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# Preeclampsia is associated with compromised maternal synthesis of long-chain polyunsaturated fatty acids, leading to offspring deficiency

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# Preeclampsia is associated with compromised maternal synthesis of long-chain polyunsaturated fatty acids, leading to offspring deficiency

## Abstract

Obesity and excessive lipolysis are implicated in preeclampsia (PE). Intrauterine growth restriction is associated with low maternal body mass index and decreased lipolysis. Our aim was to assess how maternal and offspring fatty acid metabolism is altered in mothers in the third trimester of pregnancy with PE (n=62) or intrauterine growth restriction (n=23) compared with healthy pregnancies (n=164). Markers of lipid metabolism and erythrocyte fatty acid concentrations were measured. Maternal adipose tissue fatty acid composition and mRNA expression of adipose tissue fatty acid-metabolizing enzymes and placental fatty acid transporters were compared. Mothers with PE had higher plasma triglyceride (21%,  $P<0.001$ ) and nonesterified fatty acid (50%,  $P<0.001$ ) concentrations than controls. Concentrations of major n-6 and n-3 long-chain polyunsaturated fatty acids in erythrocytes were 23% to 60% lower (all  $P<0.005$ ) in PE and intrauterine growth restriction mothers and offspring compared with controls. Subcutaneous adipose tissue -5 and -6 desaturase and very long-chain fatty acid elongase mRNA expression was lower in PE than controls (respectively, mean [SD] control 3.38 [2.96] versus PE 1.83 [1.91],  $P=0.030$ ; 3.33 [2.25] versus 1.03 [0.96],  $P<0.001$ ; 0.40 [0.81] versus 0.00 [0.00],  $P=0.038$  expression relative to control gene [square root]). Low maternal and fetal long-chain polyunsaturated fatty acid concentrations in PE may be the result of decreased maternal synthesis.

## Keywords

maternal, compromised, associated, preeclampsia, long, chain, synthesis, polyunsaturated, deficiency, fatty, acids, leading, offspring

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## Preeclampsia Is Associated With Compromised Maternal Synthesis of Long-Chain Polyunsaturated Fatty Acids, Leading to Offspring Deficiency

Vanessa A. Mackay, Shahzya S. Huda, Frances M. Stewart, Kahmeng Tham, Louise A. McKenna, Iain Martin, Fiona Jordan, E. Ann Brown, Leanne Hodson, Ian A. Greer, Barbara J. Meyer, Dilys J. Freeman

**Abstract**—Obesity and excessive lipolysis are implicated in preeclampsia (PE). Intrauterine growth restriction is associated with low maternal body mass index and decreased lipolysis. Our aim was to assess how maternal and offspring fatty acid metabolism is altered in mothers in the third trimester of pregnancy with PE (n=62) or intrauterine growth restriction (n=23) compared with healthy pregnancies (n=164). Markers of lipid metabolism and erythrocyte fatty acid concentrations were measured. Maternal adipose tissue fatty acid composition and mRNA expression of adipose tissue fatty acid–metabolizing enzymes and placental fatty acid transporters were compared. Mothers with PE had higher plasma triglyceride (21%,  $P<0.001$ ) and nonesterified fatty acid (50%,  $P<0.001$ ) concentrations than controls. Concentrations of major n–6 and n–3 long-chain polyunsaturated fatty acids in erythrocytes were 23% to 60% lower (all  $P<0.005$ ) in PE and intrauterine growth restriction mothers and offspring compared with controls. Subcutaneous adipose tissue  $\Delta$ –5 and  $\Delta$ –6 desaturase and very long-chain fatty acid elongase mRNA expression was lower in PE than controls (respectively, mean [SD] control 3.38 [2.96] versus PE 1.83 [1.91],  $P=0.030$ ; 3.33 [2.25] versus 1.03 [0.96],  $P<0.001$ ; 0.40 [0.81] versus 0.00 [0.00],  $P=0.038$  expression relative to control gene [square root]). Low maternal and fetal long-chain polyunsaturated fatty acid concentrations in PE may be the result of decreased maternal synthesis. (*Hypertension*. 2012;60:1078-1085.) • [Online Data Supplement](#)

**Key Words:** fatty acid ■ pregnancy ■ preeclampsia ■ intrauterine growth restriction

Preeclampsia (PE), a multisystem disorder particular to pregnancy, is a leading cause of maternal and neonatal morbidity and mortality. PE is characterized by widespread endothelial dysfunction, resulting in hypertension attributable to vasoconstriction, proteinuria attributable to glomerular damage, and edema secondary to increased vascular permeability. Maternal obesity, increased insulin resistance, and aberrant fatty acid metabolism are involved in its pathogenesis.<sup>1</sup> Excessive nonesterified fatty acid (NEFA) flux in PE, similar to that seen in nonalcoholic fatty liver disease, may instigate ectopic lipid accumulation in the liver and other tissues<sup>2</sup> and interfere with long-chain polyunsaturated fatty acid (LC PUFA) synthesis.<sup>3</sup> Intrauterine growth restriction (IUGR) can occur independently or simultaneously with PE. Isolated IUGR pregnancies are characterized by low maternal body mass index (BMI), low plasma lipid levels, and reduced lipolysis.<sup>4,5</sup> Few data exist on the impact of PE or IUGR on maternal fatty acid mobilization during pregnancy, and those available have focused on percentage of total fatty acids in plasma<sup>6–9</sup> rather than absolute amounts or

erythrocyte composition. These latter measures allow primary independent effects on individual fatty acids to be determined and better reflect tissue fatty acid composition and long-term nutritional status rather than recent dietary intake.<sup>10</sup>

LC PUFAs of the n–3 and n–6 series, such as docosahexaenoic acid (22:6n–3) and arachidonic acid (20:4n–6), are required for fetal growth<sup>11</sup> and brain development.<sup>12</sup> Thus, a potential long-term consequence of disturbed LC PUFA synthesis in PE is suboptimal neurodevelopment of the infants.<sup>13</sup> Maternal LC PUFAs are mobilized by week 13 of gestation,<sup>14</sup> but their source(s) are not established. The n–6 and n–3 LC PUFAs are synthesized from essential shorter chain precursors (18:2n–6 and 18:3n–3, respectively) via  $\Delta$ 5-desaturase,  $\Delta$ 6-desaturase, and elongase enzymes (Figure S1 in the online-only Data Supplement). Pregnant women accumulate adipose tissue in the early anabolic stage of pregnancy, but in later gestations, because of insulin resistance, adipose tissue fatty acid mobilization increases.<sup>15</sup> Placental transfer of LC PUFAs to the fetus is obligatory,<sup>12</sup> and both circulating NEFAs and

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fatty acids released from lipolysis of lipoprotein triglycerides are transported across the placenta by a series of fatty acid-binding and transfer proteins.<sup>16</sup> Among these, placental plasma membrane-associated fatty acid binding protein (pFABPm), a membrane transporter, and fatty acid binding protein (FABP)7, an intracellular transporter, have high affinities for 22:6n-3 and are expressed by trophoblasts.<sup>17-19</sup>

We hypothesized that, in an analogous way to nonalcoholic fatty liver disease, excessive NEFA flux in PE provokes ectopic lipid accumulation in the liver and inhibits LC PUFA synthesis. Our aim was to assess whether maternal fatty acid metabolism is altered in mothers with PE or IUGR compared with healthy pregnancy and impacts on offspring LC PUFA status. We carried out erythrocyte fatty acid compositional analysis of paired maternal and fetal samples from healthy and complicated pregnancies. Markers of lipid metabolism were also assessed to describe the gross differences in fuel metabolism. To assess whether LC PUFA status was related to the composition of stored fatty acids we analyzed both visceral and upper body subcutaneous adipose tissue fatty acid composition in a subset of the pregnancies. The mRNA expression of desaturase and elongase enzymes was quantitated in adipose tissue as a marker of LC PUFA synthesis in this and other tissues. To test whether offspring LC PUFA status was related to placental transfer of fatty acids, mRNA expression of key enzymes and transporters in LC PUFA metabolism was quantitated in placental biopsies.

## Methods

### Subjects

Subjects with PE (n=62), subjects with IUGR (n=23), and controls with uncomplicated pregnancies (n=164) in the third trimester of a singleton pregnancy were recruited. The study was approved by the Glasgow Royal Infirmary Local Research Ethics Committee, and women gave written informed consent. For further details on recruitment and biopsy sampling please see the online-only Data Supplement.

### Biochemical Analyses

Total cholesterol, triglyceride and high-density lipoprotein cholesterol assays,<sup>20</sup> and glucose and high-sensitivity C-reactive protein assays<sup>21</sup> were performed at the Department of Clinical Biochemistry, Glasgow Royal Infirmary. Other analytes were assayed using commercially available kits (please see the online-only Data Supplement). Fatty acids were extracted from erythrocyte membranes<sup>14</sup> and adipose tissue and identified by gas chromatography<sup>14,22</sup> (for details please see online-only Data Supplement).

### Messenger RNA Expression

Total RNA was isolated from placenta (control n=57, PE n=17, and IUGR n=11), subcutaneous adipose tissue (control n=50, PE n=12, and IUGR n=13), and visceral adipose tissue (control n=25, PE n=12 and IUGR n=5) and cDNA was synthesized. Target gene expression was quantitated relative to a control gene by TaqMan real-time polymerase chain reaction using commercial primer probe sets (Applied Biosystems; for details please see the online-only Data Supplement).

### Statistical Analysis

Details of statistical analysis are provided in the online-only Data Supplement. Unsaturation index is the average number of double bonds per fatty acid residue multiplied by 100; average chain length is the sum of mol% times chain length for each reported fatty acid divided by 100; C20-22 is the total percentage of LC PUFAs with  $\geq 20$  carbon units; docosahexaenoic acid deficiency index is  $22:5n-6/22:4n-6$ ; and essential fatty acid (EFA) deficiency index is  $(n-3+n-6)/(n-7+n-9)$ . Because of multiple testing, significance

levels were set at  $P < 0.005$  for plasma metabolic markers and fatty acid analysis.

## Results

### Maternal Metabolic and Inflammatory Profile

Maternal antenatal booking characteristics (please see Table S1 in the online-only Data Supplement) and third-trimester plasma profiles (please see Table S2) are shown. Mothers with PE had higher levels of triglyceride, NEFA, leptin, adiponectin, and interleukin 6 than controls, and these differences were maintained after adjustment for maternal BMI, parity, smoking status, and gestational age at sampling.

### Impact of PE and IUGR on Maternal LC PUFA Status

Maternal third-trimester erythrocyte fatty acid concentrations are shown in Table 1. There were no differences in concentrations of saturated fatty acids (SAFAs) between groups apart from a lower concentration of the minor fatty acid 22:0 in IUGR. For the monounsaturated fatty acids, there was a 19% lower concentration of 24:1n-9 in IUGR. Concentrations of all n-6 PUFAs, apart from the minor fatty acids 18:3n-6 and 22:2n-6, were 23% to 60% lower in both PE and IUGR mothers. Of the n-3 PUFAs, 22:5n-3 and 22:6n-3 were lower in PE and IUGR mothers to a similar extent as for the n-6 PUFAs. Interestingly, 20:5n-3 was not different between groups.

Summary indices of maternal fatty acid status are shown in Table 1. The percentage of SAFAs is higher and the percentage of unsaturated fatty acids and PUFAs is significantly lower in PE and IUGR. The main driver for the change in proportions is the lower PUFAs, because concentrations of SAFAs are similar between groups. The lower PUFA concentrations account for the lower unsaturation index and average chain length observed in PE and IUGR. Because concentrations of both n-6 and n-3 PUFAs are lower, the n-6/n-3 ratio is similar across groups. PE and IUGR mothers are deficient in EFAs and 22:6n-3. All observed differences were maintained after adjustment for potential confounders. Women with severe PE had significantly lower concentrations than those with mild PE for the majority of fatty acid parameters measured (please see Table S3). There was no relationship with gestational age at PE onset (data not shown).

### Offspring Metabolic and Inflammatory Profile

Cord blood total cholesterol, but not high-density lipoprotein, concentration was significantly higher in PE offspring (please see Table S2). Conversely, both total and high-density lipoprotein cholesterol concentrations were lower in IUGR compared with control offspring. Cord blood triglyceride concentrations were higher in PE and IUGR offspring compared with controls. There were no differences in cord blood NEFA, glucose, insulin, insulin resistance (homeostatic model assessment), or inflammatory marker levels between groups. Cord blood leptin (46%–68%) and adiponectin (38%–60%) levels were significantly lower in PE and IUGR offspring, associations that were lost after adjusting for maternal BMI and gestation at delivery for leptin and maternal smoking for adiponectin, as described by others.<sup>23-25</sup>

**Table 1. Maternal Erythrocyte Fatty Acid Concentrations (nmol/mL of Blood) From Third-Trimester Control, PE, and IUGR Mothers**

Fatty Acid	Control (n=164)	PE (n=62)	IUGR (n=23)	P*	Adjusted P†
<b>SAFA</b>					
12:0	0.3 (2)	1.4 (5)	0 (0)	0.026	0.099
14:0	15 (9)	17 (8)	16 (8)	0.26	0.48
16:0	516 (116)	531 (107)	447 (128)	0.010	0.016
17:0	6 (5)	5 (5)	7 (4)	0.21	0.19
18:0	318 (74)	294 (70)	294 (83)	0.055	0.021
20:0	11 (4)	10 (4)	11 (4)	0.65	0.11
22:0	22 (14) <sup>a</sup>	17 (16) <sup>a</sup>	9 (12) <sup>b</sup>	<0.001	<0.001
24:0	60 (12)	61 (17)	61 (13)	0.80	0.85
<b>MUFA</b>					
14:1n-7	0 (0)	0 (0)	0 (0)	-	-
16:1n-7	17 (10)	20 (9)	17 (10)	0.20	0.079
17:1n-7	17 (28)	15 (27)	15 (27)	0.81	0.69
18:1n-7	5 (10)	5 (11)	3 (9)	0.71	0.96
18:1n-9	288 (80)	283 (65)	238 (71)	0.012	0.007
20:1n-9	8 (5)	6 (5)	6 (4)	0.025	0.007
22:1n-9	1 (3)	1 (3)	0 (0)	0.21	0.11
24:1n-9	86 (22) <sup>a</sup>	80 (20) <sup>a,b</sup>	70 (21) <sup>b</sup>	0.002	0.004
<b>PUFA n-6</b>					
18:2n-6	171 (54) <sup>a</sup>	130 (51) <sup>b</sup>	115 (49) <sup>b</sup>	<0.001	<0.001
18:3n-6	1.8 (3)	1.3 (3)	2.1 (3)	0.43	0.38
20:2n-6	4 (5) <sup>a</sup>	2 (3) <sup>b</sup>	2 (3) <sup>b</sup>	<0.001	<0.001
20:3n-6	32 (15) <sup>a</sup>	23 (14) <sup>b</sup>	20 (11) <sup>b</sup>	<0.001	<0.001
20:4n-6	225 (89) <sup>a</sup>	142 (97) <sup>b</sup>	128 (82) <sup>b</sup>	<0.001	<0.001
22:2n-6	0.8 (1.8)	0.7 (1.5)	0.8 (1.6)	0.97	0.64
22:4n-6	43 (20) <sup>a</sup>	26 (20) <sup>b</sup>	25 (17) <sup>b</sup>	<0.001	<0.001
22:5n-6	10 (6) <sup>a</sup>	5 (6) <sup>b</sup>	4 (5) <sup>b</sup>	<0.001	<0.001
<b>PUFA n-3</b>					
18:3n-3	5 (4)	3 (3)	4 (3)	0.028	<0.001
20:3n-3	1.1 (2.8)	0.1 (0.6)	0 (0)	0.006	0.002
20:5n-3	16 (10)	16 (10)	17 (12)	0.91	0.86
22:3n-3	3.6 (5.1)	2.6 (4.0)	1.5 (2.3)	0.080	0.012
22:5n-3	32 (14) <sup>a</sup>	19 (14) <sup>b</sup>	17 (14) <sup>b</sup>	<0.001	<0.001
22:6n-3	65 (30) <sup>a</sup>	40 (35) <sup>b</sup>	39 (31) <sup>b</sup>	<0.001	<0.001
<b>Summary indices</b>					
% SAFA	49 (7) <sup>a</sup>	54 (9) <sup>b</sup>	56 (10) <sup>b</sup>	<0.001	<0.001
% MUFA	21 (2) <sup>a</sup>	23 (2) <sup>b</sup>	22 (4) <sup>a</sup>	<0.001	<0.001
% PUFA	30 (7) <sup>a</sup>	22 (9) <sup>b</sup>	22 (9) <sup>b</sup>	<0.001	<0.001
% UNSAT	51 (7) <sup>a</sup>	46 (9) <sup>b</sup>	44 (10) <sup>b</sup>	<0.001	<0.001
Unsaturation index	131 (29) <sup>a</sup>	102 (35) <sup>b</sup>	101 (36) <sup>b</sup>	<0.001	<0.001
Average chain length	18.5 (0.3) <sup>a</sup>	18.3 (0.3) <sup>b</sup>	18.3 (0.3) <sup>b</sup>	<0.001	<0.001
C 20-22	26 (6) <sup>a</sup>	20 (7) <sup>b</sup>	21 (6) <sup>b</sup>	<0.001	<0.001
n-6/n-3 ratio	4.2 (1.2)	4.5 (2.0)	4.5 (2.1)	0.21	0.21
DHA deficiency index	0.20 (0.11) <sup>a</sup>	0.13 (0.16) <sup>b</sup>	0.11 (0.11) <sup>b</sup>	<0.001	<0.001
EFA deficiency index	1.44 (0.39) <sup>a</sup>	0.98 (0.42) <sup>b</sup>	1.02 (0.41) <sup>b</sup>	<0.001	<0.001

PE indicates preeclampsia; IUGR, intrauterine growth restriction; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UNSAT, unsaturated fatty acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; BMI, body mass index.

Values are mean (SD).

\*ANOVA was used to test for differences among groups. Different superscript letters indicate differences between individual groups using post hoc Tukey test. Significance level  $P < 0.005$ .

†Adjusted for maternal BMI, parity, smoking status, and gestational age at sampling.



### Impact of PE and IUGR on Offspring LC PUFA Status

Cord blood erythrocyte SAFA concentrations were similar between groups apart from higher levels of the minor fatty acids 14:0 and 17:0 in PE and IUGR (Table 2). There were no differences in monounsaturated fatty acid concentrations between groups. There were significantly lower 20:3n-6, 22:4n-6, and 22:5n-6 concentrations in PE and IUGR, and there was a trend toward reduced 20:4n-6. Similar to their mothers, there were significantly lower concentrations of 22:5n-3 and 22:6n-3 in PE and IUGR offspring. The lower 22:5n-3 concentration was lost on adjustment for confounders, particularly parity. Interestingly, there was a trend toward higher 18:3n-3 concentration in PE and IUGR.

Summary measures for cord blood erythrocytes are shown in Table 2. The pattern in cord blood is the same as that observed in their mothers, that is, a higher proportion of SAFAs and a reduced proportion of unsaturated fatty acids and PUFAs, driven by the lower levels of PUFAs. PE offspring had lower 22:6n-3 and were classed as being EFA deficient, whereas in IUGR offspring there was only a trend toward a deficiency in 22:6n-3 and EFAs. The study was underpowered to examine the impact of severity of PE on offspring fatty acid composition. Figure 1 shows percentage of maternal and cord blood PUFA concentrations relative to the control concentration for PE (Figure 1A) and IUGR (Figure 1B). Values <100% indicate a relative fatty acid deficiency compared with controls. IUGR and PE maternal fatty acids levels are all deficient apart from 20:5n-3. Cord blood levels of PUFAs were deficient to a lesser degree than mothers. For the majority of PUFAs, the relative deficiency in the IUGR offspring is less than that in PE, but this did not reach statistical significance because of high interindividual variability.

### Maternal Adipose Tissue as a Potential Source of LC PUFAs

There were no differences in subcutaneous or visceral adipose tissue fatty acid composition among controls, PE mothers, and IUGR mothers (please see Tables S4 and S5). The predominant fatty acids in both tissues were 16:0, 18:1n-9, and 18:2n-6. LC PUFA was a minor component of adipose tissue. There were significant differences in subcutaneous adipose tissue  $\Delta$ 5-desaturase (*FADS1*) and  $\Delta$ 6-desaturase (*FADS2*) mRNA expression between groups (Figure 2A). Subcutaneous adipose tissue *FADS1* and *FADS2* expression in PE was lower than in controls (mean [SD], control 3.38 [2.96] versus PE 1.83 [1.91] square root *FADS1* expression relative to *PPIA*,  $P=0.030$  and control 3.33 [2.25] versus PE 1.03 [0.96] square root *FADS2* expression relative to *PPIA*,  $P<0.001$  respectively). All PE subcutaneous adipose tissue samples showed no detectable expression of subcutaneous very long-chain fatty acid elongase (*ELOVL2*). There was a significantly higher proportion of detectable subcutaneous adipose tissue *ELOVL2* expression in control compared with PE ( $P=0.038$ ). There were no significant differences in *FADS1*, *FADS2*, or *ELOVL2* mRNA expression in visceral adipose tissue (Figure 2B). Stearoyl CoA desaturase and long-chain fatty acid elongase

(*ELOVL6*) mRNA expression did not differ among groups in either subcutaneous or visceral adipose tissues.

### Placental Fatty Acid Metabolism and Transport Markers

To confirm whether low cord levels of 20:3n-6 in the presence of normal levels of 18:2n-6 was indicative of low  $\Delta$ 6-desaturase activity in PE and IUGR offspring,  $\Delta$ 6-desaturase (*FADS2*) mRNA expression in a fetal tissue (placenta) was quantitated and found not to be different among groups (Figure 2C). To test whether lower offspring LC PUFA levels might be attributed to lower placental expression of fatty acid transfer proteins, mRNA expression of placental fatty acid transporters (pFABPpm [*GOT2*] and *FABP7*) was assessed and found not to differ among groups (Figure 2C). The lack of difference in placental gene expression was independent of the mode of delivery.

### Discussion

Absolute amounts of maternal erythrocyte n-6 and n-3 PUFA concentrations were  $\approx 60\%$  lower in PE and IUGR compared with controls, and cord blood LC PUFA deficiency is also common to PE and IUGR. Low amounts of maternal LC PUFAs could result from inhibition of synthesis, decreased release from maternal stores, or reduced acquisition from diet.

In PE, the metabolic pattern is of high BMI and high plasma triglyceride and NEFA concentrations. Although we could not assess maternal insulin resistance because of the random nature of maternal blood samples, second-trimester insulin resistance has previously been shown to be associated with PE.<sup>26</sup> The complete biochemical profile of PE women observed here is indicative of metabolic syndrome, a biochemical manifestation of insulin resistance that is typical of PE.<sup>27</sup> The observed reduced adipose tissue  $\Delta$ 5- and  $\Delta$ 6-desaturase and *ELOVL2* expression (please see Figure S2 in the online-only Data Supplement) suggests that low LC PUFAs in PE could be attributable to decreased synthesis. We have previously hypothesized that increased NEFA flux in pregnancy can lead to mitochondrial dysfunction,<sup>2</sup> thus impairing LC PUFA synthesis, analogous to the situation in nonalcoholic fatty liver disease, which is associated with obesity, insulin resistance, and ectopic lipid accumulation in the liver. In nonalcoholic fatty liver disease, LC PUFAs in liver are depleted possibly via reduced  $\Delta$ 5- and  $\Delta$ 6-desaturase activities.<sup>3</sup> It was notable that the magnitude of reduction in LC PUFAs was greater in women with severe PE.

Mothers with IUGR are reported to have low lipolysis rates<sup>4</sup> and low BMI.<sup>28</sup> In IUGR, we observed trends toward lower maternal erythrocyte concentrations of the major fatty acids stored in adipose tissue, 18:1n-9 and 16:0,<sup>29</sup> which are consistent with lower adipocyte lipolysis. The metabolic pattern in IUGR mothers thus may indicate a primary defect in fat storage and mobilization from adipose tissue (please see Figure S2). IUGR cord leptin levels are extremely low, reflecting reduced adipose tissue depots; however, the level of cord triglyceride is high and the concentration of NEFA is normal, suggesting that, although maternal fatty acid supply may be abnormal, there is still fetal capacity for triglyceride synthesis.

**Table 2. Cord Erythrocyte Fatty Acid Concentrations (nmol/mL of Blood) From Control, PE, and IUGR Offspring**

Fatty Acid	Control (n=85)	PE (n=21)	IUGR (n=13)	P*	Adjusted P†
<b>SAFA</b>					
12:0	0 (0)	0 (0)	0 (0)	-	-
14:0	8 (4) <sup>a</sup>	11 (5) <sup>b</sup>	12 (5) <sup>b</sup>	0.001	0.044
16:0	429 (99)	446 (78)	451 (90)	0.60	0.81
17:0	3 (2) <sup>a</sup>	4 (2) <sup>a,b</sup>	5 (1) <sup>b</sup>	<0.001	0.045
18:0	275 (59)	265 (43)	272 (35)	0.74	0.86
20:0	8 (3)	8 (3)	7 (2)	0.20	0.18
22:0	21 (7)	19 (4)	17 (5)	0.08	0.044
24:0	62 (14)	59 (9)	57 (9)	0.31	0.078
<b>MUFA</b>					
14:1n-7	1.0 (3.8)	1.0 (2.2)	1.5 (3.5)	0.87	0.82
16:1n-7	10 (6)	10 (5)	8 (2)	0.34	0.53
17:1n-7	29 (24)	38 (21)	45 (16)	0.023	0.14
18:1n-7	14 (16)	9 (14)	5 (13)	0.13	0.47
18:1n-9	171 (54)	168 (38)	163 (33)	0.88	0.82
20:1n-9	1.5 (1.6)	2.4 (2.6)	2.3 (1.2)	0.087	0.85
22:1n-9	0.2 (1.1)	0 (0)	0 (0)	0.71	0.38
24:1n-9	57 (16)	57 (15)	56 (11)	0.97	0.73
<b>PUFA n-6</b>					
18:2n-6	57 (18)	55(16)	57 (17)	0.91	0.67
18:3n-6	0.4 (1.1)	0.6 (0.8)	0.9 (1.2)	0.22	0.26
20:2n-6	5 (6)	4 (6)	5 (4)	0.76	0.094
20:3n-6	41 (16) <sup>a</sup>	30 (15) <sup>b</sup>	27 (11) <sup>b</sup>	0.002	0.016
20:4n-6	239 (90)	176 (90)	193 (66)	0.007	0.008
22:2n-6	1.9 (3.6)	0.4 (1.9)	0 (0)	0.037	0.055
22:4n-6	48 (19) <sup>a</sup>	33 (18) <sup>b</sup>	37 (13) <sup>a,b</sup>	0.002	0.016
22:5n-6	18 (8) <sup>a</sup>	10 (7) <sup>b</sup>	12 (5) <sup>b</sup>	<0.001	0.006
<b>PUFA n-3</b>					
18:3n-3	0.08 (0.3)	0.39 (0.8)	0.37 (0.6)	0.011	0.087
20:5n-3	6.1 (7)	5.0 (8)	2.6 (4)	0.22	0.52
22:3n-3	0.1 (0.4)	0 (0)	0 (0)	0.67	0.16
22:5n-3	11 (5) <sup>a</sup>	7 (6) <sup>b</sup>	8 (5) <sup>a,b</sup>	0.004	0.11
22:6n-3	73 (33) <sup>a</sup>	49 (31) <sup>b</sup>	53 (29) <sup>a,b</sup>	0.003	0.03
<b>Summary Indices</b>					
% SAFA	51 (7)	56 (10)	55 (9)	0.007	0.007
% MUFA	18 (2) <sup>a</sup>	19 (4) <sup>b</sup>	19 (2) <sup>a,b</sup>	0.002	0.094
% PUFA	31 (7) <sup>a</sup>	24 (9) <sup>b</sup>	26 (9) <sup>a,b</sup>	<0.001	0.002
% UNSAT	49 (7)	44 (10)	45 (9)	0.007	0.007
Unsaturation index	141 (29) <sup>a</sup>	114 (36) <sup>b</sup>	122 (35) <sup>a,b</sup>	<0.001	0.003
Average chain length	18.7 (0.3) <sup>a</sup>	18.4 (0.3) <sup>b</sup>	18.4 (0.3) <sup>b</sup>	<0.001	<0.001
C 20-22	33 (6.2) <sup>a</sup>	27 (6.4) <sup>b</sup>	28 (6.9) <sup>b</sup>	<0.001	<0.001
n-6/n-3 ratio	4.8 (1.2)	5.1 (2.1)	5.1 (2.0)	0.63	0.98
DHA deficiency index	0.36 (0.10)	0.30 (0.12)	0.32 (0.08)	0.019	0.13
EFA deficiency index	1.84 (0.53) <sup>a</sup>	1.33 (0.55) <sup>b</sup>	1.48 (0.39) <sup>a,b</sup>	<0.001	0.001

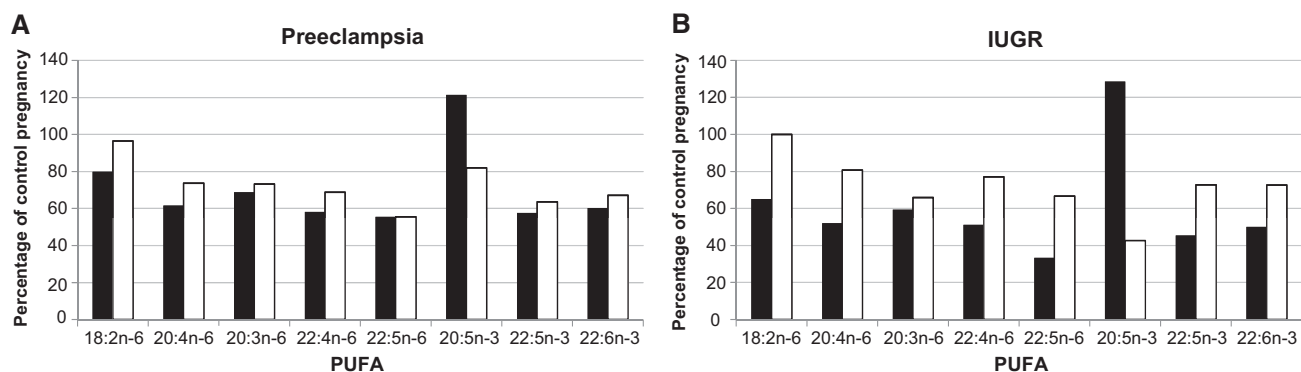
PE indicates preeclampsia; IUGR, intrauterine growth restriction; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UNSAT, unsaturated fatty acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; BMI, body mass index.

Values are mean (SD).

\*ANOVA was used to test for differences among groups. Different superscript letters indicate differences between individual groups using post hoc Tukey test.

Significance level  $P < 0.005$ .

†Adjusted for maternal BMI, parity, smoking status, and gestational age at delivery.



**Figure 1.** Maternal (■) and cord (□) plasma polyunsaturated fatty acid (PUFA) concentration expressed as a percentage of control maternal and cord fatty acid concentration, respectively, in pregnancies complicated by (A) preeclampsia and (B) intrauterine growth restriction (IUGR).

The ratio of the n-6: n-3 series remains similar among groups, suggesting that both pathways are affected equally. It is notable that PE and IUGR mothers are not deficient in 20:5n-3, which suggests that it can be synthesized from 18:3n-3. Elongation or desaturation pathways downstream of this point may be impaired (please see Figures S1 and S2). We observed that subcutaneous adipose tissue mRNA expression of  $\Delta 5$ - and  $\Delta 6$ -desaturase and very long-chain fatty acid elongase (*ELOVL2*, which acts on C20 and C22 fatty acids) was significantly lower in PE mothers, suggesting compromised synthesis downstream of C20. Adipose tissue is not a major site of LC PUFA synthesis, but expression here may reflect the expression of synthetic enzymes in inaccessible tissues, for example, the liver.

It is not known from which maternal stores LC PUFAs are mobilized in pregnancy. Potential sites are adipose tissue, liver, and membranes (including the brain). As in the nonpregnant state,<sup>29</sup> maternal adipose tissue contains reasonable quantities of 18:2n-6, but very little 18:3n-3 and minor amounts of LC PUFAs. There were also no differences in adipose tissue fatty acid composition between groups that could explain the large difference in maternal erythrocyte LC PUFA concentrations. Together, these data suggest that maternal adipose tissue does not act as a short-term store or site of synthesis of PUFAs. It is possible that maternal membrane (brain) and liver LC PUFA stores are affected in PE and IUGR, especially if synthetic pathways are impeded.

Differing diet between PE and IUGR mothers and controls is another potential explanation for reduced PUFAs. The extent of deficiency ( $\leq 60\%$ ) would suggest that diets would have to be substantially different to have that magnitude of effect on erythrocyte PUFA concentrations. Vegan mothers are not specifically susceptible to PE or IUGR<sup>30,31</sup> nor do they demonstrate a similar degree of EFA deficiency.<sup>32</sup> In our study, the ratio of 22:5n-6/22:6n-3, a measure of dietary  $\omega$ -3 or 22:6n-3 deficiency,<sup>33</sup> was not significantly different between PE cases and controls, further suggesting that diet alone is not the cause of the fatty acid changes. There is also no evidence that dietary supplementation with LC PUFAs impacts on incidence of PE or IUGR.<sup>34,35</sup>

The fetus is dependent on the mother for its EFA supply; therefore, it is not surprising that we observed reduced LC PUFAs in PE and IUGR offspring. The degree to which the

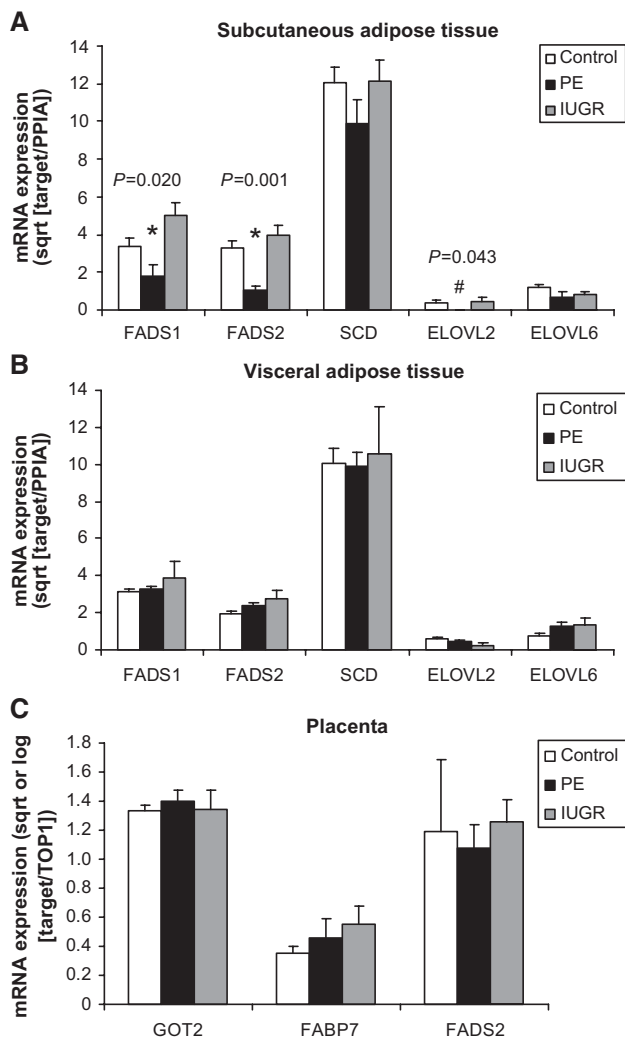
infants are deficient is less than that expected from the lack of mobilization in the mothers (Figure 1). Docosahexaenoic acid is an extremely important fatty acid for fetal neural development, and PE cord blood erythrocytes have a lesser degree of docosahexaenoic acid deficiency than EFA deficiency (Table 2). This suggests that there are mechanisms to compensate, such as upregulation of placental transport or fetal synthesis. There was no difference in placental expression of pFABPpm (*GOT2*) and FABP7 between groups. However, there is a wide array of placental fatty acid transport proteins,<sup>16</sup> and a more systematic analysis is required to eliminate the possibility of upregulation of placental fatty acid transport. There was a trend toward increased levels of 18:3n-3, the precursor of n-3 PUFAs in cord erythrocytes for PE and IUGR. However, placental  $\Delta 6$ -desaturase mRNA expression did not differ. Thus, there is no evidence for upregulation of placental or fetal fatty acid synthesis pathways. Both PE and IUGR offspring had significantly lower cord blood adiponectin levels than controls (please see Table S2), which may result from reduced n-3 LC PUFA-induced adiponectin release from adipocytes.<sup>36,37</sup>

The strength of our data is the use of absolute fatty acid concentrations, which allows us to understand which specific fatty acids are driving the differences in composition between healthy and complicated pregnancies. There are limitations to our study. Blood samples were random, and women were not all fasted, which will have an impact on some maternal variables (triglyceride and NEFA levels) but not erythrocyte fatty acid composition. No samples from prepregnancy or early gestation were available for these women. There were few IUGR subjects; the PE group was a mixture of primiparous and parous women with potentially different underlying risk factors, and there were no dietary intake data.

## Perspectives

At birth, the offspring of PE and IUGR pregnancies are deficient in essential LC PUFAs, which may have long-term consequences for their development. The mechanisms by which these deficiencies arise differ between PE and IUGR and suggest different interventions. In IUGR, LC PUFA supplementation may overcome the lack of maternal fatty acid mobilization. In PE, insulin-sensitizing treatments such as metformin may reduce ectopic fat accumulation and upregulate LC PUFA synthetic pathways.





**Figure 2.** **A**, Maternal subcutaneous adipose tissue gene expression in control (n=50), preeclampsia (PE) (n=12), and intrauterine growth restriction (IUGR) (n=13). **B**, Maternal visceral adipose tissue gene expression in control (n=25), PE (n=12), and IUGR (n=5). **C**, Placental gene expression in control (n=57), PE (n=17), and IUGR (n=11) pregnancy relative to control gene (*PPIA* for adipose tissue and *TOP1* for placenta). Means (SEs) of transformed [ $\log$ [*GOT2* and *FADS2*] or square root [*sqrt*]] target gene expression relative to control gene expression values are presented. \*Significant difference among groups on ANOVA; #Significant difference among groups on Kruskal–Wallis test.

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### Disclosures

None.

### References

- Sattar N, Gaw A, Packard CJ, Greer IA. Potential pathogenic roles of aberrant lipoprotein and fatty acid metabolism in pre-eclampsia. *Br J Obstet Gynaecol.* 1996;103:614–620.
- Jarvie E, Hauguel-de-Mouzon S, Nelson SM, Sattar N, Catalano PM, Freeman DJ. Lipotoxicity in obese pregnancy and its potential role in adverse pregnancy outcome and obesity in the offspring. *Clin Sci (Lond).* 2010;119:123–129.
- Videla LA, Rodrigo R, Araya J, Poniachik J. Oxidative stress and depletion of hepatic long-chain polyunsaturated fatty acids may contribute to non-alcoholic fatty liver disease. *Free Radic Biol Med.* 2004;37:1499–1507.
- Diderholm B, Stridsberg M, Nordén-Lindeberg S, Gustafsson J. Decreased maternal lipolysis in intrauterine growth restriction in the third trimester. *BJOG.* 2006;113:159–164.
- Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Divergent metabolic and vascular phenotypes in pre-eclampsia and intrauterine growth restriction: relevance of adiposity. *J Hypertens.* 2004;22:2177–2183.
- Cetin I, Giovannini N, Alvino G, Agostoni C, Riva E, Giovannini M, Pardi G. Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatr Res.* 2002;52:750–755.
- Mahomed K, Williams MA, King IB, Mudzamiri S, Woelk GB. Erythrocyte omega-3, omega-6 and trans fatty acids in relation to risk of preeclampsia among women delivering at Harare Maternity Hospital, Zimbabwe. *Physiol Res.* 2007;56:37–50.
- Ogburn PL Jr, Williams PP, Johnson SB, Holman RT. Serum arachidonic acid levels in normal and preeclamptic pregnancies. *Am J Obstet Gynecol.* 1984;148:5–9.
- Ortega-Senovilla H, Alvino G, Taricco E, Cetin I, Herrera E. Enhanced circulating retinol and non-esterified fatty acids in pregnancies complicated with intrauterine growth restriction. *Clin Sci.* 2010;118:351–358.
- Arab L. Biomarkers of fat and fatty acid intake. *J Nutr.* 2003;133:925S–932S.
- Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. *Am J Clin Nutr.* 2000;71(1 suppl):307S–311S.
- Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr.* 1995;74:55–68.
- Cetin I, Koletzko B. Long-chain omega-3 fatty acid supply in pregnancy and lactation. *Curr Opin Clin Nutr Metab Care.* 2008;11:297–302.
- Stewart F, Rodie VA, Ramsay JE, Greer IA, Freeman DJ, Meyer BJ. Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum. *Lipids.* 2007;42:335–344.
- Huda SS SN, Freeman DJ. Lipoprotein metabolism and vascular complications in pregnancy. *Clin Lipidol.* 2009;4:91–102.
- Cunningham P, McDermott L. Long chain PUFA transport in human term placenta. *J Nutr.* 2009;139:636–639.
- Campbell FM, Clohessy AM, Gordon MJ, Page KR, Dutta-Roy AK. Uptake of long chain fatty acids by human placental choriocarcinoma (BeWo) cells: role of plasma membrane fatty acid-binding protein. *J Lipid Res.* 1997;38:2558–2568.
- Larqué E, Krauss-Etschmann S, Campoy C, Hartl D, Linde J, Klingler M, Demmelmair H, Caño A, Gil A, Bondy B, Koletzko B. Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. *Am J Clin Nutr.* 2006;84:853–861.
- Xu LZ, Sánchez R, Sali A, Heintz N. Ligand specificity of brain lipid-binding protein. *J Biol Chem.* 1996;271:24711–24719.
- Lipid Research Clinics Program. *Lipid and Lipoprotein Analysis. Manual of Laboratory Operations.* Vol. 1. Bethesda, MD: Department of Health, Education and Welfare, National Institutes of Health; 1974:Publication No. 75–628.
- Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphie CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. *N Engl J Med.* 2000;343:1148–1155.

22. Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes*. 2002;51:2684–2690.
23. Catalano PM, Presley L, Miniium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care*. 2009;32:1076–1080.
24. Pardo IM, Geloneze B, Tambascia MA, Barros AA. Inverse relationship between cord blood adiponectin concentrations and the number of cigarettes smoked during pregnancy. *Diabetes Obes Metab*. 2005;7:144–147.
25. Stoll-Becker S, Kreuder J, Reiss I, Etspüler J, Blum WF, Gortner L. Influence of gestational age and intrauterine growth on leptin concentrations in venous cord blood of human newborns. *Klin Padiatr*. 2003;215:3–8.
26. Hautz JC, Clifton RG, Roberts JM, Myatt L, Spong CY, Leveno KJ, Varner MW, Wapner RJ, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Samuels P, Sciscione A, Tolosa JE, Saade G, Sorokin Y, Anderson GD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Maternal insulin resistance and preeclampsia. *Am J Obstet Gynecol*. 2011;204:327.e1–327.e6.
27. Rodie VA, Freeman DJ, Sattar N, Greer IA. Pre-eclampsia and cardiovascular disease: metabolic syndrome of pregnancy? *Atherosclerosis*. 2004;175:189–202.
28. Ehrenberg HM, Dierker L, Milluzzi C, Mercer BM. Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol*. 2003;189:1726–1730.
29. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res*. 2008;47:348–380.
30. Carter JP, Furman T, Hutcheson HR. Preeclampsia and reproductive performance in a community of vegans. *South Med J*. 1987;80:692–697.
31. Fikree FF, Berendes HW, Midhet F, D'Souza RM, Hussain R. Risk factors for intrauterine growth retardation: results of a community-based study from Karachi. *J Pak Med Assoc*. 1994;44:30–34.
32. Lakin V, Haggarty P, Abramovich DR, Ashton J, Moffat CF, McNeill G, Danielian PJ, Grubb D. Dietary intake and tissue concentration of fatty acids in omnivore, vegetarian and diabetic pregnancy. *Prostaglandins Leukot Essent Fatty Acids*. 1998;59:209–220.
33. Fokkema MR, Smit EN, Martini IA, Woltij HA, Boersma ER, Muskiet FA. Assessment of essential fatty acid and omega3-fatty acid status by measurement of erythrocyte 20:3omega9 (Mead acid), 22:5omega6/20:4omega6 and 22:5omega6/22:6omega3. *Prostaglandins Leukot Essent Fatty Acids*. 2002;67:345–356.
34. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. *Am J Epidemiol*. 2004;160:774–783.
35. Szajewska H, Horvath A, Koletzko B. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2006;83:1337–1344.
36. Flachs P, Mohamed-Ali V, Horakova O, Rossmeisl M, Hosseinzadeh-Attar MJ, Hensler M, Ruzickova J, Kopecky J. Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia*. 2006;49:394–397.
37. Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra GD, Moore CS, Jebb SA. Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. *Int J Obes (Lond)*. 2006;30:1535–1544.

## Novelty and Significance

### What Is New?

- This study shows that levels of a class of fats that are particularly important for fetal brain development (long-chain polyunsaturated fatty acids) are lower in maternal and cord blood in pregnancies complicated by preeclampsia and intrauterine growth restriction. We provide evidence that in preeclampsia this might be attributable to a decreased ability of the mother to make these fats from dietary precursors.

### What Is Relevant?

- The findings are important because they indicate that offspring of pregnancies complicated by preeclampsia or intrauterine growth restriction

may be at risk of impaired neural development and suggest that the approaches to reducing this risk would differ. In preeclampsia, drug interventions that may improve the mother's metabolism and ability to make long-chain polyunsaturated fatty acids are indicated, whereas in intrauterine growth restriction, supplementation of the diet with long-chain polyunsaturated fatty acids would be indicated.

### Summary

Low maternal and offspring levels of fats important for offspring neural development in preeclampsia may be attributable to an impaired ability of the mother to synthesize them.