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Placental MFSD2A expression in fetal growth restriction and maternal and fetal DHA status

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ABSTRACT

Introduction: Fetal growth restriction (FGR) may affect placental transfer of key nutrients to the fetus, such as the fatty acid docosahexaenoic acid (DHA). Major facilitator superfamily domain containing 2A (MFSD2A) has been described as a specific DHA carrier in placenta, but its expression has not been studied in FGR. The aim of this study was to evaluate for the first time the placental MFSD2A levels in late-FGR pregnancies and the maternal and cord plasma DHA.

Methods: 87 pregnant women from a tertial reference center were classified into late-FGR (N = 18) or control (N = 69). Fatty acid profile was determined in maternal and cord venous plasma, as well as placental levels of MFSD2A and of insulin mediators like phospho-protein kinase B (phospho-AKT) and phospho-extracellular regulated kinase (phospho-ERK).

Results: Maternal fatty acid profile did not differ between groups. Nevertheless, late-FGR cord vein presented higher content of saturated fatty acids than control, producing a concomitant decrease in the percentage of some unsaturated fatty acids. In the late-FGR group, a lower DHA fetal/maternal ratio was observed when using percentages, but not with concentrations. No alterations were found in the expression of MFSD2A in late-FGR placentas, nor in phospho-AKT or phospho-ERK.

Discussion: MFSD2A protein expression was not altered in late-FGR placentas, in line with no differences in cord DHA concentration between groups. The increase in the saturated fatty acid content of late-FGR cord might be a compensatory mechanism to ensure fetal energy supply, decreasing other fatty acids percentage. Future studies are warranted to elucidate if altered saturated fatty acid profile in late-FGR fetuses might predispose them to postnatal catch-up and to long-term health consequences.

1. Introduction

The fatty acid docosahexaenoic acid (DHA, 22:6 n-3) is selectively and preferentially provided by the placenta to the fetus during the last trimester of pregnancy, ensuring the proper neurodevelopment of the newborn [1]. Recently, MFSD2A (major facilitator superfamily domain containing 2A) has been described as a selective lysophospholipid carrier in both brain and placenta, being essential for DHA tissue uptake [2,

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3]. In fact, MFSD2A plays a dual role establishing integrity of the blood–brain barrier and in uptake of DHA in brain [2]. MFSD2A has been studied in different pregnancy conditions, showing lower placental expression in gestational diabetes [3] or severe preeclampsia [4], however, there is no information regarding its levels in fetal growth restriction (FGR) placentas.

FGR, defined by a condition where the fetus does not reach its full growth potential, is one of the most common obstetric complications [5–7]. It is associated with long-term health consequences, such as increased cardiovascular risk, neurodevelopmental disorders, and endocrine disruption [8]. FGR, especially in developed/Western countries, is usually related to a reduced nutritional supply to the fetus from the utero-placental circulation, despite adequate nutrition from the mother [9,10]. Many echography studies have shown a higher pulsatility index (PI) of the uterine artery (UtA) and the umbilical artery (UA) in these fetuses, supporting a high resistance to blood flow in the placenta in these pregnancies [11]. This placental insufficiency becomes more evident during the third trimester of pregnancy. FGR is classified into two groups according to the gestational age at the time of diagnosis: early-FGR (<32 weeks) and late-FGR (≥32 weeks) [12]. This classification is based on the relevant differences between these two phenotypes of FGR in severity, natural history, and Doppler findings [13]. Late-FGR is far more frequent than early-FGR, and in contrast to early-FGR, late-FGR is usually milder and less likely to be associated with preeclampsia [14]. This is the reason why the two FGR populations should not be mixed when analyzing them.

Studies about fatty acid profile in fetal plasma with FGR are controversial, as not all show a lower DHA percentage [15–17]. Placental fatty acid transport can also be affected by the insulin action [18,19]. Specifically, insulin binds to its receptor in the trophoblast membrane [20] and activates two different cascades which involve the phosphorylation of AKT (protein kinase B) and ERK (extracellular regulated kinase) [21]. These insulin mediators are essential for cellular proliferation and differentiation during gestation [22]. Both phosphorylated proteins have been correlated to some lipid carriers in placenta, such as endothelial lipase, fatty acid translocase, and fatty acid binding protein [19]. *In vitro* studies on human choriocarcinoma cell line BeWo treated with insulin pathway inhibitors also reduced these lipid carriers, confirming such effect by the insulin mediators [19]. Placental levels of phospho-AKT and phospho-ERK have been analyzed in patients with preeclampsia [23,24] or gestational diabetes [24,25], however, limited data is available in FGR. Studying the mentioned insulin mediators in FGR placentas could be crucial to better understand the molecular mechanisms affected in these pregnancies.

The main objective of this study was to evaluate in late-FGR the fatty acid profile of both maternal and fetal plasma and the placental levels of MFSD2A to better understand the polyunsaturated fatty acid alterations of this common condition. Moreover, molecular mechanisms affected in late-FGR were discerned through analysis of different placental proteins related to nutrient metabolism as insulin pathway mediators (phospho-AKT and phospho-ERK).

2. Materials and methods

2.1. Study population

This is a prospective, observational study (NCT 04047966, https://clinicaltrials.gov/study/NCT04047966) that includes 87 singleton Caucasian pregnancies delivering after 37 weeks of gestation, recruited between 32 and 36 weeks of gestation at BCNatal - Hospital Sant Joan de Déu (Barcelona, Spain). The study population included 69 controls defined by birthweight > 10th centile with no pregnancy complications and 18 late-FGR defined according to the International Federation of Gynecology and Obstetrics (FIGO) [12] by estimated fetal weight (EFW) < 3rd centile and/or EFW < 10th centile together with cerebro-placental ratio (CPR) < 5th centile and/or mean UtA PI > 95th centile. Only late-FGR cases with confirmed birthweight < 10th centile were included in the study.

The exclusion criteria were: fetal malformations, premature rupture of membranes, alcohol, tobacco, or other drugs during pregnancy, pregestational diabetes, preeclampsia, and use of antibiotics in the 3 months prior to recruitment (including those for *Streptococcus agalactiae* group B). The complete study design and the protocol have been previously published [26]. Patients were selected from women who attended the Maternal-Fetal Medicine Department at BCNatal in Barcelona from July 2018 to July 2020. Cases were recruited as a row of all the small fetuses diagnosed during that period. Controls were selected from low-risk pregnancies matched to cases by gestational age at ultrasound (±1 week). In all pregnancies, gestational age was calculated based on crown-rump length at first trimester ultrasound [27], and EFW and birthweight centiles were calculated using local reference curves [28]. Pregnancies with structural/chromosomal anomalies or evidence of fetal infection were excluded.

2.2. Perinatal data

Cases and controls underwent prenatal ultrasonographic examination at 36 weeks (±1 week) using a Siemens Sonoline Antares ultrasound system (Siemens Medical Systems, PA, United States), including EFW and standard feto-placental Doppler evaluation. Feto-placental Doppler comprised measurements of the PI of UtA, UA, and middle cerebral artery (MCA). CPR was calculated by dividing the MCA PI by the UA PI, as previously described [29].

At delivery, gestational age, mode of delivery, birthweight, birthweight centile, Apgar scores, and umbilical cord vein and artery pH were recorded.

2.3. Samples collection

Maternal venous blood was collected at 36 weeks of gestation in EDTA-coated tubes. At the time of delivery, both maternal and venous umbilical cord blood samples were also taken. Blood was centrifuged at 1400 g for 10 min at 4 °C to obtain plasma. Placenta samples were collected within 30 min after delivery generating a pool of five 1 cm^3 fragments: one sample from each placental quadrant from the maternal side and one periumbilical sample from the fetal side. These tissues were rinsed in cold 0.9 % NaCl solution. Plasma and placenta samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.4. Fatty acid analyses

Total lipids were extracted from 100 μL of plasma into chloroform: methanol (2:1 v/v) according to Folch et al. method [30]. Previous to the extraction, 0.05 mg pentadecanoic acid was added to the samples as internal standard. Fatty acid methyl esters were produced according to Stoffel et al. [31] by adding 1 mL of 3 N methanolic HCl (Supelco, Sigma-Aldrich, MO, United States) and heating at 90 °C for 1 h. The derivatives were extracted into hexane and stored at −20 °C until gas chromatographic analysis.

Fatty acid methyl esters were analyzed by gas chromatography using an SP-2560 capillary column (100 m × 0.25 mm i. d. × 20 μm) (Supelco, Sigma-Aldrich, MO, United States) in a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Madrid, Spain) equipped with a flame ionization detector [32]. The temperature of the detector and the injector was 240 °C. The oven temperature was programmed at 175 °C for 30 min and increased at 2 °C/min to 230 °C and held at this temperature for 17 min. Helium was used as the carrier gas at a pressure of 45 psi. Peaks were identified by comparison of their retention times with appropriate fatty acid methyl esters standards (Sigma-Aldrich, MO, United States) and fatty acids concentrations were determined in relation to the peak area of internal standard. In the case of α-linolenic acid

(ALA, 18:3 n-3), its peak overlaps with 20:1 n-9, so despite attempts to separate them, the quantification was not entirely accurate. Fatty acid data were represented as concentration (g/L) and/or percentage of total fatty acids (g/100 g of total fatty acids).

2.5. Protein extracts for Western blotting

Protein extracts were obtained by homogenizing 30 mg of placental tissue in 0.3 mL ice-cold lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM Na_2EDTA, 1 mM EGTA, 1 % Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin) from Cell Signaling Technology (MA, United States). A phenylmethanesulfonyl fluoride solution of 1 mM was added to the lysis buffer before homogenization [33]. Samples were homogenized using a Tissue Lyser LT device (Qiagen Iberia SL, Madrid, Spain). Protein lysates were obtained after 10 min centrifugation at 10000 g 4 °C. Protein concentration was quantified by Bradford assay [34] and samples were stored at −80 °C until Western blot analysis.

2.6. Western blot analysis

The primary antibodies used were: rabbit polyclonal anti-MFSD2A/NLS1 (Abcam, Cambridge, United Kingdom, Ref: ab177881) 1:500; rabbit monoclonal against AKT (Cell Signaling Technology, MA, United States, Ref: 4691S) 1:1000; rabbit monoclonal anti-phospho-AKT (Cell Signaling Technology, MA, United States, Ref: 4060S) 1:1000; rabbit polyclonal antibody against ERK1/2 (ProteinTech, Manchester, United Kingdom, Ref: 16443-1-AP) 1:1000; rabbit polyclonal antibody anti-phospho-ERK1/2 (ProteinTech, Manchester, United Kingdom, Ref: 28733-1-AP) 1:1500; and mouse monoclonal against β-actin (Sigma-Aldrich, MO, United States, Ref: A5441) 1:15000. The secondary antibodies used were anti-mouse (Santa Cruz Biotechnology, TX, United States, Ref: sc-516102) and anti-rabbit (Santa Cruz Biotechnology, TX, United States, Ref: sc-2357) polyclonal antibodies conjugated with horseradish peroxidase. Anti-rabbit 1:2500 for MFSD2A/NLS1, 1:5000 for AKT, phospho-AKT, and ERK1/2, and 1:7500 for phospho-ERK1/2; anti-mouse 1:25000 for β-actin.

The protein extracts (15 mg protein) diluted in sample buffer were resolved on 10 % polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany). Membranes were blocked in phosphate saline buffer with 0.05 % Tween-20 (PBS-T) containing 2 % bovine serum albumin for 1 h at room temperature. Thereafter, membranes were incubated separately with primary antibodies anti-MFSD2A, anti-phospho-AKT, and anti-phospho-ERK overnight at 4 °C. Blots were then washed with PBS-T and probed for 1 h at room temperature with the corresponding secondary antibodies conjugated with horseradish peroxidase. Finally, all membranes were stripped with Tris/HCl buffer pH 2.3 containing β-mercaptoethanol 0.1 M and re-probed with anti-β-actin to perform loading controls. In the case of phospho-AKT and phospho-ERK, the membranes were also re-probed with anti-AKT and anti-ERK in order to know their relative rate of phosphorylation.

Immunoblot signals were detected using a chemiluminescence kit according to the manufacturer's instruction (Pierce ECL Plus Western Blotting Substrate; Thermo Fisher Scientific, MA, United States, Ref: 32132). The density of all bands was determined by densitometry using ImageJ software (National Institutes of Health, MD, United States).

2.7. Statistical analyses

Results were expressed as mean ± standard error of the mean (SEM) for continuous variables that followed a normal distribution, median (interquartile range: IQR) for non-normal distributed variables, or n (%) for qualitative variables. The Kolmogorov-Smirnov normality test was used to check the normal distribution of continuous variables. To study differences between experimental groups, a *t*-test analysis was performed for normally distributed variables or the Mann-Whitney *U* test for non-normally distributed ones. In the case of qualitative variables, a Chi^2 test was performed. Analyses were adjusted by potential confounding variables by the ANCOVA test: age, pre-pregnancy body mass index (BMI), and previous pregnancies for maternal and placental variables; pre-pregnancy BMI, previous pregnancies, and sex for fetal statistical analysis. SPSS 28.0 software package (IBM Corp., NY, United States) was used for statistical analysis. Differences were considered statistically significant when the P_value < 0.05.

2.8. Ethics statement

The study protocol has been approved by the local Ethics Committee of Hospital Sant Joan de Déu (February 22, 2018; PIC-40-18) and University of Granada (March 2, 2018; 02032018), and was conducted according to the standards given in the Declaration of Helsinki and Good Clinical Practice Guidelines. The research was carried out in agreement with the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) Statement guidelines. A written informed consent was obtained from all participant pregnant women at recruitment.

Table 1
Baseline and perinatal characteristics of the study population.

	Control	Late-FGR	*P*
	N = 69	N = 18	
Mothers			
Age (years)	32 ± 1	33 ± 1	0.664
Weight pre-pregnancy (Kg)	66.8 ± 2.0	56.9 ± 2.1	**0.001**
Height (cm)	162.9 ± 0.7	159.8 ± 1.7	**0.048**
BMI pre-pregnancy	25.1 ± 0.7	22.2 ± 0.6	**0.002**
BMI in the third trimester	27.9 ± 0.6	25.4 ± 0.8	**0.029**
DHA supplementation at recruitment	45 (65 %)	8 (44 %)	0.064
Nulliparous	40 (58 %)	11 (61 %)	0.190
Gestational diabetes	1 (1.4 %)	0 (0 %)	0.608
Asthma/allergies	11 (25 %)	0 (0 %)	0.076
Ultrasound data			
Gestational age (weeks)	36 ± 1	36 ± 1	
EFW centile	52.0 (28.5,75.0)	1.5 (1.0,5.3)	**< 0.001**
Mean uterine artery PI	0.7 (0.6,0.8)	0.9 (0.7,1.1)	**0.023**
Umbilical artery PI	0.8 (0.7,0.8)	1.0 (0.9,1.2)	**0.025**
Middle cerebral artery PI	1.7 (1.5,1.8)	1.5 (1.3,1.9)	0.419
Cerebro-placental ratio	2.03 ± 0.15	1.57 ± 0.15	**0.036**
Perinatal data			
Gestational age at birth (weeks)	39.85 ± 0.15	37.62 ± 0.29	**< 0.001**
Labor induction	25 (36 %)	11 (61 %)	0.058
Caesarean section	13 (19 %)	11 (61 %)	**< 0.001**
Emergency caesarean section	2 (3 %)	3 (17 %)	0.371
Sex (males)	36 (52 %)	8 (44 %)	0.559
Birthweight (g)	3257 ± 47	2165 ± 90	**< 0.001**
Birthweight centile	35.0 (17.0,60.5)	1.0 (1.0,5.3)	**< 0.001**
Length (cm)	50.24 ± 0.32	45.09 ± 1.09	**< 0.001**
Apgar 1′	8.85 ± 0.10	8.35 ± 0.47	0.311
Apgar 5′	9.97 ± 0.02	9.65 ± 0.17	0.077
Umbilical cord artery pH	7.25 ± 0.01	7.25 ± 0.01	0.994
Umbilical cord vein pH	7.31 ± 0.01	7.29 ± 0.02	0.377

Data are expressed as mean ± SEM, median (IQR), or n (%). Significance level set at P_value < 0.05.
Apgar, activity, pulse, grimace, appearance, and respiration; BMI, body mass index; DHA, docosahexaenoic acid; EFW, estimated fetal weight; FGR, fetal growth restriction; PI, pulsatility index.

3. Results

3.1. Baseline and perinatal data

Study population and perinatal data are expressed in Table 1. Mothers from the late-FGR group presented lower BMI before pregnancy and also in the third trimester of gestation compared to control. No differences were identified when studying the proportion of nulliparous women between groups. In addition, no differences were observed regarding the number of participants consuming multivitamin supplements containing 200 mg of DHA, despite more women in the control group took these supplements at recruitment. As expected, the proportion of inductions and cesarean sections was greater in the late-FGR group due to pregnancy complications. There were no differences in the incidence of gestational diabetes between groups. Maternal asthma and allergy incidence was also similar among groups.

Late-FGR fetuses presented significantly higher UtA and UA PI at ultrasound than the control group, although values were inside the normal range, and no differences were reported when analyzing the MCA PI. In the case of CPR, it was lower in the late-FGR group compared to control. Also, as expected in FGR management protocols, gestational age at birth was significantly lower in this group, as well as birthweight and birthweight centile. Apgar scores and umbilical cord vein and artery pH were normal and showed no differences between groups.

3.2. Fatty acid profile of maternal plasma

Maternal fatty acid profile during pregnancy and at delivery are displayed in Table 2. At 36 weeks of gestation, mothers from the late-FGR group had higher eicosapentaenoic acid (EPA, 20:5 n-3) and DHA percentages (Fig. 1A) than control. This led to a significantly higher n-3 long chain-polyunsaturated fatty acid percentage and a tendency to a lower n-6:n-3 ratio in late-FGR mothers, even after adjusting for confounding variables. No changes between groups were observed in the rest of the fatty acids analyzed.

In maternal plasma at delivery some of these differences were not present. In the late-FGR group, oleic acid (OA, 18:1 n-9) percentage was significantly lower compared to control, while EPA tended again to higher values ($P = 0.077$) (Fig. 1B) as well as docosapentaenoic acid (DPA, 22:5 n-3).

3.3. Fatty acid profile of umbilical cord vein plasma

Cord vein fatty acids results are shown in Table 3. Saturated fatty acid content in the late-FGR cord plasma was significantly higher compared to control, both in percentage and concentration. Especially due to the greater values of palmitic acid (PA, 16:0) (Fig. 1C and D). This may explain the tendency to higher total fatty acid concentration in late-FGR fetuses. In addition, in the late-FGR group we reported a significantly higher fetal/maternal ratio of saturated fatty acids in concentration compared to the control group.

On the other hand, the monounsaturated fatty acid percentage was decreased in late-FGR cord. The percentage of DHA with respect to total fatty acids was also significantly lower in the late-FGR group compared to control, although a tendency was observed after adjusting by covariates ($P = 0.075$) (Fig. 1C). However, DHA concentration did not change between groups, and in fact, fetuses with late-FGR had even more concentration of both DPA and EPA (Fig. 1D). We observed a significantly lower fetal/maternal ratio using DHA percentage in late-FGR pregnancies compared to control, but no differences when using DHA concentration values.

3.4. Placental Western blot analysis

Regarding protein expression in placental tissue, after adjustment, no significant differences were found for MFSD2A levels between late-FGR and controls (Table 4). Levels of the phosphorylated form of AKT (phospho-AKT/β-actin) did not vary between experimental groups, and

Table 2
Fatty acid profile of maternal plasma at 36 weeks of gestation and at delivery.

Mothers at 36 weeks of gestation (%)					Mothers at delivery (%)			
FA	**Control**	**Late-FGR**	***P***	***P*[a]**	**Control**	**Late-FGR**	***P***	***P*[a]**
	N = 65	N = 17			N = 53	N = 14		
14:0	2.191 ± 0.172	2.044 ± 0.235	0.683	0.510	1.225 ± 0.080	1.288 ± 0.119	0.709	0.913
16:1 n-9	0.339 ± 0.014	0.356 ± 0.017	0.544	0.613	0.363 ± 0.012	0.361 ± 0.018	0.923	0.691
18:0	6.291 ± 0.139	6.251 ± 0.279	0.896	0.650	7.092 ± 0.231	7.053 ± 0.286	0.934	0.983
18:1 n-7	1.507 ± 0.034	1.477 ± 0.050	0.671	0.397	1.971 ± 0.065	1.944 ± 0.144	0.851	0.432
18:2 n-6	26.386 ± 0.482	25.818 ± 0.827	0.585	0.558	23.851 ± 0.509	23.934 ± 1.029	0.941	0.540
18:3 n-3	0.295 ± 0.013	0.283 ± 0.018	0.654	0.810	0.232 ± 0.008	0.225 ± 0.015	0.678	0.474
18:3 n-6	0.179 ± 0.020	0.182 ± 0.036	0.953	0.734	0.229 ± 0.014	0.232 ± 0.018	0.895	0.813
20:3 n-6	1.764 ± 0.054	1.905 ± 0.139	0.274	0.192	1.629 ± 0.054	1.726 ± 0.102	0.414	0.233
22:4 n-6	0.119 ± 0.023	0.134 ± 0.033	0.756	0.673	0.197 ± 0.018	0.199 ± 0.020	0.950	0.912
22:5 n-3	0.087 ± 0.015	0.106 ± 0.039	0.585	0.439	0.163 ± 0.008	0.208 ± 0.014	**0.015**	**0.004**
22:5 n-6	0.162 ± 0.019	0.156 ± 0.039	0.893	0.717	0.234 ± 0.011	0.321 ± 0.051	0.121	**0.019**
24:0	0.687 ± 0.087	0.612 ± 0.103	0.676	0.880	1.280 ± 0.114	1.115 ± 0.042	0.464	0.545
24:1 n-9	0.862 ± 0.043	0.905 ± 0.081	0.650	0.330	0.816 ± 0.019	0.775 ± 0.052	0.372	0.468
20:4 n-6/18:2 n-6	0.223 ± 0.006	0.229 ± 0.012	0.653	0.633	0.258 ± 0.016	0.247 ± 0.010	0.734	0.764
22:6 n-3/18:3 n-3	11.875 ± 0.634	13.426 ± 1.168	0.264	0.279	14.409 ± 0.833	14.848 ± 1.392	0.805	0.617
SFA	36.565 ± 0.359	36.342 ± 0.833	0.786	0.904	38.588 ± 0.474	38.496 ± 0.614	0.925	0.955
MUFA	24.717 ± 0.398	24.771 ± 0.640	0.949	0.871	24.670 ± 0.385	24.251 ± 1.270	0.756	0.138
PUFA	38.398 ± 0.464	38.657 ± 0.982	0.803	0.746	36.194 ± 0.456	36.678 ± 1.291	0.662	0.204
Trans	0.320 ± 0.041	0.229 ± 0.051	0.175	0.256	0.548 ± 0.037	0.575 ± 0.042	0.716	0.591
n-3 PUFA	3.801 ± 0.127	4.328 ± 0.223	0.058	**0.043**	3.848 ± 0.106	4.107 ± 0.353	0.493	0.232
n-6 PUFA	34.556 ± 0.487	34.291 ± 0.954	0.805	0.840	32.239 ± 0.481	32.454 ± 1.197	0.847	0.342
n-6/n-3	9.929 ± 0.436	8.262 ± 0.494	**0.015**	0.053	8.775 ± 0.309	8.624 ± 0.744	0.832	0.889
n-3 LC-PUFA	3.461 ± 0.124	4.013 ± 0.225	**0.043**	**0.034**	3.510 ± 0.104	3.780 ± 0.348	0.468	0.203
n-6 LC-PUFA	7.991 ± 0.156	8.291 ± 0.416	0.421	0.307	8.159 ± 0.198	8.288 ± 0.291	0.757	0.376
Total FA (g/L)	6.151 ± 0.150	6.509 ± 0.309	0.285	0.250	10.128 ± 0.588	8.781 ± 0.760	0.270	0.133

Data are expressed as mean ± SEM. *Analysis adjusted by potential confounding variables: age, pre-pregnancy BMI, and previous pregnancies. Significance level set at P_value < 0.05.

FA, fatty acid; FGR, fetal growth restriction; LC-PUFA, long chain-polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

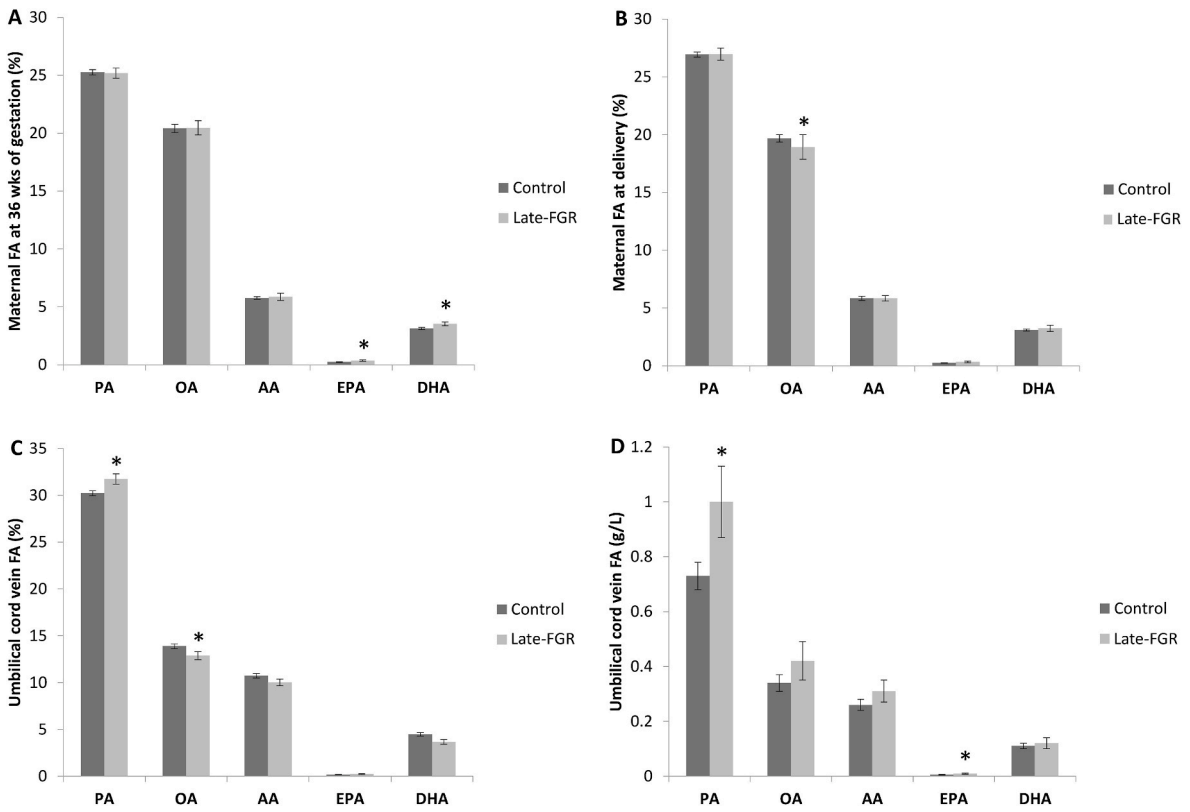

Fig. 1. Fatty acids in control and late-FGR subjects. (A) Maternal plasma at 36 weeks of gestation (%). (B) Maternal plasma at delivery (%). (C) Umbilical cord vein (%). (D) Umbilical cord vein (g/L). Analysis adjusted by potential confounding variables: age, pre-pregnancy BMI, and previous pregnancies (for mothers), and pre-pregnancy BMI, previous pregnancies, and sex (for the umbilical cord vein). Maternal EPA percentage at delivery between groups: $P = 0.077$. Fetal DHA percentage between groups: $P = 0.075$. *Indicates statistically significant differences between experimental groups (P_value < 0.05). AA, arachidonic acid; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FGR, fetal growth restriction; OA, oleic acid; PA, palmitic acid.

neither did its phosphorylation rate (phospho-AKT/AKT). Finally, there were also no differences in phospho-ERK/β-actin and phospho-ERK/ERK ratios (Table 4). Western blot results can be seen in Supplementary Fig. 1.

4. Discussion

We evaluated for the first time in late-FGR pregnancies the expression of MFSD2A, a selective DHA carrier in the placenta. We did not find differences in its levels between late-FGR and control, suggesting no major changes in the abundance of this specific DHA placental transfer protein. This agrees with no significant changes in the DHA concentration of late-FGR cord vein, although its percentage was lower compared to control. A potential explanation might be related to the higher percentage and concentration of saturated fatty acids in late-FGR cord, which may hence decrease the percentage of monounsaturated and polyunsaturated fatty acids despite normal maternal fatty acid profile. This higher content of saturated fatty acids could be a compensatory mechanism to generate a more optimal source of energy from glucose in the late-FGR fetus [35]. From a clinical point of view, our results reinforce the hypothesis of the role of fatty acid profile in growth restriction and suggest the possibility to supplement this population in the future.

In the present study, mothers from the late-FGR group had a significantly lower BMI before pregnancy and also in the third trimester of gestation compared to control. However, some studies have not found differences in that [15–17,36], although low pre-pregnancy BMI has been associated with increased risk of low birthweight [37]. In addition, a reduction of maternal BMI > 2 kg/m^2 between the first two pregnancies has been reported to increase the chance of FGR and preterm birth [38]. We corroborated that late-FGR pregnancies of our study had higher UtA and UA PI than control, something that has been extensively demonstrated [11,39], reinforcing higher resistance to blood flow in placentas [11]. Late-FGR newborns presented lower gestational age and birthweight than control ones, as has been observed in other studies [16,17,24,36]. This is in part explained by the follow-up protocols applied in this population that includes labor induction between 37 and 38 weeks of gestation in order to reduce potential complications of the late-FGR pregnancies.

During pregnancy, there is a higher requirement of n-3 polyunsaturated fatty acids (especially DHA) in the mother to ensure visual and neurological development in the baby [40,41]. In our population, late-FGR mothers did not present any detrimental alteration in their fatty acid profile. In fact, they showed higher percentages of some n-3 long chain-polyunsaturated fatty acids compared to controls, like EPA and DHA at 36 weeks of gestation, and EPA and DPA at delivery. At recruitment, more women in the control group took multivitamin supplements (containing 200 mg of DHA). However, the consumption of this supplementation was not registered at delivery. Then, the previously noted higher DHA percentage in late-FGR maternal plasma might be related to a healthier diet in these mothers. In the same line, Cetin et al. reported increased EPA and DPA percentages in FGR Italian mothers during pregnancy, although no differences were observed in the DHA percentage [16]. However, Assumpção et al. reported lower percentages of polyunsaturated fatty acids, such as AA and DHA, in maternal erythrocytes of the FGR group at delivery [15]. Recent data on the maternal proteomic profile in late-FGR pregnancies have shown alterations in lipid metabolism such as in the biological processes of the efflux of phospholipids and cholesterol [7].

Despite the adequate maternal profile of fatty acids during pregnancy, we found a trend towards a higher amount of total fatty acids in

Table 3
Fatty acid profile of umbilical cord vein plasma at delivery.

Umbilical cord vein (%)					Umbilical cord vein (g/L)			
FA	**Control**	**Late-FGR**	***P***	***P*[a]**	**Control**	**Late-FGR**	***P***	***P*[a]**
	N = 53	N = 14			N = 53	N = 14		
14:0	2.244 ± 0.166	2.754 ± 0.305	0.166	0.113	0.055 ± 0.007	0.077 ± 0.008	0.112	0.090
16:1 n-9	0.808 ± 0.041	0.847 ± 0.103	0.689	0.631	0.020 ± 0.002	0.026 ± 0.004	0.191	0.170
18:0	11.763 ± 0.161	12.074 ± 0.466	0.432	0.671	0.283 ± 0.018	0.375 ± 0.047	**0.038**	**0.045**
18:1 n-7	2.343 ± 0.039	2.193 ± 0.095	0.103	0.064	0.057 ± 0.004	0.068 ± 0.009	0.208	0.249
18:2 n-6	9.515 ± 0.375	9.958 ± 1.158	0.639	0.435	0.243 ± 0.025	0.359 ± 0.104	0.112	0.089
18:3 n-3	0.121 ± 0.011	0.121 ± 0.028	0.842	0.964	0.003 ± 0.001	0.004 ± 0.001	0.457	0.397
18:3 n-6	0.323 ± 0.010	0.228 ± 0.010	**< 0.001**	**< 0.001**	0.008 ± 0.001	0.007 ± 0.001	0.600	0.447
20:3 n-6	2.487 ± 0.080	2.220 ± 0.085	0.112	0.087	0.058 ± 0.004	0.069 ± 0.010	0.216	0.216
22:4 n-6	0.402 ± 0.018	0.358 ± 0.029	0.251	0.417	0.010 ± 0.001	0.010 ± 0.001	0.676	0.538
22:5 n-3	0.222 ± 0.011	0.238 ± 0.015	0.494	0.370	0.005 ± 0.001	0.007 ± 0.001	0.053	**0.026**
22:5 n-6	0.467 ± 0.026	0.397 ± 0.069	0.259	0.249	0.011 ± 0.001	0.010 ± 0.001	0.890	0.927
24:0	1.497 ± 0.024	1.549 ± 0.058	0.352	0.497	0.036 ± 0.002	0.047 ± 0.005	**0.048**	0.053
24:1 n-9	1.020 ± 0.023	1.084 ± 0.060	0.247	0.602	0.025 ± 0.002	0.034 ± 0.004	**0.031**	0.055
20:4 n-6/18:2 n-6	1.199 ± 0.043	1.057 ± 0.091	0.134	0.066	1.199 ± 0.043	1.057 ± 0.091	0.134	0.066
22:6 n-3/18:3 n-3	45.580 ± 3.513	33.941 ± 6.061	0.152	0.143	45.580 ± 3.513	33.941 ± 6.061	0.152	0.143
DHA F/M ratio	1.546 ± 0.098	1.104 ± 0.100	**0.028**	**0.028**	0.411 ± 0.039	0.457 ± 0.105	0.632	0.395
SFA	49.271 ± 0.484	52.052 ± 1.164	**0.016**	**0.033**	1.192 ± 0.082	1.616 ± 0.202	**0.031**	**0.034**
SFA F/M ratio	1.287 ± 0.020	1.366 ± 0.044	0.101	0.178	0.341 ± 0.026	0.497 ± 0.086	**0.025**	**0.008**
MUFA	20.487 ± 0.287	19.429 ± 0.554	0.100	**0.048**	0.501 ± 0.036	0.618 ± 0.093	0.176	0.211
PUFA	29.724 ± 0.480	28.089 ± 0.979	0.132	0.304	0.727 ± 0.052	0.927 ± 0.170	0.135	0.105
Trans	0.518 ± 0.027	0.430 ± 0.061	0.157	0.176	0.013 ± 0.001	0.012 ± 0.003	0.980	0.881
n-3 PUFA	5.298 ± 0.194	4.510 ± 0.279	0.060	0.125	0.127 ± 0.009	0.150 ± 0.025	0.319	0.219
n-6 PUFA	24.112 ± 0.403	23.358 ± 0.959	0.422	0.710	0.592 ± 0.043	0.771 ± 0.147	0.117	0.093
n-6/n-3	4.817 ± 0.182	5.432 ± 0.410	0.143	0.203	4.817 ± 0.182	5.432 ± 0.410	0.143	0.203
n-3 LC-PUFA	5.000 ± 0.196	4.233 ± 0.288	0.070	0.137	0.120 ± 0.009	0.141 ± 0.023	0.331	0.227
n-6 LC-PUFA	14.275 ± 0.292	13.172 ± 0.461	0.083	0.114	0.341 ± 0.022	0.405 ± 0.051	0.211	0.178
Total FA					2.433 ± 0.166	3.174 ± 0.453	0.068	0.069

Data are expressed as mean ± SEM. [a]Analysis adjusted by potential confounding variables: pre-pregnancy BMI, previous pregnancies, and sex. Significance level set at *P*_value < 0.05.
DHA, docosahexaenoic acid; FA, fatty acid; F/M, fetal/maternal; FGR, fetal growth restriction; LC-PUFA, long chain-polyunsaturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 4
Placental protein expression of MFSD2A, phospho-AKT, and phospho-ERK.

Protein expression (a.u.)	Control	Late-FGR	*P*	*P*[a]
	N = 54	N = 15		
MFSD2A/β-actin	1.08 ± 0.08	1.17 ± 0.16	0.576	0.511
Phospho-AKT/β-actin	1.91 ± 0.09	1.63 ± 0.12	0.128	0.130
Phospho-AKT/AKT	0.73 ± 0.05	0.62 ± 0.09	0.339	0.190
Phospho-ERK/β-actin	0.39 ± 0.02	0.44 ± 0.06	0.283	0.226
Phospho-ERK/ERK	0.98 ± 0.05	0.95 ± 0.09	0.811	0.865

Data are expressed as mean ± SEM. [a]Analysis adjusted by potential confounding variables: age, pre-pregnancy BMI, and previous pregnancies. Significance level set at *P*_value < 0.05.
a.u., arbitrary units; FGR, fetal growth restriction.

the late-FGR umbilical cord vein compared to control, especially due to higher saturated fatty acid levels (mainly PA) in both percentage and concentration. These results are consistent with those of greater saturated fatty acid percentages in other studies with FGR human fetuses [15,16]. In this condition there is poor placental blood circulation that may generate an insufficient nutrient supply to the fetus; the greater percentage and concentration of saturated fatty acids could be a compensatory response from the fetus to generate a more optimal energetic source from glucose. FGR fetuses try to adapt to the hypoglycemic environment in utero by developing mechanisms that maintain fetal energy stores to ensure their survival, specifically increasing glycogen and fat stores [35]. Limesand et al. in a study in sheep demonstrated that glycogen stores are higher in the liver and skeletal muscle of FGR fetuses compared to controls [35]. These authors proposed that the underlying mechanism of this phenomenon might be the increased insulin sensitivity in the mentioned tissues that would enhance glucose storage [35]. In fact, FGR umbilical cord vein has been reported to present lower glucose and oxygen concentrations compared to controls, but higher glucose/O_2 metabolic quotient [36]. This might indicate that glucose is not only being oxidized to produce ATP but some of it is being used for lipogenesis, which would be in line with the higher fetal/maternal ratio of saturated fatty acids in the late-FGR group. This adaptive mechanism could predispose FGR fetuses to greater fat deposition when exposed to high-sugar and high-fat diets later in life [35] and also to catch-up on infancy [42].

Regarding cord vein unsaturated fatty acids, we found a tendency to a lower DHA percentage in the late-FGR group compared to control. Caesarean section was not associated with cord vein DHA (β = −0.131, P = 0.304) or saturated fatty acids concentration (β = 0.024, P = 0.853) in the present study. Additionally, other authors have reported even lower oxidation in caesarean respect to labor [43,44]. A lower DHA percentage has been also reported by Assumpção et al. in umbilical cord erythrocytes in FGR [15], while other authors have not found changes between groups [16,17]. The percentage values referred to the proportion of one fatty acid to total fatty acids, however, since total fatty acid concentration tended to be higher in cord of the late-FGR group, it is also important to consider not only percentage values but also the absolute amount of the different fatty acids, specially, in the cord blood. When we analyzed the concentration of DHA in umbilical cord, there were no differences between groups, even being the mean value higher in the late-FGR (but not significant). This is consistent with other studies in animal models [45].

Although no differences in DHA concentration were observed in the present study, the lower percentage of DHA in late-FGR fetuses might imply a reduced DHA tissue bioavailability since the proportion of DHA respect to other fatty acids was decreased. However, this would need confirmation in future long-term studies. Cetin et al. reported that FGR fetuses presented a lower fetal/maternal ratio of DHA percentage and suggested a possible placental transfer problem in these subjects [16].

We also observed a significantly lower fetal/maternal ratio using DHA percentage in late-FGR fetuses compared to control, but no differences when using DHA concentration values. The discrepancy between percentage and concentration of DHA in late-FGR fetuses could be mainly explained by the increase in the total fatty acid amount (especially saturated fatty acids), and not by specific DHA placental transfer problems.

To detect a possible disturbed DHA placental transfer, we analyzed for the first time the protein expression of MFSD2A in late-FGR placentas since this is a specific carrier for this fatty acid. We did not find any changes between groups, indicating no major changes in the abundance of this placental DHA carrier in late-FGR pregnancies. The Pearson correlation between placental MFSD2A and cord vein DHA concentration was not significant ($R = 0.122$ and $P = 0.366$), which is in line with no differences in cord DHA concentration. Toufaily et al., in a little study with only 6 placentas, analyzed the expression of MFSD2A due to its important role in trophoblast fusion. They found lower levels of this protein in placentas with severe preeclampsia, but no differences in moderate preeclampsia samples [4]. Thus, previously reported differences about MFSD2A placental expression in preeclampsia do not contradict the findings in this paper (as preeclampsia was an exclusion criterion in our study).

In a study with stable isotopes after birth, it was found that infants with FGR have impaired formation of DHA from DPA [46]. Cetin et al. also reported a decreased proportion of AA and DHA respect to their precursors (LA and ALA, respectively) [16]. We confirmed a trend to lower AA synthesis from LA in the fetuses with late-FGR of the present study compared to control, although due to the limitation in the quantification of ALA (as indicated in 'Materials and methods'), we did not report differences in the DHA synthesis ratio.

Concerning the placental expression levels of insulin mediators, we did not find significant changes in phospho-AKT or phospho-ERK between late-FGR and controls. However, it has been reported that rats with dexamethasone-induced FGR present a decrease in the activation of AKT and ERK, and consequently in their downstream effectors [22,47]. In humans, results are inconclusive. Tsai et al., in a small group of 10 FGR placentas, found lower levels of phospho-AKT and higher of phospho-ERK, as well as of their phosphorylated downstream effectors [24]. The discrepancies observed between human results and those reported in previous animal studies could be related to the higher severity of these models compared to human FGR condition.

This study has some strengths and limitations that merit comment. The main strength of our study lies in its groundbreaking analysis of placental levels of MFSD2A in late-FGR pregnancies and the DHA status of both mothers and fetuses. As well, our study population was comprised by a very well-characterized cohort of small fetuses according to EFW, UtA, and CPR. However, there are certain limitations in our research that should be mentioned. First, information about maternal DHA supplementation was only available at inclusion, lacking this information at the time of delivery. In addition, we are aware of the relatively limited size of this study, what difficult to adjust the results at birth for gestational age. It is possible that the lower gestational age at birth of the late-FGR babies may, at least in part, contribute to some of the differences observed in the fatty acid profile of cord venous blood.

In conclusion, our results indicate that late-FGR placentas do not present alterations in their MFSD2A levels. This agrees with no significant changes in DHA concentration of late-FGR cord, although its percentage tended to be lower. Future studies are warranted to elucidate if higher saturated fatty acid content in FGR fetuses might predispose them to postnatal catch-up and to long-term cardiovascular risk and neurodevelopment impairment. These children could benefit from monitoring adequate supply/supplementation of omega-3 fatty acids at long-term.

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CRediT authorship contribution statement

Valentina Origüela: Writing – original draft, Methodology, Data curation. **Patricia Ferrer-Aguilar:** Writing – review & editing, Methodology. **Antonio Gázquez:** Writing – review & editing, Methodology, Data curation. **Miriam Pérez-Cruz:** Writing – review & editing, Methodology. **María Dolores Gómez-Roig:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Carolina Gómez-Llorente:** Writing – review & editing, Funding acquisition, Conceptualization. **Elvira Larqué:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2024.04.002.

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