Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus

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1Physiology Department, Faculty of Biology, University of Murcia, Murcia, Spain; 2Gynecology Service, Virgen de la Arrixaca Hospital, Murcia, Spain; and 3Division of Nutrition and Metabolic Diseases, LMU University, Munich, Germany

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Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus. Am J Physiol Endocrinol Metab 305: E826–E833, 2013. First published August 6, 2013; doi:10.1152/ajpendo.00291.2013.—Better knowledge on the disturbed mechanisms implicated in materno-fetal long-chain polyunsaturated fatty acid (LC-PUFA) transfer in pregnancies with gestational diabetes mellitus (GDM) may have potentially high implications for later on in effective LC-PUFA supplementation. We studied in vivo placental transfer of fatty acids (FA) using stable isotope tracers administrated to 11 control and 9 GDM pregnant women (6 treated with insulin). Subjects received orally [13C]palmitic, [13C]oleic and [13C]linoleic acids, and [13C]docosahexaenoic acid (13C-DHA) 12 h before elective caesarean section. Maternal blood samples were collected at 12, 0, –2, and –1 h, delivery, and +1 h. Placental tissue and venous cord blood were also collected. FA were quantified by gas chromatography (GC) and 13C enrichments by GC-isotope ratio mass spectrometry. [13C]FA concentration was higher in total lipids of maternal plasma in GDM vs. controls, except for [13C]DHA. Moreover, [13C]DHA showed lower placenta/maternal plasma ratio in GDM vs. controls and significantly lower cord/maternal plasma ratio. For the other studied FA, ratios were not different between GDM and controls. Disturbed [13C]DHA placental uptake occurs in both GDM treated with diet or insulin, whereas the last ones also have lower [13C]DHA in venous cord. The tracer study pointed toward impaired placental DHA uptake as critical step, whereas the transfer of the rest of [13C]FA was less affected. GDM under insulin treatment could also have higher fetal fat storage, contributing to reduce [13C]DHA in venous cord. DHA transfer to the fetus was reduced in GDM pregnancies compared with controls, which might affect the programming of neurodevelopment in their neonates.

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders of pregnancy, affecting up to 5% of all pregnancies (2). This disorder is characterized by an abnormal glucose tolerance diagnosed for the first time during pregnancy due to a decreased insulin sensitivity combined with insufficient insulin secretion (5). The increased insulin resistance induces alterations in lipid metabolism that lead to dyslipidemia in GDM women (12). Moreover, women with a history of GDM have a higher risk of developing type 2 diabetes mellitus later in life (14). Maternal diabetes during pregnancy might affect behavioral and intellectual development of the offspring (26); pregestational and gestational diabetes mellitus were found to adversely affect attention span and motor functions of offspring at school age (21). Since long-chain polyunsaturated fatty acids (LC-PUFA), especially docosahexaenoic acid (DHA), are of critical importance for the fetal development of the central nervous system, adverse effects of GDM on their transfer would be of major relevance.

The placental supply of maternal LC-PUFA to the fetus is critical since the ability of both the fetus and the human placenta to synthesize LC-PUFA from essential fatty acids (FA) by desaturation and elongation is limited (15). In pregnancies complicated by GDM, some studies have shown normal levels of LC-PUFA in maternal plasma lipids (36) and even higher values for DHA in maternal triglycerides and phospholipids (19, 32). In contrast, lower values of DHA and other LC-PUFA were observed in cord blood of GDM neonates (19, 33). According to these results, Wijendran et al. (35) suggested an impaired materno-fetal LC-PUFA transfer during GDM that might lead to adverse fetal neurological programming of the offspring. Reduced LC-PUFA percentages in cord blood during GDM might result from augmented de novo synthesis of saturated and monounsaturated fatty acids in the fetus from the abundantly available glucose and hence, decreasing proportions of essential fatty acids and their derivatives. Another hypothesis could be a disturbed placental LC-PUFA transfer or even higher LC-PUFA accretion by fetal adipose tissue in GDM babies. It is important to discern the reason for the lower levels of DHA in GDM babies to identify the best strategy of supplementation with LC-PUFA in the mother, the baby, or both.

We studied in vivo the placental transfer of FA using stable isotope tracers administrated to control and GDM pregnant women 12 h before elective caesarean section. We aimed to evaluate the transfer of FA from the mother to the fetus in this pathology and elucidate the distribution of the stable isotope tracers in the different maternal, placental, and fetal compartments.

MATERIALS AND METHODS

Subjects. We recruited 11 healthy pregnant women (control group) and nine women diagnosed with GDM (6 were treated with insulin and 3 with diet only) in the Obstetrical Service of the Hospital Virgen de la Arrixaca, Murcia (Spain). Control group subjects belonged to a previously published study (11).

Participants satisfied the following inclusion criteria: singleton pregnancy with term delivery, age 18–40 yr, and a fetal Doppler scan within the normal reference range (23) on the day before the caesarean section. Ecographical measurements were not significantly different between GDM and controls (results not shown). Smoking subjects, subjects reporting health problems or pregnancy complications, and...
Thus, the results of 13C-DHA concentrations were calculated for only the first days of the study process by the supplier company changed during the study. The concentration of DHA, a dose of 0.1 mg/kg DHA (10% 13C enrichment) stable isotope tracers was given. Because of the lower plasma concentrations of palmitic acid (PA), oleic acid (OA), and linoleic acid (LA) are relatively high, 0.5 mg/kg body wt of the corresponding labeled in all of their carbons (Martek Biosciences, Columbia, MD). 3 h: 145 mg/dl). Glucose oral challenge. A positive screening result (1-h plasma glucose concentration >140 mg/dl) was followed by a 3-h oral glucose tolerance test with a 100-g glucose load and further serum glucose analyses at 1, 2, and 3 h after glucose intake. The test was considered positive if two of the four glucose values collected were above the normal range (basal: 105 mg/dl; 1 h: 190 mg/dl; 2 h: 165 mg/dl; and 3 h: 145 mg/dl).

During pregnancy, GDM women treated with insulin received low doses of rapid-acting insulin (≤10 units) during meals. Subjects from this study took their last dose of fast insulin with their last meal (10–11 h before the caesarean section), and they kept fasting until the surgery. At delivery, anthropometrical maternal and neonatal characteristics were recorded (Table 1). Birth weight and length z-score of neonates were calculated according to the data of Carrascosa Lezcano et al. (6).

**Stable isotope tracer administration.** The FA were uniformly 13C labeled in all of their carbons (Martek Biosciences, Columbia, MD) and they were given orally to the pregnant women 12 h before the time of elective caesarean section, and they keep fasting until the surgery. Subjects were recruited the night before the cesarean section, and the consumption of fish during the last meal received was excluded. The women remained fasted until the time of caesarean section. Because the plasma concentrations of palmitic acid (PA), oleic acid (OA), and linoleic acid (LA) are relatively high, 0.5 mg/kg body wt of the corresponding stable isotope tracers was given. Because of the lower plasma concentrations of DHA, a dose of 0.1 mg/kg DHA (10% 13C enrichment) was adequate. Nevertheless, five subjects from the control group received 0.1 mg/kg 13C-DHA with 100% 13C enrichment (the synthesis process by the supplier company changed during the study); thus the results of 13C-DHA concentrations were calculated for only six controls vs. nine GDM. Applied tracer doses per kilogram of body weight were identical between the groups and thus any group differences could be ascribed to different metabolism.

**Blood and placenta sampling and analysis.** We collected 4 ml of maternal blood at basal time (12 h before the caesarean section), at each hour during the 3 h before delivery, at the time of delivery, and 1 h after delivery. Directly after birth, 2 ml each of cord blood from the vein and the artery were collected. Samples of placental tissue (5 g) were cut from the central cotyledon of the placenta, rinsed with cold isotonic NaCl solution to eliminate contamination with blood, immediately frozen in liquid nitrogen, and stored at −80°C. All blood samples were taken with EDTA-containing tubes, and after <2 h they were centrifuged for 3 min at 1,200 g to separate plasma and blood cells.

FA from individual lipid fractions in plasma and placental tissue were extracted as reported by Gil-Sánchez et al. (11) and stored in hexane-containing butylated hydroxytoluene as antioxidant at −20°C until gas chromatographic analysis. Gas chromatography was performed on a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a BPX70 column (SGE, Weiterstadt, Germany) with 60 m length and 0.32 mm inner diameter (8).

The 13C enrichment of individual FA methyl esters was measured by gas chromatography-combustion–isotope ratio mass spectrometry (GC-IRMS; Hewlett-Packard GC interfaced to a Finnigan MAT delta S mass spectrometer; Hewlett-Packard, Bremen, Germany) (8). Expression of results. From the 13C/12C ratio of the samples measured by GC-IRMS, the 13C atom percent excess (APE) representing the 13C enrichment was calculated (4). Concentrations of stable isotope tracers (μmol 13C/l or μmol 13C/g) were calculated by multiplying absolute concentrations of FA obtained by gas chromatography by their 13C APE values obtained by GC-IRMS. APE enrichment values indicate the percentage of tracer 13C to the total carbon in a pool, whereas concentration values represent the absolute amount of tracer 13C in a compartment.

From maternal plasma concentrations of 13C, we calculated the area under the tracer concentration curve (AUC; μmol 13C·l−1·h−1) by integrating the measured tracer concentration until delivery over time according to the trapezoidal rule. AUC of tracer concentration instead of tracer concentration at the time of delivery was chosen because it is considered to be a better proxy of tracer available for transfer than the concentration at a single time point, even though the limited number of sampling time points possible may not allow for describing the true AUC from tracer intake until delivery. Since we measured 13C/12C ratio by GC-IRMS in each lipid fraction, the 13C enrichment in total lipids of maternal plasma, placenta, and cord plasma was calculated indirectly as the tracer/tracer ratio (TTR) by the sum of the 13C FA concentrations (μmol 13C/l or μmol 13C/g) from each lipid fraction divided by the sum of FA concentration obtained by gas chromatography (μmol/l or μmol/g) and multiplied by 100.

The distribution ratio of the tracer FA between mother and fetus was estimated by calculating the percentage of tracer concentration (μmol 13C/l) in venous cord blood relative to AUC (μmol 13C·l−1·h−1) in maternal plasma. The distribution ratio of 13C tracers between placental tissue and AUC in maternal plasma was calculated as the percentage of the tracer concentration in placenta (nmol 13C/g) relative to AUC (μmol 13C·l−1·h−1) in maternal plasma until delivery. Statistical analysis. The results are expressed as means ± SE. GDM and control groups were compared using Student’s t-test. The significance level was set to P < 0.05. All analyses were performed with the statistical software SPSS, version 15.0 (SPSS, Chicago, IL).

Table 1. Anthropometric characteristics of the mothers and neonates at delivery

<table>
<thead>
<tr>
<th>Value</th>
<th>Control (n = 11)</th>
<th>GDM (n = 9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, yr</td>
<td>33.00 ± 1.26</td>
<td>34.50 ± 1.25</td>
<td>0.408</td>
</tr>
<tr>
<td>Maternal weight, kg</td>
<td>78.03 ± 2.64</td>
<td>84.50 ± 2.24</td>
<td>0.078</td>
</tr>
<tr>
<td>Maternal height, cm</td>
<td>160.90 ± 1.81</td>
<td>163.10 ± 2.77</td>
<td>0.515</td>
</tr>
<tr>
<td>Maternal BMI, kg/m²</td>
<td>30.12 ± 0.81</td>
<td>31.90 ± 1.07</td>
<td>0.202</td>
</tr>
<tr>
<td>Maternal glucose, mg/dl</td>
<td>70.33 ± 3.29</td>
<td>74.40 ± 4.60</td>
<td>0.484</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.8 ± 0.42</td>
<td>38.19 ± 0.19</td>
<td>0.004</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>617.00 ± 40.69</td>
<td>648.00 ± 38.20</td>
<td>0.585</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>0.42 ± 0.23</td>
<td>0.88 ± 0.33</td>
<td>0.262</td>
</tr>
<tr>
<td>Birth length z-score</td>
<td>0.05 ± 0.28</td>
<td>0.17 ± 0.27</td>
<td>0.756</td>
</tr>
<tr>
<td>Cephalic circumference at birth, cm</td>
<td>34.70 ± 0.39</td>
<td>35.00 ± 0.5</td>
<td>0.641</td>
</tr>
<tr>
<td>Abdominal circumference at birth, cm</td>
<td>34.50 ± 0.96</td>
<td>33.44 ± 0.53</td>
<td>0.363</td>
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</tbody>
</table>

Results are expressed as means ± SE. GDM, gestational diabetes mellitus; BMI, body mass index. Significantly different at P < 0.05.
RESULTS

Maternal BMI at delivery was similar between GDM and controls as well as anthropometrical measurements of neonates (Table 1). Glucose levels were no different between groups at the time of delivery. Moreover, insulin levels at delivery in GDM subjects did not differ between women treated only by diet and those with insulin treatment (17.43 ± 4.64 vs. 16.43 ± 4.67 µU/mL, respectively) probably because of the low number of subjects. The length of gestation was significantly shorter in GDM than in controls (Table 1) because of a trend toward scheduling caesarean sections earlier in GDM to avoid potential complications of infant macrosomia.

In GDM [13C]DHA, either concentration (Fig. 1D) or enrichment (Table 2) was significantly lower in total lipids of maternal plasma at the time of delivery than in controls, and AUC in maternal plasma tended to be reduced in GDM (Fig. 1D). However, TTR enrichment of non-LC-PUFA was similar among groups (Table 2), although

Table 2. [13C] enrichment of fatty acids and unlabeled fatty acid concentration in total lipids of maternal plasma, placenta, and cord plasma

<table>
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<tbody>
<tr>
<td></td>
<td>Control (n = 11)</td>
<td>GDM (n = 9)</td>
<td>Control (n = 11)</td>
<td>GDM (n = 9)</td>
</tr>
<tr>
<td>[13C] enrichment (% [13C]APE)</td>
<td>0.02 ± 0.002</td>
<td>0.026 ± 0.002</td>
<td>0.021 ± 0.002</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>Maternal plasma (mean)</td>
<td>0.029 ± 0.003</td>
<td>0.031 ± 0.003</td>
<td>0.019 ± 0.005</td>
<td>0.005 ± 0.003*</td>
</tr>
<tr>
<td>Placental tissue</td>
<td>0.006 ± 0.0004</td>
<td>0.008 ± 0.0004*</td>
<td>0.009 ± 0.0008</td>
<td>0.01 ± 0.0008</td>
</tr>
<tr>
<td>Venous cord plasma</td>
<td>0.08 ± 0.002*</td>
<td>0.082 ± 0.002</td>
<td>0.005 ± 0.003</td>
<td>0.011 ± 0.001*</td>
</tr>
<tr>
<td>Artery cord plasma</td>
<td>0.003 ± 0.0002</td>
<td>0.004 ± 0.0002*</td>
<td>0.003 ± 0.003</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>Ratio placenta/maternal plasma</td>
<td>0.01 ± 0.001</td>
<td>0.01 ± 0.001</td>
<td>0.001 ± 0.001</td>
<td>0.002 ± 0.005*</td>
</tr>
<tr>
<td>Ratio venous cord/maternal plasma</td>
<td>0.021 ± 0.001</td>
<td>0.021 ± 0.001</td>
<td>0.021 ± 0.001</td>
<td>0.021 ± 0.001</td>
</tr>
<tr>
<td>Unlabeled fatty acid concentration</td>
<td>0.16 ± 0.016</td>
<td>0.16 ± 0.016</td>
<td>0.16 ± 0.016</td>
<td>0.16 ± 0.016</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. APE, atom % excess. *Significantly different at P < 0.05.
AUC concentrations of $^{13}$C[PA and $^{13}$C[OA were significantly higher in GDM than in controls, and $^{13}$C[LA also showed this trend in GDM ($P = 0.096$; Fig. 1, A–C). The higher $^{13}$C[FA concentration of non-LC-PUFA in GDM was due mainly to the maternal hyperlipidemia in these subjects (mostly triglycerides), whereas for $^{13}$C[DHA its lower concentration was due mainly to lower $^{13}$C enrichment values in GDM subjects (Table 2).

$^{13}$C[PA and $^{13}$C[OA concentrations were significantly higher in maternal plasma triglycerides of GDM than of controls (Fig. 2, A and B), whereas $^{13}$C[LA and $^{13}$C[DHA concentrations showed a similar sense of change (Fig. 2, C and D). $^{13}$C[DHA showed lower relative incorporation into maternal plasma phospholipids and higher incorporation into triglycerides in GDM compared with controls (Fig. 2D), which could influence availability for placental uptake. In fact, AUC of $^{13}$C[DHA concentrations tended to be lower in all maternal plasma lipid fractions of GDM except in triglycerides, although the differences were not statistically significant (Fig. 2D). In addition, the appearance as nonesterified FA (NEFA) was significantly lower for all $^{13}$C[FA in maternal plasma of GDM than in controls (Fig. 2).

In total lipids of both placental tissue (Fig. 3A) and venous cord blood (Fig. 3B), we again found significantly lower concentrations of $^{13}$C[DHA in GDM than in controls and also lower enrichments (Table 2). All other $^{13}$C[FA showed no significant group differences in placenta, except for $^{13}$C[PA, which was significantly higher in GDM, in agreement with higher concentration in placenta phospholipids (Table 3). In venous cord blood, non-LC-PUFA showed similar concentrations between groups, with a significantly higher concentration for $^{13}$C[OA in GDM (Fig. 3B). $^{13}$C[DHA tended toward lower concentrations in cord triglycerides of GDM (Table 3), showing significant differences in venous cord phospholipids; $^{13}$C[PA and $^{13}$C[OA were significantly higher in venous cord cholesterol esters and $^{13}$C[LA in phospholipids of GDM than in controls (Table 3).

The ratio between the $^{13}$C[FA concentration in placenta and the maternal plasma AUC concentration tended toward lower $^{13}$C[DHA accretion in GDM subjects ($P = 0.110$) (Fig. 3C). If we consider all control subjects ($n = 11$) to calculate this ratio (within each subject, the dose of $^{13}$C[DHA consumed by the mothers affects both compartments equally), GDM showed a significantly lower ratio of $^{13}$C[DHA in placental tissue to maternal AUC than controls ($P = 0.021$). The ratio between $^{13}$C[DHA concentration in venous cord blood and the AUC in maternal plasma was significantly lower in GDM than in controls, whereas for the other tracer FA, the ratios were not different between the groups (Fig. 3D). Similar results were found if the ratios were estimated from $^{13}$C enrichment values (Table 2).

Although only six GDM subjects received insulin treatment, whereas three GDM subjects received only dietary treatment, we attempted to see whether there were differences between these two groups. It was surprising that, despite no differences being found in the placental uptake between the groups (Fig. 4A), the
The ratio of $[^{13}C]$DHA concentration in cord with respect to maternal plasma was significantly lower in GDM subjects treated with insulin with respect to those treated with diet (Fig. 4B).

**DISCUSSION**

In the present study, women with GDM showed significantly lower $[^{13}C]$DHA concentrations in total lipids of maternal plasma, placenta, and venous cord plasma than healthy pregnant women. Furthermore, the ratio between $[^{13}C]$DHA concentration in both placenta and cord blood and maternal plasma AUC was lower in GDM than in controls, which indicates both lower materno-placental transfer and lower materno-fetal transfer of DHA in GDM. Because transfer-related ratios were not different between groups for the other studied FA, this indicates an effect of GDM specific for DHA.

**Maternal plasma.** At the time of the maternal blood sampling (beginning 9 h after ingestion), most of the lipids containing stable isotope tracers had been hydrolyzed by lipoprotein lipase (LPL), and the majority of the tracer had been taken up by adipose tissue. However, significant amounts remained in triglycerides (probably chylomicron remnants for the most part) and phospholipids, with smaller amounts in NEFA (de-

![Fig. 3. A: concentration of tracer in total lipids of placental tissue. B: concentration of tracer in total lipids of venous cord blood. C: ratio (%) between placental concentration and maternal plasma AUC of $[^{13}C]$FA. D: ratio (%) between fetal concentration and maternal plasma AUC of $[^{13}C]$FA. Control group (black bars), $n = 11$ (except for $[^{13}C]$DHA, in which $n = 6$); GDM group (open bars), $n = 9$. Results are expressed as means ± SE. *Significant differences, $t$-test with $P < 0.05$.](http://ajpendo.physiology.org/)

### Table 3. Concentration of $[^{13}C]$fatty acids in total lipids of placenta and venous cord plasma

<table>
<thead>
<tr>
<th></th>
<th>$[^{13}C]$Palmitic Acid</th>
<th>$[^{13}C]$Oleic Acid</th>
<th>$[^{13}C]$Linoleic Acid</th>
<th>$[^{13}C]$Docosahexaenoic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placenta, nmol/g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ($n = 11$)</td>
<td>6.40 ± 0.48</td>
<td>8.59 ± 0.54*</td>
<td>3.28 ± 0.39</td>
<td>12.37 ± 1.58</td>
</tr>
<tr>
<td>GDM ($n = 9$)</td>
<td>3.38 ± 0.25</td>
<td>13.97 ± 1.27</td>
<td>13.03 ± 0.24</td>
<td>0.97 ± 0.18</td>
</tr>
<tr>
<td>PL</td>
<td>6.40 ± 0.48</td>
<td>8.59 ± 0.54*</td>
<td>3.28 ± 0.39</td>
<td>12.37 ± 1.58</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.41 ± 0.15</td>
<td>0.32 ± 0.07</td>
<td>0.33 ± 0.10</td>
<td>1.03 ± 0.24</td>
</tr>
<tr>
<td>TG</td>
<td>0.30 ± 0.06</td>
<td>0.22 ± 0.03</td>
<td>0.37 ± 0.05</td>
<td>0.51 ± 0.07</td>
</tr>
<tr>
<td>CE</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Venous cord, µmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.26 ± 0.03</td>
<td>0.32 ± 0.04</td>
<td>0.08 ± 0.01</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>TG</td>
<td>0.21 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.16 ± 0.03</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>CE</td>
<td>0.06 ± 0.01</td>
<td>0.09 ± 0.01*</td>
<td>0.14 ± 0.02</td>
<td>0.20 ± 0.10</td>
</tr>
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</table>

Results are expressed as means ± SE. PL, phospholipids; NEFA, nonesterified fatty acids; TG, triglycerides; CE, cholesterol esters; ND, not detectable. *Significantly different at $P < 0.05$.  

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rived from incomplete tissue uptake of fatty acids after LPL hydrolysis) and cholesterol esters. The higher AUC of concentrations of the non-LC-PUFA in total lipids and triglycerides of GDM maternal plasma could be due to the lower clearance of maternal plasma triglycerides in gestational diabetes that contributes to the hyperlipidemia in these subjects. In type 2 diabetes mellitus, insulin resistance causes high postprandial levels of triglyceride-rich lipoproteins and their prolonged residence in the circulation (24). The hyperlipidemia could affect the rate of fatty acid transfer across the placenta in GDM subjects with respect to controls. However, the smaller AUC of $^{13}$C[DHA concentration in total lipids of GDM compared with controls ($P = 0.057$) might indicate different effects of GDM on the metabolism of triglycerides and phospholipids, which might even cause faster plasma clearance of $^{13}$C[DHA.

We found a trend toward reduced $^{13}$C[DHA incorporation into maternal phospholipids ($P = 0.068$) with respect to maternal triglycerides (Fig. 2D). Some have reported increased percentages of DHA in maternal plasma phospholipids and triglycerides in women with GDM (19, 32, 36); in contrast to plasma, red cells of the GDM women had significantly lower levels of arachidonic acid and DHA, particularly in choline phospholipids (18). Thomas et al. (32) suggested a potential failure to incorporate LC-PUFA into the red cell phospholipids in addition to a defect in placental transport. Our results using stable isotope tracers support a disturbed incorporation of DHA into maternal plasma phospholipids and into further maternal lipid fractions in GDM but not in plasma triglycerides, whereas for the other labeled fatty acids this effect was not observed.

GDM has been associated with higher maternal plasma NEFA content (37) because of the higher insulin resistance and increased rate of lipolysis of adipose tissue (12); nevertheless, some studies did not detect such an increase of maternal plasma NEFA (25, 28). In the current study, we have observed a trend toward lower appearance of all studied $^{13}$C[FA in plasma NEFA in the GDM group and also lower enrichment (results not shown). This could indicate that most of the NEFA analyzed in this study were provided by lipolysis from adipose tissue, where $^{13}$C[FA would be too highly diluted in GDM to cause measurable $^{13}$C enrichment in NEFA after relipolysis.

**Placental tissue.** The results of the present study showed a significantly lower concentration of $^{13}$C[DHA in placental total lipids of women with GDM compared with control subjects but not for the other fatty acids. The decrease in $^{13}$C[DHA concentrations in the placenta of GDM tended to occur in all placental lipid fractions, although the differences were not statistically significant. Rats made diabetic by injection of streptozocin that received intragastrically $^{14}$C[OA had significantly higher radioactivity 24 h later in placenta compared with control rats (30); the treatment of GDM subjects during pregnancy with diet or insulin seems to ameliorate these differences. An increase in total lipids of human placenta in GDM (3, 9) and a higher accumulation of DHA in placental phospholipids, but no differences of DHA in placental triglycerides, were reported (3). Phospholipids contribute about 80–90% to total placental lipids (13), and we found a significant increase in $^{13}$C[PA in placental phospholipids but no trend for $^{13}$C[DHA. Thus, our results do not support the concept that LC-PUFA uptake by the placenta is increased by GDM. There is more intensive esterification of fatty acids into lipids in GDM placenta, in agreement with higher accumulation of lipid droplets (25), which leads to a smaller NEFA pool in GDM placenta with fast turnover. Moreover, a consistent activation of the expression of several genes involved in placental lipid biosynthesis pathways was found in GDM (25). Recently, Visiedo et al. (34) reported by in vitro studies in diabetic and healthy placental explants that hyperglycemia reduces fatty acid oxidation and increases triglyceride accumulation in human placenta.

GDM subjects showed a lower ratio of $^{13}$C[DHA concentration in placenta to maternal plasma than controls. This agrees with a lower ratio of $^{13}$C[DHA enrichment in placenta to maternal plasma (Table 2), indicating that a lower proportion of the placental DHA is maternally derived. Thus, not only were the levels of $^{13}$C[DHA in maternal circulation lower, but also its uptake by the placenta was reduced by GDM. Nevertheless, as reported previously for the healthy subjects (11), there was a preferential placental uptake of DHA relative to the other studied fatty acids in GDM. Available comparisons of the activity of enzymes that release fatty acids from circulating maternal lipids for placental uptake (LPL and endothelial lipase) between GDM and uncomplicated pregnancies are inconclusive. No differences (10, 17), increased endothelial lipase but not LPL activity (16), and even a reduction in placental LPL (25) to counteract excessive placental uptake of fatty acids in GDM have been reported. If the concentration of $^{13}$C[DHA in maternal plasma phospholipids is smaller in...
GDM, we could speculate about a high relevance of endothelial lipase for DHA release and transfer in these subjects. Because less DHA is available in maternal plasma of GDM, this leads to a lower transfer of DHA from the mother to the placenta in GDM. Scholler et al. (29) reported an increased expression of phospholipid transfer protein in the endothelial cells of the fetal placental surface of GDM subjects, although no differences were found in fetal plasma. Phospholipid transfer protein has a role in plasma lipoprotein metabolism and enhances transfer and exchange of phospholipids between cells, but its role in LC-PUFA metabolism in GDM is uncertain.

Umbilical cord plasma. We confirm an impaired LC-PUFA transfer from the mother to venous cord blood in GDM. Moreover, we found lower [13C]DHA in cord blood phospholipids, which are synthesized mainly by the fetal liver from the available NEFA received from the mother. Other studies have reported that neonates born to mothers with GDM had decreased percentages of DHA in both plasma (19, 33) and red blood cells (19, 35). The reduced ratio of [13C]DHA concentrations between fetal and maternal circulation was consistent with lower absolute transfer of [13C]DHA to venous cord plasma in GDM subjects; moreover, when this ratio was calculated from 13C enrichment values, a lower proportion of maternal plasma in GDM subjects; moreover, when this ratio was calculated from 13C enrichment values, a lower proportion of maternal plasma concentration ratio for non-LC-PUFA13C concentrations in the offspring of GDM women was similar to controls and even higher for [13C]OA; thus it seems that transfer of non-LC-PUFA is not affected by GDM in a relevant way, which agrees with similar fetal/maternal plasma concentration ratio for non-LC-PUFA between controls and GDM. This is new information that can only be carried out by isotope labeling. Because of the difficulty in finding pregnant women who accept intake tracer isotope during pregnancy, the number of patients is limited but in the usual range compared with other studies with these isotopes (31). Moreover, control subjects belonged to a previous study, which could be considered a limitation.

GDM is associated with higher fetal fat mass (7) that could be related to higher incorporation of circulating fatty acids into fetal adipose tissue. It is interesting to note that placental uptake of [13C]DHA was similar between GDM subjects treated with diet or insulin (Fig. 4A), whereas materno-fetal transfer ratio of [13C]DHA was significantly lower in GDM subjects treated with insulin vs. only diet (Fig. 4B). Moreover, [13C]DHA concentration in venous cord blood was significantly reduced in GDM subjects treated with insulin vs. GDM subjects treated with diet (0.025 ± 0.007 vs. 0.062 ± 0.004 μmol/l, respectively, P = 0.009). Thus, in addition to disturbed placental function by GDM, fetal insulin on GDM subjects with more severe hyperglycemia could enhance fatty acid accretion by fetal adipose tissue, also contributing to the reduction of [13C]DHA in cord blood. Our results are in accord with the negative correlation between cord triglycerides and fetal growth in GDM offspring but not in healthy controls, as reported by Schaefer-Graf and colleagues (27, 28). Moreover, Ortega-Senovilla et al. (22) found lower percentages of arachidonic acid and DHA in umbilical arterial but not in venous plasma of neonates of GDM subjects than those from controls, pointing toward an altered handling or metabolism of these fatty acids in neonates of GDM mothers. In our study, we did not find changes in the umbilical artery or vein difference between GDM subjects treated with diet or insulin (results not shown) or between GDM vs. controls, probably because 12 h was not enough time to detect differences in the fatty acid uptake by fetal tissues or because the major differences occur in NEFA, and the 13C determination in NEFA is less precise than in other lipid fractions due to the small NEFA concentrations in cord blood. Thus, with this tracer approach, we cannot exclude enhanced accumulation of [13C]DHA in fetal adipose tissue by GDM as reason for the lower fetalmaternal [13C]DHA ratio. It would be interesting to evaluate LC-PUFA supplementation to neonates of GDM mothers with respect to neurodevelopmental and other outcomes.

In conclusion, the concentrations of labeled LC-PUFA were significantly lower in maternal, placental, and fetal compartments in GDM compared with controls, reflecting differences in the DHA metabolism between healthy mothers and mothers with GDM. Placental LC-PUFA uptake is impaired in GDM and in combination with enhanced fetal fat accretion in more severe GDM under insulin treatment can well explain the reduced DHA levels in cord blood. More studies are needed to elucidate the benefit for LC-PUFA supplementation during this time of development and to establish the best strategy to supplement the mother, the fetus, or both.

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DISCLOSURES

There are no conflicts of interest, financial or otherwise, declared by any of the authors.

AUTHOR CONTRIBUTIONS

A.P., M.T.P.-S., J.E.B.-C., and A.G.-S. performed the experiments; A.P. and A.G.-S. analyzed the data; A.P., A.G.-S., H.D., and E.L. interpreted the results of the experiments; A.P. prepared the figures; A.P., M.T.P.-S., J.E.B.-C., A.G.-S., H.D., and E.L. drafted the manuscript; J.J.P., H.D., B.K., and E.L. contributed to the conception and design of the research; J.J.P., H.D., B.K., and E.L. approved the final version of the manuscript; H.D., B.K., and E.L. edited and revised the manuscript.

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