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Enhanced circulating retinol and non-esterified fatty acids in pregnancies complicated with intrauterine growth restriction

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ABSTRACT

IUGR (intrauterine growth restriction) increases the incidence of perinatal complications and, although several placental transport functions have been shown to be altered in pregnancies complicated by IUGR, the mechanism behind it is not well understood. The aim of the present study was to investigate factors in maternal and cord blood plasma from normal and IUGR-complicated pregnancies associated with the body weight of newborns. At the time of Caesarean section, 24 women with IUGR pregnancies were compared with a group of 30 normal controls with AGA (appropriate gestational age) fetuses who were studied at Caesarean section, which took place 5 weeks later than IUGR pregnancies, and also to a group of 25 non-delivered gestational age-matched control pregnant women (AGA-35wk). Maternal plasma retinol, γ- and α-tocopherol, NEFAs (non-esterified fatty acids), and palmitic, palmitoleic, γ-linolenic and arachidonic acids were higher in women with IUGR pregnancies than in AGA-35wk controls, whereas stearic and α-linolenic acids were lower. Smaller differences were found when comparing these variables for IUGR and AGA women. However, umbilical vein plasma γ-tocopherol, cholesterol, triacylglycerols and NEFAs were higher in the IUGR group than in the AGA group, whereas arachidonic acid was lower. Maternal plasma retinol and NEFAs were the only variables negatively correlated with birthweight when multiple linear regressions were analysed. In conclusion, the increased levels of circulating retinol and NEFAs in maternal plasma are negatively associated with birth and placental weights, which may reflect an impaired placental transfer in IUGR pregnancies. As retinoids are involved in the control of gene transcription, it is proposed that a decrease in placental transfer of retinol could underlie the metabolic dysfunction of IUGR pregnancies.

INTRODUCTION

The human fetus is dependent on adequate placental transport of fatty acids from the maternal circulation, in addition to many other nutrients, for normal development and growth. Lipids are essential components of cell membranes, function as important energy sources and are also precursors to cellular signalling molecules. In

Key words: fatty acid, intrauterine growth restriction (IUGR), long-chain polyunsaturated fatty acid (LCPUFA), non-esterified fatty acid (NEFA), pregnancy, retinol.

Abbreviations: AA, arachidonic acid; AGA, appropriate gestational age; AGA-35wk, AGA at 35 weeks of pregnancy; ALA, α-linolenic acid; BMI, body mass index; CV, coefficient of variation; DHA, docosahexaenoic acid; EFA, essential fatty acid; GLA, γ-linolenic acid; IUGR, intrauterine growth restriction; LA, linoleic acid; NEFA, non-esterified fatty acid; PUFA, polyunsaturated fatty acid; LCPUFA, long-chain PUFA; RBP, retinol-binding protein; CRBP, cellular RBP; SCD, stearyl-CoA desaturase.

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particular, the EFAs (essential fatty acids) LA (linoleic acid; $C_{18:2n-6}$) and ALA (α-linolenic acid; $C_{18:3n-3}$), and their LCPUFA [long-chain PUFA (polyunsaturated fatty acid)] derivatives, play a critical role in fetal development [1]. Thus, although LCPUFAs are ubiquitous components in every mammalian cell, a significant accretion of them has been observed in fetal brain and retina during the last trimester of pregnancy [2]. LCPUFAs can be synthesized endogenously from precursor EFAs in a process that involves Δ^6- and Δ^5-desaturases, constituting the $n-6$ and $n-3$ PUFA metabolic pathways respectively [3]. This process is thought to occur at an adequate rate in healthy human adults but not in fetuses. Moreover, the placenta lacks the desaturase enzymes to convert EFAs [4] and, therefore, LCPUFAs should derive predominantly from the mother via placental transfer during fetal life.

IUGR (intrauterine growth restriction) is characterized by the failure of the fetus to reach its growth potential. In the short term, IUGR increases the incidence of perinatal complications, including neonatal death, impaired neurological development and respiratory distress syndrome [5]. Several placental transport functions are altered in pregnancies complicated by IUGR [6,7] and, consequently, this dysfunction can markedly affect normal fetal growth and development. However, the mechanism behind this is not well understood.

There are studies that suggest micronutrients have direct effects or may be markers of other underlying determinants of pregnancy outcomes [8]. It is known that vitamin A (retinol) is a lipid-soluble micronutrient required for normal mammalian reproduction and embryonic [9] and fetal development [10–12]. Moreover, through interactions with nuclear receptors, retinoic acid, the biologically active form of vitamin A, can modulate gene transcription rates involved in the regulation of cell proliferation and differentiation [13]. In plasma, vitamin A is transported in combination with carrier molecules such as the RBP (retinol-binding protein)–transthyterin complex [14]. Placental uptake of retinol involves the specific interaction of serum RBP with a plasma membrane receptor at the extracellular surface [15–17], followed by ligand transfer to cytoplasmic CRBP (cellular RBP) in a membrane-dependent manner [15]. Thus the fetus acquires vitamin A from the maternal circulation. Experiments *in vitro* have shown that the fatty acid status of cells are determinants of retinol uptake [18] not only because they control bilayer fluidity and thereby influence membrane transport and function [19,20], but also because they induce CRBP expression [21].

In order to attain a better understanding of the factors that may be influencing placental function and consequent fetal growth in IUGR-complicated pregnancies, the aim of the present study was to analyse the circulating levels of lipids and lipophilic vitamins during normal and IUGR pregnancies, in both maternal and umbilical vein plasma.

MATERIALS AND METHODS

Subjects

In this retrospective case-control study, samples were obtained from pregnant women admitted to the Obstetrics and Gynecology Unit of the San Paolo Hospital, Milan, Italy and to the Obstetrics and Gynecology Unit of the Clinica Mangiagalli of Milan University, Milan, Italy over a 4-year period (October 2000–June 2005). The University of Milan Ethical Board approved the study protocol, and informed consent was obtained from all of the subjects.

Women with maternal diseases known to affect fetal growth, such as autoimmune and endocrine disease, chronic hypertension or pregnancy-induced hypertension, were excluded. Maternal alcohol or drug consumption was also an exclusion criterion. None of the women were taking nutritional supplements that contained specific fatty acids or lipid-soluble vitamins. A nutritional questionnaire was completed in order to analyse the nutritional intake [22]. This is a semi-quantitative validated questionnaire composed of pictures of the most common Italian foods in various portions. All pregnant women had to indicate the frequency of each dish consumed during the month prior to sampling. Data were entered into software designed to calculate nutrient quantities.

Control patients had an ultrasound scan at 30–32 weeks of gestation that confirmed a normal fetal growth pattern, and gave birth to healthy term neonates with a birthweight between the 10 and 90th percentile, according to Italian standards [23]. IUGR was identified as a reduction in fetal growth rate by ultrasound measurements of abdominal circumferences below the 10th percentile for fetuses of similar ages [24] or by a decrease of more than 40 percentiles from the growth curve. Growth restriction was confirmed at birth if the neonatal weight was below the 10th percentile [25], according to Italian birthweight and gestational age standards. In all cases, pregnant women were subjected to elective Caesarean section: in women with IUGR, a Caesarean section was performed because of deteriorating fetal or maternal conditions, whereas elective Caesarean section took place in the control group because of cephalopelvic disproportion, repeat Caesarean section or breech presentation. All pregnancies were singleton and none of the babies had any malformations, abnormal karyotypes or signs of distress at delivery. As expected, IUGR pregnancies were delivered at an average gestational age significantly lower than that for normal control pregnancies [AGA (appropriate gestational age)]. Therefore, in order to compare women with an IUGR fetus with gestational age-matched controls, we included a second

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Table 1 Maternal and neonatal characteristics of the study population

Values are means ± S.E.M. For the maternal characteristics, Tukey's test was used to determine differences among groups after one-way ANOVA. In the neonate characteristics, a Student's t test was used to compare values between the IUGR and AGA groups. Different superscripted letters within a row indicate significant differences ($P < 0.05$).

Characteristic	AGA-35wk ($n = 25$)	IUGR ($n = 24$)	AGA ($n = 30$)
At initial of gestation			
Maternal age (years)	30.8 ± 0.9^{a}	32.8 ± 0.9^{a}	32.3 ± 1.0^{a}
BMI (kg/m^2)	20.7 ± 0.4^{a}	23.6 ± 1.0^{b}	22.3 ± 0.7^{ab}
At delivery by Caesarean section			
BMI (kg/m^2)	24.9 ± 0.6^{a}	27.0 ± 1.1^{a}	26.1 ± 0.7^{a}
Weeks of gestation	35.2 ± 0.4^{a}	33.8 ± 0.7^{a}	38.9 ± 0.1^{b}
Neonate weight (g)	–	1543 ± 114^{a}	3318 ± 57^{b}
Placenta weight (g)	–	244 ± 17^{a}	514 ± 20^{b}
Placenta weight (g)/gestational age (week)	–	7.18 ± 0.47^{a}	13.2 ± 0.5^{b}
Neonate weight (g)/placenta weight (g)	–	6.57 ± 0.43^{a}	6.64 ± 0.20^{a}

control group corresponding to non-delivered pregnant women with normal pregnancies of similar gestational age (AGA-35wk) compared with those with IUGR, whereas IUGR fetuses were compared only with AGA controls. Gestational age was calculated from the last menstrual period and confirmed by an ultrasound examination performed at 20 weeks of gestation.

Sampling

Fasting blood samples from pregnant women were obtained from the maternal radial vein. Umbilical vein blood samples were obtained from a segment of the cord doubly clamped immediately after delivery. Both samples were collected in tubes containing EDTA and were kept on ice until centrifugation (1500 g at 4 °C for 25 min). Plasma was aliquoted and immediately stored at − 80 °C until analysis.

Analytic methods

Plasma cholesterol and triacylglycerols (Spinreact Reactives) and NEFAs (non-esterified fatty acids) (Wako Chemicals) were determined enzymatically using commercial kits. Adequate control plasma with different and certified concentrations were used in all determinations to verify the accuracy of the assays (Accutrol; Sigma; Precinorm and Precipath; Roche Diagnostics). The inter-assay CVs (coefficients of variation) were as follows: 0.9 % for cholesterol, 1.3 % for triacylglycerol and 1.9 % for NEFAs.

Plasma α-tocopherol, γ-tocopherol and retinol were measured by gradient HPLC (Beckman Instruments) after extraction with hexane, as described previously [26]. Retinol acetate and tocopherol acetate were used as internal standards. The standard reference material SRM 968c from the National Institute of Standards and Technology was used as a control. The inter-assay CVs were as follows: 4.3 % for α-tocopherol, 10.6 % for γ-tocopherol and 3.8 % for retinol.

Plasma lipids were extracted in chloroform/methanol (2:1) [27] containing 0.005 % BHT (butylated hydroxytoluene). Fatty acids were transesterified with methanolic hydrochloride and analysed on a PerkinElmer gas chromatograph (Autosystem) as reported previously [28].

Statistics

Results are expressed as means ± S.E.M. Statistical difference between groups was determined by ANOVA after adjusting for maternal BMI (body mass index) and gestational age; when differences were statistically significant, multiple comparisons were performed using Tukey's post-hoc test. A paired Student's t test was used to determine significant differences between maternal and umbilical plasma. Given their skewed distributions, concentrations of triacylglycerol, NEFAs and γ-tocopherol were log-transformed before statistical comparison. Correlations were tested with Pearson's method using the log-transformed data as indicated. To ascertain the independent predictors of neonatal birthweight, stepwise multiple regression with backward selection analysis was performed. All statistical analysis was performed using a computer software package (Statgraphics Centurion XV version 15.2.06; Statistical Graphics).

RESULTS

As shown in Table 1, although maternal age did not differ between the groups at the beginning of gestation, the mean BMI was significantly higher in women who developed IUGR than in the AGA group matched by gestational age (AGA-35wk), but not different from that observed in the AGA group studied at delivery. IUGR pregnancies were delivered on average 5 weeks earlier than normal pregnancies. Consequently, both the neonatal and placental weights were significantly lower in the IUGR group than in the AGA group and, although

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Table 2 Concentration of lipophilic vitamins (μmol/l) and plasma lipids (mmol/l) in maternal plasma of control pregnant women at 35 weeks of pregnancy (AGA-35wk), and in maternal and umbilical vein plasma of IUGR and control (AGA) groups at the time of Caesarean section

Values are means ± S.E.M. Adjustments were made for maternal BMI and gestational age. In the maternal vein, Tukey's test was used to determine differences among groups after one-way ANOVA. Different superscript uppercase letters indicate significant differences ($P < 0.05$) between the maternal values. In the umbilical vein, different superscripted lowercase letters indicate significant differences ($P < 0.01$) between the umbilical values (as determined using a Student's t test). $^{***}P < 0.001$ compared with the respective maternal vein value (as determined using a Student's t test). Log-transformed skewed data for γ-tocopherol, triacylglycerol and NEFA were used for statistical comparisons.

	Maternal vein plasma			Umbilical vein plasma	
Parameter	AGA-35wk ($n = 25$)	IUGR ($n = 24$)	AGA ($n = 30$)	IUGR ($n = 24$)	AGA ($n = 30$)
Vitamin (μmol/l)					
Retinol	1.00 ± 0.070^{A}	1.63 ± 0.08^{B}	0.884 ± 0.076^{A}	$0.504 \pm 0.057^{a***}$	$0.748 \pm 0.052^{a***}$
γ-Tocopherol	1.42 ± 0.12^{A}	1.78 ± 0.13^{B}	1.51 ± 0.13^{AB}	$0.451 \pm 0.056^{a***}$	$0.274 \pm 0.056^{b***}$
α-Tocopherol	27.7 ± 2.6^{A}	40.1 ± 3.0^{B}	39.7 ± 2.9^{B}	$6.72 \pm 0.83^{a***}$	$6.75 \pm 0.76^{a***}$
Lipid (mmol/l)					
Cholesterol	6.31 ± 0.29^{A}	6.86 ± 0.33^{A}	6.39 ± 0.32^{A}	$1.61 \pm 0.16^{a***}$	$1.30 \pm 0.14^{b***}$
Triacylglycerol	2.34 ± 0.23^{A}	2.37 ± 0.26^{A}	2.15 ± 0.25^{A}	$0.366 \pm 0.044^{a***}$	$0.349 \pm 0.038^{b***}$
NEFA	0.329 ± 0.042^{A}	0.948 ± 0.067^{B}	0.599 ± 0.050^{C}	$0.166 \pm 0.022^{a***}$	$0.134 \pm 0.013^{b***}$

the ratio of placental weight to gestational age was lower in IUGR than in AGA, the neonate weight/placental weight did not differ between the two groups.

The results of the nutritional questionnaire showed no significant differences between the two groups in either the total daily caloric intake or in the total amount of proteins, carbohydrates or lipids. In addition, no significant intergroup differences were found for any of the vitamins in the diet (vitamins A, C, D and E; results not shown).

Table 2 shows that the plasma retinol concentration was significantly higher in pregnant women who developed IUGR compared with either of the two healthy control groups. Values for retinol in umbilical vein plasma did not differ between the IUGR and AGA neonates, and they were always significantly lower than in maternal plasma. However, the umbilical/maternal plasma retinol ratio was much lower in the IUGR group than in the AGA group (0.376 ± 0.050 compared with 0.824 ± 0.047 respectively; $P < 0.0000$). In maternal plasma, the concentration of γ-tocopherol and α-tocopherol was significantly higher in the IUGR group than in the control group of the same gestational age (AGA-35wk), although they did not differ in the IUGR and AGA groups.

Cholesterol and triacylglycerol levels in maternal plasma were not different among the groups (Table 2); however, NEFA levels in maternal plasma were higher in women with IUGR pregnancies than in the two controls. In umbilical vein plasma, concentrations of cholesterol, triacylglycerols and NEFAs were significantly lower than in maternal plasma, but values in IUGR neonates were significantly higher than in those in AGA neonates. Although there were no correlations between maternal and fetal cholesterol, the concentration of both triacylglycerols and NEFAs in maternal plasma had a statistically significant positive linear correlation with the respective concentration of these analytes in umbilical vein plasma ($r = 0.4976$, $P = 0.0002$ for triacylglycerol, and $r = 0.4234$, $P = 0.0101$ for NEFAs).

Table 3 shows the percentage of the most relevant fatty acids. In maternal plasma, palmitic acid was higher and stearic acid was lower in IUGR pregnancies than in the AGA-35wk group, but were similar to those in the AGA group. However, palmitoleic acid was significantly higher in the plasma of women with IUGR pregnancies than those in either the AGA-35wk or AGA group, whereas oleic acid did not differ between the groups. Both the palmitoleic acid/palmitic acid and oleic acid/stearic acid ratios, which may be estimated as an indirect index of SCD (stearyl-CoA desaturase) activity, were higher in maternal plasma from the IUGR group than in the AGA-35wk and AGA groups, although, in the case of the oleic acid/stearic acid ratio, the difference between IUGR and AGA pregnancies did not reach statistical significance. In cord blood, the proportion of palmitic acid and palmitoleic acid did not differ from that in maternal plasma, but the proportion of stearic acid was higher and that of oleic acid was lower. However, no differences were observed in any of these fatty acids in cord blood between the two groups studied. The percentage of LA was lower in the maternal plasma of IUGR pregnancies than in either of the two control groups, whereas the percentages of LCPUFA products of LA [GLA (γ-linolenic acid) and AA (arachidonic acid)] were higher in maternal plasma in the IUGR group than in the controls. The GLA/LA ratio, which is an indirect estimation of Δ^6-desaturase activity, was significantly higher in maternal plasma of IUGR pregnancies than in the control groups, indicating a higher conversion of LA into its product. Moreover,

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Table 3 Plasma fatty acid composition (g/100 g of fatty acids) in maternal plasma of controls (AGA-35wk), and in maternal and umbilical vein plasma of IUGR and control (AGA) groups at the time of Caesarean section

Values are means ± S.E.M. Adjustments were made for maternal BMI and gestational age. Fatty acid results are expressed as a percentage (% w/w) of all of the detected fatty acids with a chain length in the range of 12–24 carbon atoms. In the maternal vein, a Tukey's test was used to determine differences among groups after one-way ANOVA. Different superscript uppercase letters indicate significant differences ($P < 0.05$) between the maternal values. In the umbilical vein, different superscript lowercase letters indicate significant differences ($P < 0.01$) between the umbilical values (as determined using a Student's t test). $^{***}P < 0.001$ compared with the respective maternal vein value (as determined using a Student's t test). n.d., not detected.

	Maternal vein plasma			Umbilical vein plasma	
Fatty acid	AGA-35wk ($n = 25$)	IUGR ($n = 24$)	AGA ($n = 30$)	IUGR ($n = 24$)	AGA ($n = 30$)
$C_{16:0}$ (palmitic acid)	24.3 ± 0.6^{A}	28.9 ± 0.7^{B}	27.2 ± 0.6^{B}	27.8 ± 1.3^{a}	25.8 ± 1.2^{a}
$C_{18:0}$ (stearic acid)	9.74 ± 0.51^{A}	6.35 ± 0.59^{B}	7.11 ± 0.57^{B}	$15.5 \pm 2.6^{a***}$	$20.2 \pm 2.2^{a***}$
$C_{16:1n-7}$ (palmitoleic acid)	2.34 ± 0.21^{A}	4.77 ± 0.24^{B}	3.04 ± 0.23^{C}	4.53 ± 0.40^{a}	3.37 ± 0.34^{a}
$C_{18:1n-9}$ (oleic acid)	25.3 ± 0.6^{A}	26.3 ± 0.7^{A}	26.5 ± 0.7^{A}	$18.9 \pm 0.9^{a***}$	$20.0 \pm 0.8^{a***}$
$C_{16:1n-7}/C_{16:0}$	$0.096 \pm 0.00\ ^{A}$	0.168 ± 0.009^{B}	0.111 ± 0.009^{C}	0.162 ± 0.012^{a}	0.126 ± 0.010^{a}
$C_{18:1n-9}/C_{18:0}$	2.83 ± 0.28^{A}	4.53 ± 0.32^{B}	4.26 ± 0.31^{B}	$1.56 \pm 0.21^{a***}$	$1.33 \pm 0.18^{a***}$
$C_{18:2n-6}$ (LA)	24.4 ± 0.8^{A}	19.7 ± 0.9^{B}	22.1 ± 0.9^{A}	$8.23 \pm 0.44^{a***}$	$7.72 \pm 0.38^{a***}$
$C_{18:3n-6}$ (GLA)	0.080 ± 0.028^{A}	0.283 ± 0.032^{B}	0.136 ± 0.031^{A}	n.d.	n.d.
$C_{20:4n-6}$ (AA)	4.61 ± 0.22^{A}	5.57 ± 0.25^{B}	5.16 ± 0.24^{AB}	$10.6 \pm 0.5^{a***}$	$12.3 \pm 0.4^{b***}$
$C_{18:3n-6}/C_{18:2n-6}$	0.004 ± 0.001^{A}	0.015 ± 0.002^{B}	0.006 ± 0.001^{A}	—	—
$C_{20:4n-6}/C_{18:2n-6}$	0.196 ± 0.013^{A}	0.289 ± 0.016^{B}	0.243 ± 0.015^{A}	$1.33 \pm 0.10^{a***}$	$1.64 \pm 0.08^{b***}$
$C_{18:3n-3}$ (ALA)	0.423 ± 0.072^{A}	0.210 ± 0.082^{B}	0.435 ± 0.079^{AB}	n.d.	n.d.
$C_{22:6n-3}$ (DHA)	2.10 ± 0.12^{A}	2.27 ± 0.14^{A}	2.55 ± 0.13^{A}	$3.99 \pm 0.21^{a***}$	$3.84 \pm 0.18^{a***}$

the overall conversion of LA into AA, calculated by the AA/LA ratio, was also significantly higher in women with IUGR pregnancies than in either of the control groups. In umbilical vein plasma, LA values were lower and AA were higher than in maternal plasma, also causing a higher AA/LA ratio in the former. The proportion of the different $n-6$ fatty acids were similar in the umbilical veins of IUGR and AGA neonates, except in the case of AA, whose values were significantly lower in the umbilical vein of the IUGR group than in the AGA group. Concerning $n-3$ fatty acids in maternal plasma, only the proportion of ALA was lower in the IUGR group than in the AGA-35wk group. In umbilical vein plasma, the proportion of EPA (eicosapentaenoic acid; results not shown) and DHA (docosahexaenoic acid; Table 3) was higher than in maternal plasma, but no difference was found between the IUGR and AGA groups.

When the birthweight predictors compared with all of the variables studied were analysed by multiple linear regression and backward selection, only the maternal plasma retinol and NEFAs concentrations were selected (Table 4).

Table 4 Multiple regression analysis of maternal contributors to birthweight

The model included the following independent variables: cholesterol, triacylglycerol, NEFAs, $C_{16:0}$, $C_{16:1n-7}$, $C_{18:0}$, $C_{18:1n-9}$, $C_{18:3n-3}$, $C_{20:5n-3}$, $C_{22:6n-3}$, $C_{18:2n-6}$, $C_{18:3n-6}$, $C_{20:4n-6}$, retinol, γ-tocopherol and α-tocopherol. In the backward selection, variables were removed if F values were lower than 4. Only statistically significant predictors are shown. Log-transformed skewed data for triacylglycerol, NEFA and γ-tocopherol were used.

Selected predictor	β	P value	R^2
NEFA	− 514.6	0.0008	52.1
Retinol	− 1227.4	0.0000	—

DISCUSSION

In the present study, the factors that could be related to the development of IUGR pregnancies were investigated. A potential bias in these types of studies is related to the different gestational age at the time of Caesarean section in IUGR compared with control pregnancies which hampers the use of appropriate controls. We attempted to overcome this problem by using a second group of AGA controls (AGA-35wk), which was matched by gestational age with the IUGR pregnancies, allowing the comparison of maternal variables at the same gestational age. However, parameters in umbilical vein plasma of IUGRs, which are born prematurely, had to be compared with those of newborns delivered 5 weeks later.

We have reported previously that during normal pregnancy retinol concentrations in maternal plasma fall in the third trimester of gestation [29,30], which may result from either its enhanced placental transfer or its increased uptake by the mammary gland, known to occur around parturition [31]. However, in the present study we show for the first time that plasma retinol levels in women

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with IUGR pregnancies are higher than those in pregnant controls of either the same gestational age or at term. Most cases of IUGR are associated with placental insufficiency, reflecting an underlying pathology resulting in an inability of the placenta to supply the metabolic demands of the rapidly growing fetus [32]. This finding agrees with previous reports showing that the IUGR placenta may be smaller and have abnormal vascular development [33]. We found the placental size in IUGR pregnancies was in the order of 50 % lower than in AGA pregnancies, even when corrected for gestational age. Abnormal placental development could be responsible for a reduced retinol transfer to the fetus, thus contributing to the higher levels found in the maternal circulation and the lower umbilical levels. This hypothesis is supported by the fact that, although the proportion of cord plasma retinol was around 65 % of that found in maternal plasma in controls, in agreement with previous reports [34–36], it falls to 35 % in IUGRs.

Other lipophilic vitamins such as γ-tocopherol and α-tocopherol were also found in the present study to be augmented in the plasma of pregnant women who developed IUGR when compared with gestational age-matched controls. The difference is probably not due to changes in dietary intake as food frequency questionnaires did not reveal differences in the daily intake between the groups. This would indicate that the placental transfer of these lipid moieties is also impaired in IUGR.

The augmented ratio of mono-unsaturated/saturated fatty acids found in women with IUGR pregnancies indicates an increase in SCD activity. Retinol has been shown to be an inducer of SCD expression [37–39], indicating its role in controlling fatty acid metabolism. Any deregulation of SCD expression would result in changes in the oleic acid/stearic acid ratio, as was found in the present study in women with IUGR pregnancies when compared with their gestational age-matched controls. Physiologically, such a ratio has been reported as being an important determinant in controlling cell growth and differentiation through its effects on cell membrane bilayer fluidity and function [19,40] and, therefore, its altered value in women with IUGR pregnancies could contribute in some way to this pathological condition.

Other fatty acids may be implicated in the development of IUGR. As reported previously, EFA status in pregnant women has been related to premature delivery [41] and, in agreement with previous reports [42,43], a lower proportion of EFAs in plasma was found in the present study in women with IUGR pregnancies than in controls. As the human placenta has a low activity of both Δ^6- and Δ^5-desaturases [44,45] and the conversion of EFAs into their LCPUFA derivatives in the human fetus does not satisfy its requirements, LCPUFAs in fetal circulation must be derived primarily from maternal plasma. Therefore a deficiency in EFAs in pregnant women appears to be a contributory factor to abnormal gestational outcome development. In spite of this, the conversion of EFAs into LCPUFAs appeared more effective in IUGR pregnancies because their proportion in plasma was either unchanged, as in the case of DHA, or even higher, as in the case of AA, compared with those found in the AGA group. The results of the present study do not show decreased DHA in umbilical vein plasma of IUGR relative to AGA neonates, as has been reported by others [46], but a decreased utilization by the fetus could compensate for its reduced transfer. The efficient synthesis of AA in IUGR pregnancies at the maternal site, as suggested by the enhanced AA/LA ratio, contrasts with the situation observed in cord plasma, where both AA and the AA/LA ratio were significantly lower in the IUGR group than in the AGA group, indicating a reduced placental transfer of AA in the former, which could be associated with fetal growth retardation [43].

In the present study, we found a highly significant negative correlation between maternal retinol concentration and birthweight. Although we did not find precedents for the existence of such a correlation in a group of pregnant women which included women with IUGR pregnancies, Mathews et al. [47] in a prospective study with healthy pregnant women reported that high retinol and haemoglobin concentrations in late pregnancy were associated with low birthweight. Together with retinol, we observed in the present study an increase in NEFA concentrations in the maternal circulation that could be related to an enhanced lipolytic activity and/or to a decrease in placental weight and fetal development. It is hypothesized that both changes could be a consequence of the impaired placental function present in IUGR pregnancies, causing a proportional enrichment of both retinol and NEFAs on the maternal side.

In conclusion, the increased concentrations of retinol, NEFAs and antioxidant vitamins, as well as the altered fatty acid profile found in the plasma of women with IUGR pregnancies, could be a consequence of impaired placental function. The increase in circulating retinol and NEFAs in women with IUGR pregnancies are, however, the only modified variables that appear to be directly related to decreased fetal development. Previous studies have demonstrated that retinol concentrations decrease throughout normal pregnancy [29], so abnormally high retinol levels in mid-pregnancy could be a useful biochemical predictor marker in IUGR pregnancies. The key role of retinoids in controlling gene transcription involved in cell differentiation and proliferation, including SCD expression, warrants further investigation to fully understand their implication in this pathology.

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