Clinical Research

Placental nutrient and transport system in fetus with small for gestational age and growth restriction compared to appropriate for gestational age

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ABSTRACT

BACKGROUND Fetal growth restriction (FGR) has multifactorial etiology, including nutrition. Fetal nutrient status depends not only on an adequate supply of key nutrients but also optimal delivery, served by the placenta as a major metabolic and transport organ. This study aimed to evaluate the status of placental oxygen and nutrient concentration and their transporters between appropriate for gestational age (AGA), small for gestational age (SGA), and FGR.

METHODS This cross-sectional study was conducted at Cipto Mangunkusumo Hospital, Jakarta, from July 2018 to December 2020. Patients were divided into 3 groups, namely AGA, SGA, and FGR. The placental samples were taken following delivery. Placental concentration of glucose, amino acids (AAs), and fatty acids (FAs) were measured by calorimetric assay, liquid chromatography-tandem mass spectrometry, and gas chromatography-mass spectrometry, respectively. Placental concentration of vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT1), system y^+L, and fatty acid transport protein 1 (FATP1) were examined using enzyme-linked immunosorbent assay.

RESULTS A total of 57 subjects participated in the study. Compared with the AGA group, the SGA and FGR groups had lower placental AA concentration (p = 0.004), higher placental FA concentration (p = 0.048), higher placental expression of VEGF (p = 0.003), system y^+L (p = 0.07), and FATP1 (p = 0.021). No difference in placental glucose and GLUT1 concentration was observed among all groups (p = 0.301).

CONCLUSIONS The similar profile of macronutrient concentration with increased expression of oxygen and several nutrient transporters in SGA and FGR groups might indicate similar pathogenesis between these groups.

KEYWORDS fetal growth restriction, nutrient, placenta, small for gestational age, transport system

Fetal growth restriction (FGR) is a global concern that causes short- and long-term complications. The short-term complications include prematurity-related morbidity, whereas the long-term effects are stunting, neurobehavioral impairment, metabolic, and cardiovascular diseases (CVDs).[1–3] In Indonesia, the prevalence of FGR is 19.8%.[4,5]

The multifactorial nature of FGR poses a challenge in addressing proper treatment for patients. The maternal nutrient supply is a major factor in fetal growth, as is the placental delivery of nutrients to the fetus. This function is served by syncytiotrophoblasts, particularly the microvillus plasma membrane on the maternal side and the basal membrane on the fetal side. Nutrient transport mechanisms include passive, facilitated, and active mechanisms. Placental delivery of key nutrients, such as oxygen, glucose, amino acids (AAs), and fatty acids (FAs), depends on maternal supply, placental morphology, vascularization, and transporter protein.[6] Oxygen crosses the placenta

by passive diffusion, which is marked by vascular endothelial growth factor (VEGF) expressed by trophoblasts, reflecting placental oxygen transport capacity.[7,8] Glucose transporter 1 (GLUT1) is the major fetal glucose transporter, with stable expression during pregnancy. There have been controversies regarding whether GLUT1 concentrations differ between appropriate for gestational age (AGA) and FGR cases.[9,10] The AA transporter has 28 subtypes, including systems A, L, and y^+L. The expression of these transporters is altered in FGR because maternal intravenous administration does not restore the lower concentrations of some AAs in FGR plasma, e.g., leucine and phenylalanine.[11–13] Free fatty acids cross the placenta through facilitated endocytosis via fatty acid transport proteins (FATPs). In FGR, the expressions of FATP1, FATP2, and FATP4 increase to maintain the supply of long-chain polyunsaturated fatty acids (PUFAs) to the fetus.[14,15]

To date, FGR management remains unsatisfactory and challenging because of the difficulty in determining the exact fetal nutrient deficiency or altered nutrient transport. However, no study has assessed the transport of placental multinutrients. Therefore, this study aimed to evaluate the placental concentrations of macronutrient substrates, namely glucose, AAs, and FAs, as well as nutrient transporters among groups, where VEGF represents oxygen transport, GLUT1 represents glucose transport, system y^+L represents AA transport, and FATP1 represents FA transport.

METHODS

This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: 1254/UN2.F1/ETIK/2018). Written informed consent was obtained from all patients.

Study design and population

This cross-sectional study was conducted from July 2018 to December 2020 at Cipto Mangunkusumo Hospital, Jakarta. The inclusion criteria were pregnant women at >37 weeks' gestational age with three fetal growth statuses: AGA, small for gestational age (SGA), and FGR based on ultrasonography findings; and consent to participate in this study. Patients with congenital anomalies, infection, anemia, multiple pregnancies, abnormal placental morphology, preeclampsia, CVD, gestational diabetes mellitus (GDM), chronic infection (tuberculosis/AIDS), smoking history, psychiatric disorders, or uterine abnormalities were excluded. Patients were selected consecutively through history-taking and physical and ultrasound examinations. The sample size was calculated using an independent comparative formula, resulting in a minimum of 26 participants in each group.[16]

Definitions and data collection

Fetal biometry included biparietal diameter, head circumference (HC), abdominal circumference (AC), femur length, estimated fetal weight (EFW), HC/AC ratio, amniotic fluid index (AFI), and Doppler evaluation consisting of the uterine artery pulsatility index (UtA-PI), umbilical artery pulsatility index (UA-PI), middle cerebral artery pulsatility index, cerebroplacental ratio (CPR), and ductus venosus (DV) wave.

SGA was defined as fetal weight at 5–10th percentile with normal AFI, Doppler measurements, and symmetrical proportion of biometry. FGR was defined by a solitary parameter of AC/EFW <3rd percentile or combined parameters of fetal AC/EFW <10th percentile with at least one of the following: AC/EFW crossing percentiles >2 quartiles on growth percentiles, AFI <10th percentile, abnormal Doppler measured by CPR ≤5th percentile, UtA-PI >95th percentile, UA-PI >95th percentile, or absent/reverse end-diastolic flow of umbilical artery or DV. Gestational age was verified

Table 1. Definitions of AGA, SGA, and FGR

AGA	SGA	FGR
AC at 20th–80th centile with: - Normal AFI - Normal blood flow	AC at 5th–10th centile with: - Normal AFI - Normal blood flow	AC ≤5th centile, or AC ≤10th centile with either: - Abnormal AFI - Abnormal blood flow

AC=abdominal circumference; AFI=amniotic fluid index; AGA=appropriate for gestational age; FGR=fetal growth restriction; SGA=small for gestational age

The definitions are based on Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016;48(3):333–9

based on the last menstrual period, fetal biometry, and serial ultrasonography findings. The definitions of AGA, SGA, and FGR based on ultrasonography findings are summarized in Table 1.[1]

Specimens were obtained from the maternal plasma and placenta. Maternal vein blood (10 cc) was obtained and placed into a container without anticoagulant, centrifuged for 15 min at 3,000 rpm, and stored at –80°C. After birth and weight measurements, a placental sample (20 g) was collected from the central area (30 mm × 30 mm × 30 mm), immersed in phosphate-buffered saline, kept at 4°C for a maximum of 24 hours, and stored at –80°C. The placental sample was homogenized twice with the Precellys Homogenizer (Bertin Technologies, France) at 5,000 rpm for 11 sec twice.[17]

Homogenate samples were prepared and managed according to the standard operating procedure of the

Table 2. Maternal, fetal, and neonatal profiles of the AGA, SGA, FGR groups

Clinical characteristics	AGA (N = 24)	SGA (N = 13)	FGR (N = 20)	*p*
Maternal profile				
Age (years), median (IQR)	29 (24–34)	27 (23–39)	31 (16–40)	0.40*
GA (weeks), median (IQR)	39 (37–41)	38 (37–40)	37 (37–41)	0.15*
Gestational weight gain (g/week), median (IQR)	392 (200–729)	243 (179–538)	255 (135–640)	**0.04***
Pre-pregnancy BMI, n (%)				
Underweight	3 (13)	6 (46)	10 (50)	0.05†
Normoweight	12 (50)	4 (31)	7 (35)	0.12‡
Overweight	9 (38)	3 (23)	3 (15)	0.24‡
Fetal ultrasound profile				
Biometry, median (IQR)				
HC (mm)	340 (316–366)	315 (296–330)	310 (269–336)	**0.02**
AC (mm)	340 (301–360)	300 (278–314)	276.5 (206–301)	**0.03**
EFW (g)	3,393 (2,707–3,900)	2,528 (2,250–2,850)	2,103 (1,100–2,500)	**0.01**
AFI, n (%)				
Normal (5–25 cm)	24 (100)	13 (100)	4 (20)	**0.01**§
Oligohydramnios	0 (0)	0 (0)	16 (80)	
Blood flow, n (%)				
Normal	24 (100)	13 (100)	13 (65)	**0.01**§
Abnormal	0 (0)	0 (0)	7 (35)	
Neonatal profile				
Anthropometric				
Birth weight (g)	3,408 (2,990–3,832)	2,600 (2,300–2,850)	2,135 (1,300–2,560)	**0.001***
Birth weight centile	52 (18–89)	7 (0.5–23)	1 (0.1–12)	**0.015***
AC (mm)	315 (300–350)	290 (270–300)	260 (210–290)	**0.001***
Placental weight (g)	636 (450–850)	485 (348–658)	372 (216–585)	**0.01***
Ward admission, n (%)				
Postnatal care unit	21 (88)	11 (85)	5 (25)	**0.001**§
Neonatal SCN, n (%)	3 (13)	2 (15)	13 (65)	**0.001**‡
NICU, n (%)	0 (0)	0 (0)	2 (10)	**0.001**‡
Length of hospital stay	3 (2–5)	3 (2–9)	9 (3–34)	**0.001**¶

AC=abdominal circumference; AFI=amniotic fluid index; AGA=appropriate for gestational age; BMI=body mass index; EFW=estimated fetal weight; FGR=fetal growth restriction; GA=gestational age; HC=head circumference; IQR=interquartile range; NICU=neonatal intensive care unit; SCN=special care nursery; SGA=small for gestational age

*Kruskal–Wallis test; †chi-square test; ‡combined statistical test; §Fisher test; ¶Mann–Whitney test

PRODIA Clinical Laboratory, Jakarta. The measurement will be conducted using the sandwich enzyme-linked immunosorbent assay (ELISA) method. The glucose measurement was performed using calorimetric methods with Glucose Colorimetric Assay Kit, Cat. No. 100009582 (Cayman Chemical, USA) and was read by Bio-Rad Model 680 Microplate Reader (Bio-Rad Inc., USA); AAs using liquid chromatography tandem-mass spectrometry method with Agilent 6460 Triple Quad LC/MS (Agilent, USA); FAs using gas chromatography-mass spectrometry with Agilent 5977A MSD (Agilent). The measurement of GLUT1 concentration was performed using a Human SLC2A1/GLUT-1 ELISA Kit, Cat. No LS-F11591 (LifeSpan BioSciences, Inc., USA) reagent and Bio-Rad Model 680 Microplate Reader (Bio-Rad Inc.,); y^+L system using Human SLC7A7 ELISA Kit, Cat. No LS-F9473 (LifeSpan BioSciences, Inc.) reagent and Bio-Rad Model 680 Microplate Reader (Bio-Rad Inc.); FATP1 concentration using Human SLC27A7 ELISA Kit, Cat. No OKEH05587 (Aviva Systems Biology, USA) reagent; and VEGF using Quantikine® ELISA human VEGF, Cat. No DVE00 (R&D Systems, USA) reagent.

Statistical analysis

Normally distributed data are presented as mean and standard deviation, whereas abnormally distributed data are presented as median and minimum–maximum. One-way analysis of variance was used to analyze data with a normal distribution, and the Kruskal–Wallis test was used to analyze data with an abnormal distribution. Data were analyzed using SPSS software version 20 (IBM Corp., USA).

RESULTS

Of the 57 patients, 24 were in the AGA group, 13 were in the SGA group, and 20 were in the FGR group. Maternal, fetal, and neonatal profiles are shown in Table 2. Significant differences were found in gestational age, pre-pregnancy body mass index (BMI), gestational weight gain, fetal biometry, biometric proportion, and fetal blood flow. Nearly half of the patients in the SGA and FGR groups had an underweight pre-pregnancy BMI with inadequate gestational weight gain (243 [179–538] and 255 [135–640] g/week, respectively). The FGR group had poorer neonatal outcomes, such as increased necessity for special or intensive care, ventilation support, parenteral nutrition, and length of hospital stay, than the SGA group.

The concentrations of transport proteins in the placenta, total metabolic substrates, AAs, and FAs in the maternal serum and placenta are summarized in Table 3. Higher VEGF, system y^+L, and FATP1 concentrations were observed in the SGA and FGR groups than in the AGA group. No significant differences in GLUT1 concentrations were observed among groups. The glucose concentration showed no difference among groups, whereas total placental AAs were lower in the SGA and FGR groups than in the AGA group. Interestingly, total placental FA concentrations were higher in the SGA and FGR groups than in the AGA group. Nine essential AAs and 10 non-essential AAs were measured. Most placental AA concentrations in the AGA group were higher than those in the SGA and FGR groups, whereas maternal serum concentrations showed inconsistent results. The SGA and FGR groups also showed variable results in terms of the concentrations of AAs; most essential AAs in the placenta were lower in the FGR group than in the SGA group. In general, FA concentrations in the SGA and FGR groups were lower in the maternal serum but higher in the placental serum. The FGR group had significantly higher concentrations of three saturated fatty acids (SFA), two monounsaturated fatty acids (MUFA), and two PUFAs in the placental tissue than the AGA group.

DISCUSSION

Maternal nutrient status is a major risk factor for FGR. Nearly half of the patients in the SGA and FGR groups had an underweight pre-pregnancy BMI with inadequate gestational weight gain, which was consistent with the findings of several studies.[18] Inadequate gestational weight gain, ideally ranging from 400–500 g/week for low pre-pregnancy BMI, corresponds to inadequate calorie intake, leading to a negative calorie balance.[19]

Fetal growth is represented by fetal biometry, AFI, and blood flow. Asymmetrical biometry, oligohydramnios, and abnormal blood flow indicated fetal hypoperfusion. None of these features were present in the AGA and SGA groups; however, the FGR group had 90% asymmetrical biometry, 80% oligohydramnios, and 35% abnormal blood flow. These findings aligned with the increased need for advanced neonatal care in the FGR group (75%) compared with the AGA and SGA groups (12.5% and 15.4%, respectively).[20,21]

Table 3. Concentration of transport protein in placenta and total metabolic substrates levels, AAs, and FAs in placenta and maternal serum among the AGA, SGA, and FGR groups

Variables	Median (range)			*p*
	AGA (N = 24)	SGA (N = 13)	FGR (N = 20)	
Transport protein				
VEGF (pg/mg)	0.40 (0.04–1.51)	0.91 (0.34–1.96)	0.89 (0.13–2.50)	**0.003**
GLUT1 (ng/mg)	0.06 (0.02–0.15)	0.07 (0.05–0.15)	0.07 (0.03–0.12)	0.301
System y^+L (ng/mg)	0.14 (0.05–0.31)	0.21 (0.14–0.69)	0.21 (0.06–0.53)	0.07
FATP1 (pg/mg)	4.1 (2.2–13)	6.8 (3.9–11.4)	5.7 (2.6–12.7)	**0.021**
Metabolic substrates				
Glucose				
Placenta (µg/g)	615 (438–1,197)	569 (345–1,000)	657 (429–2,170)	**0.008**
Total AAs				
Placenta (µmol/g)	308 (232–583)	257 (181–370)	275 (19–389)	**0.004**
Maternal serum (µmol/l)	2,001 (1,394–2,569)	2,112 (1,678–3,267)	2,170 (634–3,718)	0.528
Total FAs				
Placenta (nmol/g)	915 (525–1,455)	1,147 (693–1,765)	1,122 (682–1,506)	**0.048**
Maternal serum (µmol/l)	17,839 (10,507–28,852)	15,707 (12,590–19,310)	14,349 (3,553–21,230)	**0.017**
AAs				
Essential AAs				
Valine				
Placenta (µmol/g)	17.5 (14–29)	14 (11–32)	14.4 (9–19)	**0.003**
Maternal serum (µmol/l)	164 (90–223)	175 (124–211)	186 (40–235)	0.173
Isoleucine				
Placenta (µmol/g)	7 (5–16)	6 (4–16)	6.2 (5–11)	0.164
Maternal serum (µmol/l)	58 (42–89)	76 (42–110)	65 (16–96)	0.069
Leucine				
Placenta (µmol/g)	18.6 (12–33)	15 (11–44)	15.9 (10–23)	**0.025**
Maternal serum (µmol/l)	115 (81–176)	144 (79–194)	134 (37–216)	**0.041**
Phenylalanine				
Placenta (µmol/g)	7.3 (5–14)	6 (4–16)	6.7 (4–10)	**0.017**
Maternal serum (µmol/l)	74 (60–126)	87 (67–113)	79 (28–228)	0.144
Methionine				
Placenta (µmol/g)	4.5 (3–9)	3.8 (2–10)	4 (2–6)	**0.02**
Maternal serum (µmol/l)	21 (15–30)	24 (12–42)	20 (8–40)	0.67
Lysine				
Placenta (µmol/g)	42 (26–75)	35 (22–87)	39 (21–48)	0.126
Maternal serum (µmol/l)	142 (67–214)	146 (73–181)	166 (35–475)	0.155
Threonine				
Placenta (µmol/g)	19.6 (12–33)	15 (10–34)	15 (9–21)	**0.005**
Maternal serum (µmol/l)	183 (101–294)	163 (114–415)	180 (44–526)	0.743
Histidine				
Placenta (µmol/g)	5 (3–9)	4 (3–10)	3.7 (2–7)	**0.001**
Maternal serum (µmol/l)	78 (42–111)	76 (59–164)	82 (19–141)	0.977

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Table 3. (Continued)

Variables	Median (range)			*p*
	AGA (N = 24)	SGA (N = 13)	FGR (N = 20)	
Arginine				
Placenta (µmol/g)	20 (6–42)	14.8 (10–37)	14.1 (10–27)	**0.012**
Maternal serum (µmol/l)	112 (59–184)	111 (76–165)	125 (37–216)	0.852
Non-essential AAs				
Glutamate				
Placenta (µmol/g)	25.5 (13–58)	16.9 (10–38)	21 (11–40)	**0.046**
Maternal serum (µmol/l)	93.5 (47–163)	102 (60–514)	94 (25–140)	0.768
Aspartate				
Placenta (µmol/g)	14.4 (9–32.4)	10.1 (7–22.5)	9.3 (6–18)	**0.002**
Maternal serum (µmol/l)	36 (21–73)	39 (25–162)	28 (11–48)	**0.019**
Serine				
Placenta (µmol/g)	36 (26–62.8)	33 (23–82)	29 (22–53.4)	0.096
Maternal serum (µmol/l)	121 (84–168)	119 (80–117)	105 (41–234)	0.326
Glycine				
Placenta (µmol/g)	30 (21.5–59)	24.0 (18–43.7)	24.5 (16.1–33.1)	**0.004**
Maternal serum (µmol/l)	177 (116–300)	199 (132–504)	214 (76–577)	0.374
Cysteine				
Placenta (µmol/g)	0.18 (0.07–0.63)	0.19 (0.04–0.38)	0.20 (0–0.69)	0.708
Maternal serum (µmol/l)	9 (3–35)	8 (3–30)	8 (2–75)	0.453
Alanine				
Placenta (µmol/g)	39.5 (24–68)	30 (20–72.2)	32.6 (17.9–47.1)	**0.023**
Maternal serum (µmol/l)	424 (242–585)	416 (296–885)	394 (132–727)	0.483
Citrulline				
Placenta (µmol/g)	1 (0–1.4)	0.3 (0–1.2)	0.5 (0–1.0)	0.177
Maternal serum (µmol/l)	10 (6–19)	9 (5–21)	14 (2–37)	0.542
Proline				
Placenta (µmol/g)	19 (12–79)	16 (11–33.6)	17 (8–51)	0.134
Maternal serum (µmol/l)	92 (63–155)	97 (74–197)	104 (20–185)	0.737
Ornithine				
Placenta (µmol/g)	4.9 (3.1–28)	5.2 (1.8–9)	6.5 (2.5–19)	0.655
Maternal serum (µmol/l)	33 (20–78)	35 (20–50)	34 (7–116)	0.892
Tyrosine				
Placenta (µmol/g)	7 (5–12)	5.3 (4–11)	5.1 (4–8)	**0.011**
Maternal serum (µmol/l)	40 (28–61)	48 (26–61)	42 (16–63)	0.457
FAs				
SFAs				
Myristic C14				
Placenta (nmol/g)	4 (2–12)	7 (3.2–10)	5.7 (4–9)	**0.046**
Maternal serum (µmol/l)	276 (82–492)	168 (58–440)	122 (16–385)	**0.031**
Palmitic C16				
Placenta (nmol/g)	273 (181–383)	302 (216–442)	315 (176–432)	**0.016**
Maternal serum (µmol/l)	5,146 (2,512–9,575)	4,647 (3,195–6,397)	3,938 (1,114–7,405)	0.07

Table continued on next page

Table 3. (Continued)

Variables	Median (range)			*p*
	AGA (N = 24)	SGA (N = 13)	FGR (N = 20)	
Stearic C18				
Placenta (nmol/g)	43 (29–75)	55 (43–86)	58 (32–80)	**0.011**
Maternal serum (µmol/l)	876 (504–1,239)	812 (63–1,060)	716 (304–1,073)	**0.025**
MUFAs				
Palmitoleic C16:1 w7				
Placenta (nmol/g)	23 (10–35)	26 (16–53)	30 (14–42)	0.05
Maternal serum (µmol/l)	671 (252–3,154)	578 (254–1,428)	578 (83–4,146)	0.442
Oleic C18:1 w9				
Placenta (nmol/g)	107 (50–166)	137 (93-213)	132 (87–170)	**0.037**
Maternal serum (µmol/l)	4,089 (2,525–6,867)	3,613 (2,509–4,301)	2,990 (661–5,465)	0.15
PUFAs				
LA C18:2 w6				
Placenta (nmol/g)	154 (69–256)	199 (99–249)	162 (101–160)	0.339
Maternal serum (µmol/l)	4,927 (2,958–8,348)	4,581 (3,834–5,324)	4,068 (251–5,746)	**0.002**
GLA C18:3 w6				
Placenta (nmol/g)	7 (3–11)	10 (4–16)	9 (5–18)	**0.048**
Maternal serum (µmol/l)	23 (1–51)	19 (5–119)	14 (1–165)	0.943
DGLA C20 w6				
Placenta (nmol/g)	30 (17–52)	35 (25–79)	38 (15–56)	**0.018**
Maternal serum (µmol/l)	263 (97–497)	237 (117–466)	238 (50–369)	0.841
Arachidonic acid C20:4 w6				
Placenta (nmol/g)	225 (114–415)	258 (146–484)	288 (144–493)	**0.042**
Maternal serum (µmol/l)	632 (293–1,000)	583 (498–921)	637 (237–1,140)	0.918
ALA C18:3 w3				
Placenta (nmol/g)	1 (0.9–3)	1.5 (0.9–3)	1.4 (0.6–4)	0.349
Maternal serum (µmol/l)	65 (14–198)	56 (28–107)	38 (1–138)	**0.028**
EPA C20:5 w3				
Placenta (nmol/g)	3.1 (0–13)	5 (1.4–8)	2.6 (0.5–13)	0.421
Maternal serum (µmol/l)	17 (2–106)	23 (4–1,030)	15 (2–112)	0.381
DHA C22:6 w3				
Placenta (nmol/g)	67.5 (40–108)	77 (45–124)	73 (40–112)	0.322
Maternal serum (µmol/l)	405 (75–681)	374 (165–707)	336 (86–610)	0.157

AAs=amino acids; AGA=appropriate for gestational age; ALA=alpha linolenic acid; DGLA=dihomo-gamma-linolenic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; FAs=fatty acids; FATP1=fatty acid transport protein 1; FGR=fetal growth restriction; GLUT1=glucose transporter 1; GLA=gamma-linolenic acid; LA=linoleic acid; MUFAs=monounsaturated fatty acids; PUFAs=polyunsaturated fatty acids; SFAs=saturated fatty acids; SGA=small for gestational age; VEGF=vascular endothelial growth factor

Most studies used a cut-off point at the 5th percentile to determine pathologically small neonates. Here, the SGA and FGR groups had mean birth weights in the 7th and 1st percentiles, respectively. This difference was consistent with the severity of neonatal outcomes, where a smaller percentile was associated with greater morbidity and mortality rates. The severity of FGR includes the onset and duration of the disease, nutritional status, metabolic status, maternal comorbidities, inflammatory state, adaptive response of the placenta, and fetal cardiovascular system.[22–24]

Our findings demonstrated that several nutrient transporters related to oxygen, AA, and FA delivery are associated with FGR, namely, VEGF, system

y^+L, and FATP1. The SGA and FGR groups showed significantly higher VEGF concentrations than the AGA group, suggesting a change in vascular formation to compensate for malnutrition. Increased VEGF activity indicates increased angiogenesis; however, VEGF data alone are insufficient to determine the balance between pro- and anti-angiogenic factors. Several studies have reported various results regarding VEGF concentration in FGR, with some showing no correlation, an increase, or a decrease.[25–27] These findings indicate that placental vascularization is caused by multiple factors, namely the net balance of anti- and angiogenic factors. In the present study, it remained unclear whether altered VEGF levels were caused by hypoxia in infants with SGA and FGR or if initially diminished VEGF levels contributed to the development of hypoxia and, subsequently, SGA and FGR.

The SGA and FGR groups had increased system y^+L concentrations compared with the AGA group, suggesting a similar pattern of change in both groups. The system y^+L transports AAs to and from the placenta, and heterodimeric AA binds to cationic and neutral AAs, e.g., lysine, arginine, glutamine, histidine, methionine, and leucine.[28] One study reported apparent differences in system y^+L concentrations between FGR and preeclampsia among 46 transporters.[13] This increase was a part of the adaptive response to the low level of AAs in the placenta. Additionally, patients with FGR and preeclampsia have increased expression of system L and mammalian targets of rapamycin in the placental microvillus membrane as a compensatory mechanism to limit nutritional resources due to abnormal remodeling of the uterine spiral arteries.[29]

The present study showed significantly higher concentrations of FATP1 in the SGA and FGR groups than in the AGA group. This was also part of an adaptive response to the low availability of FAs to ensure an adequate supply to the fetus. This finding was in line with that of Assumpção et al's[30] study, which showed increased expressions of FATP1, FATP2, and FATP4, as well as fatty acid- binding protein (FABP) 1 and FABP3, in FGR. FATP1 is extensively expressed in the placental basal membrane and facilitates FA transportation. FATP1 and FATP4 directly correlate with docosahexaenoic acid (DHA) in the placenta and maternal plasma. Interestingly, the expressions of FATP1 and FATP4 were decreased in mothers with obesity and GDM, suggesting an adaptive mechanism to prevent excessive supply of FAs to the fetus.[31] Higher concentrations of VEGF, system y^+L, and FATP1 observed in the SGA and FGR groups suggested similar pathophysiology, leading to the hypothesis that SGA is not normal but rather a response that can lead to FGR if compensation fails.

Interestingly, the present study demonstrated no differences in GLUT1 expression among groups. This is consistent with findings in a study by Chang et al,[32] who showed no difference in GLUT1 expression in fetuses with FGR. GLUT1 expression in trophoblasts is correlated with low oxygen levels, suggesting that the change is correlated with chronic tissue hypoxia, e.g., that observed in preeclampsia.[10] Therefore, fetal or neonatal hypoglycemia is not attributed to GLUT1 change but rather to other factors, such as glucose availability, vascular changes, or placental metabolic changes. As a major glucose transporter in the human body, GLUT1 is insensitive to changes in blood glucose levels. Further studies evaluating other glucose levels, such as those of GLUT3, are warranted. Several studies have shown that GLUT3 expression is upregulated in patients with late-stage FGR, and this upregulation, along with increased hypoxia-inducible factor 1-alpha, suggests an adaptive response to hypoxic conditions in FGR.[32]

The concentrations of glucose showed no difference in all groups. Glucose, the main energy source of the fetus, is derived from maternal circulation. The mechanism of maternal-fetal glucose transfer involves a concentration gradient, placental transport capacity, concentration of protein transfer, and maternal-fetal endocrine signaling. Mothers with restricted calorie reserves have limited glucose availability, thus reducing the fetal supply.[33,34]

The transport capacity of nutrients is associated with the placental phenotype. Placentas with smaller sizes or weights have lower nutrient transport capacity, as observed in the SGA and FGR groups here. Under normal conditions, low transport capacity results in low glucose concentration. Under conditions of deficiency, there is an increase in placental glucose concentration to compensate for the low glucose transport capacity due to the small size of the placenta, which explains the indifferent glucose concentrations in this study.[6]

The current study demonstrated that the placental concentrations of AAs in the SGA and FGR groups were lower than those in the AGA group. The increased

consumption of AAs, inadequate maternal supply, and low fetal protein metabolism may explain these findings. AAs are involved in reproductive functions, protein synthesis, RNA and DNA synthesis, tissue building blocks, cell signaling, and hormonal and metabolic regulation. Deficiency of AAs, particularly essential ones, might impair homeostasis, as the body cannot self-produce them.[35,36]

Twelve AA profiles differed between the AGA and FGR groups, including seven essential AAs (valine, leucine, phenylalanine, methionine, threonine, histidine, and arginine) and five non-essential AAs (aspartate, serine, glycine, alanine, and tyrosine). The SGA group also had lower concentrations of 11 AAs, consisting of 6 essential AAs (valine, leucine, phenylalanine, methionine, histidine, and arginine) and 5 non-essential AAs (glutamate, aspartate, glycine, alanine, and tyrosine). These findings showed that most essential AAs were lower in both groups, suggesting a similar pathomechanism between infants with SGA and those with FGR. However, it is unknown whether this low concentration of AAs causes fetal AA deficiency. However, the small phenotype of the fetus suggests impaired tissue synthesis attributable to deranged bodily function due to a deficiency of the main substrate, one of which is AA.

The various types of AA deficiency suggest that in cases of SGA and FGR, the problem is not only the small phenotype but also a more complex bodily impairment. However, the SGA and FGR groups exhibited different AA concentrations. For instance, histidine, arginine, aspartate, serine, and tyrosine levels were higher in the SGA group than in the FGR group. These differences showed, albeit with similar small phenotypes, that the SGA and FGR groups had different metabolic pathways and transport capacities for the AAs.

Each AA has a unique metabolic rate, transfer capacity, and interconversion capability. For instance, glutamate is converted to glutamine in the placenta and delivered to the fetus at a higher rate than other AAs. In contrast, alanine in the fetus is metabolized in the placenta rather than from the maternal source, which has a slow transfer rate. The transport rate also depends on the transporter, which includes systems A, L, and y+L.[37,38]

This study found no differences in AA concentrations in the maternal serum between groups. This finding suggests that maternal deficiency was not the cause of low placental concentration; however, the measurement of AA was performed following delivery, that is, in the third trimester. Hence, it did not reflect the maternal condition at an earlier gestational age, which is a crucial period when placentation occurs. Other possible factors include increased placental oxidative stress and the activation of maternal-fetal endocrine signaling, leading to protein degradation.[39]

The placental FA concentrations in the SGA and FGR groups were higher than those in the AGA group. This was believed to be due to increased absorption from the maternal circulation or increased synthesis of non-essential FAs in the placenta. In contrast, FA concentrations in the maternal serum were significantly lower in the FGR group than in the AGA group, negating the first hypothesis.

Compared with the AGA group, the FGR group had different profiles of placental FAs in seven FAs: SFAs, myristate, palmitate, and stearate; MUFAs, palmitoleate and oleate; and PUFA, gamma-linolenic and dihomo-gamma-linolenic). In this study, there was no difference in DHA and eicosapentaenoic acid between groups, which are important for brain development.[40] However, linoleic acid, the precursor of AA, and linolenic acid, the precursor of DHA, were lower in the maternal serum of the FGR group than in the AGA group; yet, there was no difference in the placenta. In contrast to our study, Meher et al[41] reported no difference in MUFA concentrations in the maternal plasma of fetuses with FGR and healthy fetuses. This suggests that FA transfer to the fetus has an active mechanism that does not depend on maternal supply but rather fetal need, with enhanced placental concentration serving as a metabolic adaptation for thermogenic function and an alternative fetal energy source. This theory is supported by Perazzolo et al,[42] who reported a constant concentration of FAs in the fetal umbilical vein that was not influenced by maternal concentration.

This study has several limitations. Sample recruitment was performed during the coronavirus disease pandemic, affecting the hospital's referral system and reducing the patient load. Placental homogenates were measured using ELISA instead of evaluating gene expression at separate maternal and fetal interfaces. Although this method could visualize the overall placental transport system, it could not analyze the transporter at each maternal and fetal

interface, which may provide valuable information regarding the dynamic properties of the transporter. The variables measured in this study were only a fraction of the numerous nutrient-related transporters found in the placenta and could not be used to determine the overall placental function. Thus, further studies are required to evaluate other nutrient-related placental transporters and their roles in FGR.

In conclusion, the SGA and FGR groups had different placental transport system profiles, namely, angiogenic factors, AA transporters, and FA transporters. There was no difference in glucose transporter levels between groups. The increased expression of these transporters in the SGA and FGR groups might indicate a similar pathogenesis, which requires further study.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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