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Enhanced circulating retinol and nonesterified fatty acids in pregnancies complicated with intrauterine growth restriction.

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ABSTRACT

Intrauterine growth restriction (IUGR) 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. This study investigated factors in maternal and cord blood plasma from normal and IUGR complicated pregnancies associated with newborns' body weight. At the time of caesarean section, twenty-four women with IUGR pregnancies were compared to a group of thirty normal controls with appropriate gestation age (AGA) fetuses that were studied at caesarean, which took place 5 weeks earlier than IUGRs and also to a group of twenty-five nondelivered gestational age-matched control pregnant women (AGA-35 wks). Maternal plasma retinol, γ- and α-tocopherol, non-esterified fatty acids (NEFA), palmitic-, palmitoleic-, γ-linolenic- and arachidonic-acid were higher in women with IUGR pregnancies than in AGA-35 wks controls, whereas stearic- and α-linolenic-acid were lower. Smaller differences were found when comparing these variables for IUGR and AGA women. However, umbilical vein plasma γ-tocopherol, cholesterol, triacylglycerols and NEFA were higher in the IUGR group than in AGA, whereas arachidonic acid was lower. Maternal plasma retinol and NEFA were the only variables negatively correlated with birth weight when multiple linear regressions were analyzed. In conclusion, the increased circulating retinol and NEFA in maternal plasma are negatively associated with birth and placental weights, which may reflect an impaired placental transfer in IUGR pregnancies. Since retinoids have been involved in the control of gene transcription, it is proposed that a decreased placental transfer of retinol could underlie the metabolic dysfunction of IUGR pregnancies.

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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 signaling molecules. In particular, the essential fatty acids (EFA), linoleic acid (LA, C18:2n-6) and α-linolenic acid (ALA, C18:3n-3) and their long-chain polyunsaturated fatty acid (LCPUFA) derivatives, play a critical role in fetal development [1]. Thus, although LCPUFA 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]. LCPUFA can be endogenously synthesized from precursor EFAs in a process that involves delta-6 and delta-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, placenta lacks the desaturase enzymes to convert EFAs [4], and therefore LCPUFAs should derive predominantly from the mother via placental transfer during fetal life.

Intrauterine growth restriction (IUGR) 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 for 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 retinol binding protein (RBP)-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 the cytoplasmatic cellular retinol binding protein (CRBP) in a membrane-dependent manner [15]. Thus the fetus acquires vitamin A from 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, this study analyzes 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 and to the Obstetrics and Gynecology Unit of the Clinica Mangiagalli of Milan University, during a four year period (October 2000-June 2005). The University of Milan Ethical Board approved the study protocol. Informed consent was obtained from all 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 analyze the nutritional intake [22]. This is a semi-quantitative validated questionnaire composed of pictures of 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 in software designed to calculate nutrient quantities.

Control patients had an ultrasound scan at 30–32 wk of gestation that confirmed a normal fetal growth pattern, and gave birth to healthy term neonates with a birth weight between the 10th 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 10^{th} 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 10^{th} percentile [25] according to Italian birth weight 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, while elective caesarean took place in the control group because of cephalo-pelvic disproportion, repeat caesarean or breech presentation. All pregnancies were singleton and none of the babies showed 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 (appropriate gestational age, AGA). Therefore, in order to compare IUGR mothers with gestational age-matched controls, we included a second control group corresponding to nondelivered pregnant women with normal pregnancies of similar gestational age (AGA-35 weeks) than IUGR's; 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 of mothers 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-Na_2 and kept on ice until centrifugation (1500 x g at 4℃ for 25 min). Plasma was portioned and immediately stored at -80℃ until analysis.

Analytic methods

Plasma cholesterol, triacylglycerols (TG) (Spinreact Reactives, Spain) and non-esterified fatty acids (NEFA) (Wako Chemicals, Germany) were determined enzymatically by commercial kit. Adequate control plasmas with different and certified concentrations were used in all determinations to verify the accuracy of the assays (Accutrol, Sigma, Spain; Precinorm and Precipath, Roche Diagnostics, Spain). The interassay CVs 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, Palo Alto, CA) after extraction with hexane, as described elsewhere [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 (Gainthersburg, MD) was used as a control. The interassay CVs were as follows: α-tocopherol, 4.3%; γ-tocopherol, 10.6% and retinol, 3.8%.

Plasma lipids were extracted in chloroform/methanol (2:1) [27] containing 0.005 % BHT. Fatty acids were transesterified with methanolic hydrochloride and analyzed on a Perkin Elmer gas chromatograph (Autosystem; Norwalk, CT) as previously reported [28].

Statistics

Results are expressed as means ± SEM. Statistical difference between groups was determined by analysis of variance (ANOVA) after adjusting for maternal BMI and gestational age; when differences were statistically significant, multiple comparisons were performed using Tukey's post hoc test. Paired Student's *t*-test was used to determine significant differences between maternal and umbilical plasma. Given their skewed distributions, concentrations of TG, NEFA 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 neonate birth weight, 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 Corp.).

RESULTS.

As shown in **Table** 1, whereas 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), although not differing with that seen 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 IUGR than in the AGA group, and whereas 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 show no significant differences between the two groups in either the total daily caloric intake or in the total amount of proteins, carbohydrates or lipids. Also, no significant intergroup differences were found for any of the vitamins in the diet (vitamins A, C, D and E) (data not shown).

As shown in **Table 2**, plasma retinol concentration was significantly higher in pregnant women who developed IUGR as compared to either of the two healthy controls. Values of retinol in umbilical vein plasma did not differ between 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 groups (0.376±0.050 *vs* 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 those with the same gestational age (AGA-35wk), although they did not differ in the IUGR and AGA groups.
Cholesterol and TG levels in maternal plasma showed no differences among the groups (**Table 3**). However, NEFA levels in maternal plasma were higher in those with IUGR pregnancies than in the two controls. In umbilical vein plasma, concentrations of cholesterol, TG and NEFA were significantly lower than in maternal plasma, but values in IUGR fetuses were significantly higher than in those of AGA. Although there were no correlations between maternal and fetal cholesterol, the concentration of both TG and NEFAs in maternal plasma showed a statistically significant positive lineal correlation with the respective concentration of these analytes in umbilical vein plasma (r=0.4976, p=0.0002 for TAG and r=0.4234, p=0.0101 for NEFAs).

Table 4 shows the percentage of the most relevant fatty acids. In maternal plasma, palmitic acid was higher and stearic acid lower in IUGR pregnancies than in AGA-35wk but similar to that in AGA. However, palmitoleic acid was significantly higher in plasma of women with IUGR pregnancies than those in either AGA-35wk or AGA, whereas oleic acid did not differ between the groups. Both the palmitoleic/palmitic acid and oleic/stearic acid ratios, which may be estimated as an indirect index of stearyl-coenzyme A desaturase (SCD) activity, were higher in maternal plasma of the IUGR group than in the AGA-35wk and AGA groups, although in the case of the oleic/stearic acid ratio, the difference between IUGR pregnancies and AGA 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 while that of oleic acid lower. However, no differences were observed in any of these fatty acids in cord blood between the two groups studied. The percentage of linoleic acid (LA) was lower in maternal plasma of IUGR pregnancies than in either of the two control groups studied, whereas the percentages of long-chain polyunsaturated fatty acid products of LA (γ-linolenic, GLA, and arachidonic acid, AA) were higher in maternal plasma of 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 to its product. Moreover, 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 AGA-35wk or AGA. In umbilical vein plasma, LA values were lower and AA higher than in maternal plasma, also causing a higher AA/LA ratio in the former. The proportion of the different n6 fatty acids showed similar values in umbilical vein of IUGR and AGA neonates, except in the case of AA, whose values were significantly lower in umbilical vein of IUGR than in AGA. Concerning n3 fatty acids in maternal plasma, only the proportion of α-linolenic acid (ALA) was lower in the IUGR group than in AGA-35wk. In umbilical vein plasma, the proportion of eicosapentanoic (EPA) (not shown) and docosahexaenoic acid (DHA) was higher than in maternal plasma but no difference was found between IUGR and AGA.

When we analyzed the birth weight predictors versus all the studied variables by multiple linear regression and backward selection, only the maternal plasma retinol and NEFAs concentrations were selected (**Table 5**).

DISCUSSION

This study addresses factors that could be related to the development of IUGR pregnancies. A potential bias in these types of studies is related to the different gestational age at the time of caesarean section in IUGR compared to control pregnancies that 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 premature, had to be compared with those of newborns delivered five weeks later.
We have previously reported that during normal pregnancy retinol concentration in maternal plasma falls 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, we show here for the first time that plasma retinol levels in women 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 display abnormal vascular development [33]. We found the placental size in IUGR pregnancies was in the order of 50% lower than in AGA pregnant, even when corrected by gestational age. The abnormal placental development could be responsible for a reduced retinol transfer to the fetus, thus contributing to the higher levels found in maternal circulation and the lower umbilical levels. This hypothesis is supported by the fact that whereas 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 here to be augmented in the plasma of pregnant women who developed IUGR when compared to their gestational age-matched controls. The difference is probably not due to changes in dietary intake since 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 monounsaturated-/saturated-fatty acids found in women with IUGR pregnancies indicates an enhanced 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 in SCD expression would result in changes in the oleic acid/stearic acid ratio, as was found here in women with IUGR pregnancies when compared to 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 previously reported, EFA status in pregnant women has been related to premature delivery [41], and in agreement with previous reports [42, 43], a lower proportion of EFA in plasma was found in women with IUGR pregnancies than in controls. Since human placenta has a low activity of both delta-6 and delta-5 desaturases [44, 45], and the conversion of EFA to their LCPUFA derivatives in human fetus does not satisfy its necessities, LCPUFA in fetal circulation must be derived primarily from maternal plasma. Therefore, a deficiency of EFA in the mothers appears to be a contributory factor to abnormal gestational outcome development. In spite of this, the conversion of EFA to LCPUFAs appeared more effective in IUGR pregnancies because their proportion in plasma was either unchanged, as was the case for DHA, or even higher, as was the case of AA, compared with those found in the AGA group. Present data do not show decreased DHA in umbilical vein plasma of IUGR relative to AGA, as has been reported by other authors [46], but a reduced utilization by the fetus could compensate for its reduced transfer. The efficient synthesis of AA in IUGR at the maternal site, 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 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 birth weight. Although we did not find precedents for the existence of such a correlation in a group of pregnants which included women with IUGR pregnancies, Mathews *et al* [47] in a prospective study with healthy pregnants reported that high retinol and hemoglobin concentrations in late pregnancy were associated with low birth weight. Together with retinol, we observed in our study an increase in NEFA concentration in 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 IUGRs, causing a proportional enrichment of both retinol and NEFA on the maternal side.

In conclusion, the increased concentrations of retinol, NEFA 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 increased circulating retinol and NEFA 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 concentration 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 research to fully understand their implication in this pathology.

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TABLE 1.
Maternal and neonatal characteristics of the study population [1].

Characteristics.	AGA-35wk (n=25)	IUGR (n=24)	AGA (n=30)
At initial of gestation			
Maternal age (y)	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			
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 (wk)	-	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]

[1] All values are mean ± SEM. In maternal characteristics, Tukeys's test was used to determine differences among groups after one-way ANOVA. In neonate characteristics, Student´s *t*-test was used to compare values between IUGR and AGA groups. Different superscript letters within a row mean significant differences, $P<0.05$.

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TABLE 2.
Concentration of lipophilic vitamins (μmol/l) in maternal plasma of control pregnants 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 [1].

	MATERNAL VEIN PLASMA			UMBILICAL VEIN PLASMA	
	AGA-35wk	IUGR	AGA	IUGR	AGA
Vitamin	(n=25)	(n=24)	(n=30)	(n=24)	(n=30)
Retinol	1.00±0.070 ^{A}	1.63± 0.08 ^{B}	0.884±0.076 ^{A}	0.504±0.057 $^{a, ***}$	0.748±0.052 $^{b, ***}$
γ-tocopherol [2]	1.42±0.12 ^{A}	1.78±0.13 ^{B}	1.51±0.13 ^{AB}	0.451±0.056 $^{a, ***}$	0.274±0.056 $^{b, ***}$
α-tocopherol	27.7±2.6 ^{A}	40.1±3.0 ^{B}	39.7±2.9 ^{B}	6.72±0.83 $^{a, ***}$	6.75±0.76 $^{a, ***}$

[1]All values are mean ± SEM. Adjusted for BMI at initial of gestation and for gestational age.
In maternal vein, Tukeys's test was used to determine differences among groups after one-way ANOVA. Different superscript capital letters mean significant differences between maternal values, $P<0.05$. In umbilical vein, different superscript small letters mean significant differences between umbilical values (Student's *t* test), $P<0.01$. Asterisk indicate differences from its respective maternal vein (Student's *t* test) , * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
[2] log-transformed skewed data for statistical comparisons.

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TABLE 3.
Plasma lipid concentrations (mmol/l) in maternal plasma of control pregnants 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 [1].

	MATERNAL VEIN PLASMA			UMBILICAL VEIN PLASMA	
	AGA-35wk	IUGR	AGA	IUGR	AGA
Lipid	(n=25)	(n=24)	(n=30)	(n=24)	(n=30)
Cholesterol	6.31±0.29 ^{A}	6.86±0.33 ^{A}	6.39±0.32 ^{A}	1.61±0.16 $^{a, ***}$	1.30±0.14 $^{b, ***}$
Triacylglycerols [2]	2.34±0.23 ^{A}	2.37±0.26 ^{A}	2.15±0.25 ^{A}	0.366±0.044 $^{a, ***}$	0.349±0.038 $^{b, ***}$
NEFA [2]	0.329±0.042 ^{A}	0.948±0.067 ^{B}	0.599±0.050 ^{C}	0.166±0.022 $^{a, ***}$	0.134±0.013 $^{b, ***}$

[1]All values are mean ± SEM. Adjusted for BMI at initial of gestation and for gestational age.
In maternal vein, Tukeys's test was used to determine differences among groups after one-way ANOVA. Different superscript capital letters mean significant differences between maternal values, $P<0.05$. In umbilical vein, different superscript small letters mean significant differences between umbilical values (Student's *t* test), $P<0.01$. Asterisk indicate differences from its respective maternal vein (Student's *t* test) , * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
[2] log-transformed skewed data for statistical comparisons.

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TABLE 4.
Plasma fatty acid composition (g/100 g fatty acids) in maternal plasma of control pregnants 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 [1].

	MATERNAL VEIN PLASMA			UMBILICAL VEIN PLASMA	
	AGA-35wk	IUGR	AGA	IUGR	AGA
Fatty acid	(n=25)	(n=24)	(n=30)	(n=24)	(n=30)
C16:0 (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}
C18:0 (Stearic Acid)	9.74±0.51 ^{A}	6.35±0.59 ^{B}	7.11±0.57 ^{B}	15.5±2.6 $^{a, ***}$	20.2±2.2 $^{a, ***}$
C16:1n-7 (Palmitoleic)	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}
C18:1n-9 (Oleic Acid)	25.3±0.6 ^{A}	26.3±0.7 ^{A}	26.5±0.7 ^{A}	18.9±0.9 $^{a, ***}$	20.0±0.8 $^{a, ***}$
C16:1n-7 /C16:0	0.096±0.008 ^{A}	0.168±0.009 ^{B}	0.111±0.009 ^{C}	0.162±0.012 ^{a}	0.126±0.010 ^{a}
C18:1n-9/C18:0	2.83±0.28 ^{A}	4.53±0.32 ^{B}	4.26±0.31 ^{B}	1.56±0.21 $^{a, ***}$	1.33±0.18 $^{a, ***}$
C18:2n-6 (LA)	24.4±0.8 ^{A}	19.7±0.9 ^{B}	22.1±0.9 ^{A}	8.23±0.44 $^{a, ***}$	7.72±0.38 $^{a, ***}$
C18:3n-6 (GLA)	0.080±0.028 ^{A}	0.283±0.032 ^{B}	0.136±0.031 ^{A}	n.d.	n.d.
C20:4n-6 (AA)	4.61±0.22 ^{A}	5.57±0.25 ^{B}	5.16±0.24 ^{AB}	10.6±0.5 $^{a, ***}$	12.3±0.4 $^{b, ***}$
C18:3n-6/C18:2n-6	0.004±0.001 ^{A}	0.015±0.002 ^{B}	0.006±0.001 ^{A}	-	-
C20:4n-6/C18:2n-6	0.196±0.013 ^{A}	0.289±0.016 ^{B}	0.243±0.015 ^{A}	1.33±0.10 $^{a, ***}$	1.64±0.08 $^{b, ***}$
C18:3n-3 (ALA)	0.423±0.072 ^{A}	0.210±0.082 ^{B}	0.435±0.079 ^{AB}	n.d.	n.d.
C22:6n-3 (DHA)	2.10±0.12 ^{A}	2.27±0.14 ^{A}	2.55±0.13 ^{A}	3.99±0.21 $^{a, ***}$	3.84±0.18 $^{a, ***}$

Fatty acids results were expressed as a percentage (% w/w) of all detected fatty acids with a chain length in the range of 12-24 carbon atoms.
[1]All values are mean ± SEM. Adjusted for BMI at initial of gestation and for gestational age.
In maternal vein, Tukeys's test was used to determine differences among groups after one-way ANOVA. Different superscript capital letters mean significant differences between maternal values, $P<0.05$. In umbilical vein, different superscript small letters mean significant differences between umbilical values (Student's *t* test), $P<0.01$. Asterisk indicate differences from its respective maternal vein (Student's *t* test) , * $P<0.05$; ** $P<0.01$; *** $P<0.001$.
n.d. non detected.

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TABLE 5
Multiple regression analysis of maternal contributors to birth weight.

Selected predictors	β	P	R^2
NEFA[1]	-514.6	0.0008	52.1
retinol	-1227.4	0.0000	

The model included the following independent variables: cholesterol, triacylglycerol[1], NEFA[1], C16:0, C16:1n-7, C18:0, C18:1n-9, C18:3n-3, C20:5n-3, C22:6n-3, C18:2n-6, C18:3n-6, C20:4n-6, retinol, γ-tocopherol[1] and α-tocopherol[1].
In the backward selection, variables were removed for F values lower than 4.
Only statistically significant predictors are shown.
[1] log transformed skewed data.