous and umbilical veno-arterial pO_2 differences, with O_2 delivery and utilization decreased by 50% in growth-restricted human fetuses. We also report significantly lower umbilical venous and umbilical veno-arterial glucose differences,

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207 together with a significant reduction of approximately 1/3 in umbilical glucose uptake in FGR

208 compared with healthy term pregnancy. These novel data support the hypothesis tested in this

209 study that the human fetus in FGR pregnancy triggers compensatory mechanisms to reduce its

210 metabolic rate, decreasing the proportion of substrate consumption relative to oxygen delivery as

211 a survival strategy.

212 **Oxygen delivery and consumption**. Since fetal growth is accompanied by a progressive increase

213 in both the fetal demand and the placental supply of nutrients with advancing gestation, it is

214 striking that fetal oxygen consumption is significantly reduced on a per kg body weight basis in

215 human FGR compared to fetuses of Control pregnancy, in spite of very low oxygen delivery rates,

216 and increased levels of mitochondrial content in placenta and cord blood (18,19).

217 These data are novel for human pregnancy and are in good agreement with data obtained from

218 experimental animal models of FGR. In an ovine hyperthermic pregnancy model of FGR, Regnault

219 *et al.* (20) reported a 25% reduction in fetal oxygen uptake and also suggested that placental

220 oxygen utilization may represent a limiting step in fetal growth restriction, restricting oxygen

221 delivery to the fetus (20). Similarly, we have previously reported an increase in the uterine-

222 umbilical oxygen gradient in human FGR pregnancy (21), together with increased placental

223 mitochondrial DNA content and changes in the mitochondrial function of cytotrophoblast and

224 mesenchymal stromal cells in FGR pregnancy (18;22), confirming that placental oxygen

225 consumption may have a limiting role in the delivery of oxygen to the fetus.

226 Although growth restriction will likely limit long term survival, it seems to provide opportunities

227 for metabolic compensations, which increase hypoxia tolerance (23). This is similar to what

228 happens in small immature neonates that are able to reduce their oxygen demand in response to

229 impaired supply, a condition known as "hypoxic hypometabolism" (24).

230 The present data and results reported by Regnault *et al.* (20) are different from data reported by

231   Zamudio *et al.* (25) in a comprehensive study of human pregnancy at high altitude. They reported

232   that human fetuses during high altitude pregnancy were able to extract sufficient oxygen to result

233   in similar fetal oxygen delivery and consumption rates. In an experimental study (26), growth

234   restricted ovine fetuses obtained by reduction in uterine blood flow were normoxic and

235   normoglycemic, i.e. showing an adaptive response to the reduced uterine blood flow. These data

236   illustrate differential fetal strategies to withstand reductions in oxygenation of varying magnitude.

237   The chronic fetal hypoxia at high altitude is likely much milder compared to the significant chronic

238   fetal hypoxia associated with severe FGR. Therefore, the capacity of the human fetus to extract

239   oxygen to maintain oxygen delivery as an adequate compensatory response may have a threshold

240   set by the severity of impaired oxygenation, beyond which it must also recruit reduced oxygen

241   consumption as a survival strategy to maintain viability.

242   **Glucose consumption and metabolic quotient**. Additional data in the present study show

243   reduced glucose consumption in the FGR human fetus, even when correcting for fetal body weight.

244   These data are also consistent with results from an ovine model of hyperthermic fetal growth

245   restriction, that reported a reduced substrate uptake as a consequence of decreased fetal oxidative

246   metabolism, and thereby lower fetal substrate demand (27). In human high altitude pregnancy,

247   Zamudio, Illsley and colleagues also reported that the preferential anaerobic placental

248   consumption of glucose spares oxygen but limits glucose availability for fetal growth (28). In a

249   recent paper, Michelsen et al employed a four-vessel sampling technique in term human

250   pregnancies and showed that uteroplacental glucose uptake correlates with placental glucose

251   consumption which modulates maternal to fetal glucose transfer and fetal glucose consumption

252   such that high placental use of glucose limits fetal glucose delivery and consumption (8).

253   Interestingly, in the present study, while umbilical glucose uptake corrected for fetal body weight

254   was significantly lower in FGR fetuses, the glucose/oxygen metabolic quotient was increased in

255   FGR pregnancy. This is consistent with human (29) and experimental animal data (30) in FGR

256   pregnancy that show a reduced glucose transport capacity proportionally lower than the rates of

257   placental and fetal glucose utilization (29, 30). These findings combined suggest that the utilization

258   of glucose also depends on the availability of oxygen, which is reduced in the FGR fetus. This is

259   confirmed in the present study by the significant positive relationship between fetal glucose

260   consumption and oxygen uptake per kg body weight, irrespective of healthy or complicated

261   pregnancy. In addition, in the present study, values for fetal glucose clearance, the rate at which

262   fetal metabolism clears glucose from the fetal circulation, was significantly reduced in fetuses from

263   FGR compared to those of control pregnancy. This would tend to increase the fetal maternal ratio

264   for glucose, possibly further contributing to impaired glucose placental transfer in human FGR

265   pregnancy.

266   **Impact of decreased oxygen and glucose metabolism in the human FGR fetus.** The human FGR

267   fetus is likely to be delivered much earlier than at term, when higher fetal metabolic rates are

268   expected due to the higher rates of protein synthesis when fetal growth demands are maximal (31).

269   In addition, the FGR fetus is likely to trigger a circulatory brain sparing effect, in relation to chronic

270   fetal hypoxia, preferentially redistributing oxygen and glucose delivery to the central nervous

271   system (32). However, considering that glucose is the main substrate utilized by the fetal brain, and

272   that the fetus is not able to increase its own blood glucose concentration by glucogenesis (33), the

273   fetal brain, with proportionally higher nutritional demands, may be particularly affected by these

274   reductions in oxygen and glucose availability.

275   Placental transfer of other relevant energy sources, such as amino acids and lipids, has also been

276   shown to be altered in FGR (2), further reducing fetal nutrient availability.

277   **Study Limitations.** Limitations are mostly inherent to the impossibility of complete

278   standardization of the experimental conditions in the human clinical situation. The first is related

279   to the estimation of umbilical blood flow by Doppler velocimetry. A second limitation is the

280   potential confounding effects of anesthesia on the maternal cardiovascular physiology. Finally,

281   differences in gestational age between Control and FGR human pregnancies could also limit the

282   interpretation of results. This potential bias is difficult to overcome in human pregnancy, since

283   preterm delivery of babies from compromised FGR pregnancy is needed to prevent potential severe

284   morbidity and/or intrauterine demise. Potential 'control' subjects delivered prematurely are not

285   likely to be "electively" delivered by cesarean section (in the absence of labor) and their clinical

286   condition would also introduce other confounding factors.

287   Experimental animal models suggest that fetal metabolism is higher in the preterm compared to

288   the term fetus, likely associated with accelerated rates of protein synthesis (34,35). In the present

289   study, although we did not find significant differences in umbilical oxygen or glucose consumption

290   in human FGR fetuses of different severity, we cannot exclude that differences may have been

291   masked by the differences in gestational age between FGR groups. However, if we were able to

292   correct for gestational age by using the mean flow value reported for 31 weeks in healthy

293   pregnancy (36), multiplied by the mean umbilical veno-arterial O_2 or glucose difference measured

294   in our term fetuses from Control pregnancy, the hypothetical, "corrected for gestational-age" values

295   would make the difference between fetuses of Control and FGR human pregnancy even more

296   striking. Therefore, the potential error in this study is likely in the under-estimation of differences

297   between human infants of Control and FGR pregnancy related to oxygen and glucose umbilical

298   uptake.

299   **Perspective.** We provide novel insight into the nature of fetal metabolic compensations in human

300   pregnancy complicated by FGR. The human FGR fetus shows a strikingly reduced umbilical uptake

301   of both oxygen and of glucose independent of fetal body weight. Therefore, the human fetus in FGR

302   pregnancy triggers compensatory mechanisms to reduce its metabolic rate, matching glucose

303   consumption to glucose delivery in relation to oxygen availability.

304

313   **Conflict of interest statement:**

314   The authors declare no conflict of interest.

315

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316

Novelty and Significance

1. What Is New

- the human FGR fetus has a strikingly reduced umbilical delivery of both oxygen and glucose

- umbilical oxygen and glucose delivery and uptake are reduced both as absolute values and normalized for fetal body weight

- The glucose/oxygen quotient is significantly increased while glucose clearance is significantly decreased in FGR pregnancies

2. What Is Relevant?

- this study highlights potential compensatory intrauterine mechanisms predisposing to metabolic syndrome and hypertension in later life

3. Summary

- The human FGR fetus triggers compensatory mechanisms to reduce its metabolic rate, matching the proportion of substrate consumption relative to oxygen delivery as a survival strategy

329 **Figure 1.** Relationship between umbilical venous blood flow and gestational age (A), placental

330 weight (B) and fetal weight (C) in Control (white rhombuses) and FGR human pregnancy according

331 to severity (FGR1: grey squares; FGR 2+3: black triangles).

332 **Figure 2.** Fetal O_2 delivery/kg and umbilical O_2 uptake/kg in Control (white) and FGR human

333 pregnancy according to severity (FGR1, grey; FGR 2+3, black). (A) Fetal O_2 delivery/kg; (B)

334 umbilical O_2 uptake/kg. Bars represent mean ± SEM. $p < 0.001$ for umbilical oxygen delivery per-

335 kg: $F_{(2, 57)} = 7.9$. Post-hoc comparisons using the Tukey HSD test: mean score for the Control

336 group (0.35 ± 0.03 mmol·min^{-1}·kg^{-1}) significantly different from both FGR 1 (0.19 ± 0.04 mmol·min^{-}

337 ^{1}·kg^{-1}) and FGR 2+3 (0.17 ± 0.04 mmol·min^{-1}·kg^{-1})*.* FGR 1 not significantly different from FGR 2+3.

338 $p < 0.001$ for umbilical oxygen uptake per-kg: $F_{(2, 55)} = 8.5$. Post-hoc comparisons using the Tukey

339 HSD test: mean score for the Control group (248.1 ± 21.9 µmol·min^{-1}·kg^{-1}) significantly different

340 from both FGR 1 (138.3 ± 32.7 µmol·min^{-1}·kg^{-1}) and FGR 2+3 (123.1 ± 20.5 µmol·min^{-1}·kg^{-1}). FGR

341 1 not significantly different from FGR 2+3.

342 **Figure 3.** Relationship between umbilical O_2 extraction and fetal O_2 delivery (A) and between

343 umbilical O_2 extraction and umbilical artery pO_2 (B) in Controls (white) and FGR (black) human

344 pregnancy. Linear regression lines refer to controls (solid) and to FGR (broken). The slope and

345 intercept of regression lines for controls and FGR pregnancies are not significantly different.

346 **Figure 4.** (A) Relationships between umbilical glucose uptake/kg and umbilical O_2 uptake/kg and

347 (B) between umbilical glucose uptake/kg and fetal O_2 delivery in Controls (white) and FGR (black)

348 human pregnancy. The slope and intercept of regression lines for controls and FGR pregnancies are

349 not significantly different. Linear regression lines refer to the total population.

350

Table 1. Maternal and fetal characteristics for control and FGR pregnancies.

Variable	Controls (n=30)	FGR (n=32)	p value
Maternal age (years)	35.3 ± 0.7	35.3 ± 1.1	n.s.
Body mass index (Kg/m^2)	21.1 ± 0.4	22.3± 0.8	n.s.
Gestational age at birth (weeks)	38.8± 0.1	32.7 ± 0.5	p<0.001
Neonatal weight (g)	3340.8 ± 52.9	1313.3 ± 90.2	p<0.001
Neonatal weight centile (°)	50.6 ± 3.0	7.3 ± 0.8	p<0.001
Placental weight (g)	520.0 ± 23.7	252.1 ± 20.1	p<0.001
Feto/placental ratio	6.6 ± 0.2	5.6 ± 0.3	p<0.01

Fetal sex (female/male)	17 / 13	17 / 15	n.s.

Values are presented as mean ± SE

Independent-samples *t*-test: FGR vs controls: n.s. not significant

Table 2. Umbilical blood flow, O_2 and glucose utilization in control and FGR fetuses.

Variable	Controls (n=30)	FGR (n=32)	p value
uv flow (ml·min^{-1})	263.3 ± 15.8	98.2 ± 10.9	p< 0.001
uv flow/Kg (ml·min^{-1}·Kg^{-1})	78.4 ± 4.3	74.8 ± 5.6	n.s.
O_2 delivery (μmol·min^{-1})	1182.5 ± 117.8	254.8 ± 45.3	p< 0.001
O_2 delivery/Kg (μmol·min^{-1}·Kg^{-1})	354.9 ± 35.1	179.7 ± 26.1	p< 0.001
O_2 cont D (uv-ua) (mmol·l^{-1})	3.1 ± 0.2	1.7 ± 0.2	p< 0.001

umb O_2 uptake ($\mu mol \cdot min^{-1}$)	808.5 ± 70.6	181.9 ± 35.3	p< 0.001
umb O_2 uptake/Kg ($\mu mol \cdot min^{-1} \cdot Kg^{-1}$)	243.1 ± 21.7	126.5 ± 18.3	p< 0.001
Fetal O_2 extraction (%)	71.0 ± 2.8	70.7 ± 4.2	ns
Glucose delivery ($\mu mol \cdot min^{-1}$)	942.1 ± 70.6	305.8 ± 35.1	p< 0.001
Glucose delivery/kg ($\mu mol \cdot min^{-1} \cdot Kg^{-1}$)	281.6 ± 18.5	237.4 ± 20.7	ns
Glucose D (uv-ua) ($mmol \cdot l^{-1}$)	0.79 ± 0.06	0.61 ± 0.05	p<0.05

umb glucose uptake (µmol·min⁻¹)	210.9 ± 23.4	58.6 ± 9.3	p<0.001
umb glucose uptake/kg (µmol·min⁻¹·Kg⁻¹)	63.2 ± 6.7	44.1 ± 4.9	p<0.05
Glucose clearance (ml·min⁻¹)	81.84 ± 11.13	24.12 ± 3.71	p<0.001
glucose D (uv-ua) / O₂ cont D (uv-ua)	0.27 ± 0.03	0.57 ± 0.11	p<0.05
Glucose/O₂ metabolic quotient	1.65 ±0.18	3.45 ± 0.70	p<0.05

Values are presented as mean ± SE

Unpaired *t* test and GLM   FGR *vs.* controls: n.s. not significant

351