

Maternal plasma DHA levels increase prior to 29 days post-LH surge in women undergoing frozen embryo transfer: a prospective, observational study of human pregnancy

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Context: Docosahexaenoic acid (DHA) is an important fatty acid required for neurological development but its importance during early fetal neurological organogenesis is unknown.

Objective: To assess plasma fatty acid changes in early pregnancy in women undergoing natural cycle-frozen embryo transfer as a means of achieving accurately-timed periconceptual sampling.

Design: Women undergoing frozen embryo transfer were recruited and serial fasting blood samples were taken pre-luteinising hormone (LH) surge, and at days 18, 29 and 45 post-LH surge and fatty acids were analysed using gas chromatography.

Setting: Assisted Conception Unit, Glasgow Royal Infirmary, Scotland

Main outcome measures: Plasma fatty acid concentrations, influence of twin pregnancies on DHA plasma concentration.

Results: In pregnant women, there was a rapid, early increase in the maternal rate of change of plasma DHA concentration observed by 29 days post-LH surge (mean \pm SD, from 0.1 ± 1.3 to 1.6 ± 2.9 nmol DHA per mL plasma per day). This early pressure to increase plasma DHA concentration was further emphasised in twin pregnancies where the increase in DHA concentration over 45 days was two-fold higher than in singleton pregnancies (mean \pm SD increase, 74 ± 39 nmol/mL versus 36 ± 40 nmol/mL). An index of delta-6 desaturase activity increased 30% and positively correlated with the rate of change of DHA concentration between day 18 and 29-post LH surge (R-squared adjusted = 41%, $P=0.0002$). DHA was the only fatty acid with a continual accelerated increase in plasma concentration and a positive incremental area under the curve (mean \pm SD, 632 ± 911 nmol/mL x day) over the first 45 days of gestation.

Conclusions: An increase in maternal plasma DHA concentration is initiated in human pregnancy prior to neural tube closure which occurs at 28 days' gestation.

The long chain polyunsaturated fatty acids (LC PUFA), docosahexaenoic acid (DHA) and arachidonic acid (AA), are important in neurological development as these lipids are key membrane components of nervous tissues (1, 2). The fetal-placental circulation is established around 9–13 weeks of gestation, after which the growing fetus preferentially acquires DHA and AA from maternal plasma via placental transport (3, 4). In contrast, the essential dietary fatty acids linoleic acid (LA) and α -linolenic acid (ALA), which are the precursors of LC PUFA, are at best minimally transferred to the fetus from maternal plasma (3, 5). We have previously shown that maternal erythrocyte DHA concentrations (an indicator of the previous 3 months' change in concentration) increased 17% by the end of the first trimester and continued to significantly increase throughout pregnancy to 26% above postpartum (nonpregnant) concentrations by the third trimester (6). AA concentrations did not change but the concentration of nervonic acid important in myelination, increased by 5% and 22% by the end of the first and third trimester respectively (6). Thus plasma concentrations of fatty acids essential for brain development are increased by the end of the first trimester and are available for placental transport to the fetus.

The sources of LC PUFA for this increase in plasma concentration or “mobilization” in pregnant women have not been fully identified and could include ingestion in maternal diet, maternal synthesis from shorter chain precursors or release from maternal membrane stores and adipose tissue. Our previous study suggested that ALA, but not LA, is mobilized for LC PUFA synthesis in pregnancy (6). Humans possess the necessary enzymes to metabolize these two precursor fatty acids to LC PUFA (Supplemental Figure 1). Healthy, nonpregnant individuals can convert LA to AA by desaturation (δ -6 desaturase/*FADS2*), elongation (*ELOVL5*) and further desaturation (δ -5 desaturase/*FADS1*) in the liver (7). These same enzymes, in parallel, also convert ALA to eicosapentaenoic acid (EPA) and ultimately DHA but these conversions, particularly to DHA, are limited. Interestingly this pathway is more efficient in women than in men suggesting gender-specific roles for this pathway (8). The dominance of the omega-6 LC PUFA pathway can be explained by the competition of LA and ALA for the same δ -6 desaturase enzyme, given that dietary LA intake is 8 fold higher than that of ALA (9).

The critical requirement of DHA for fetal brain development, and the poor efficiency of its synthesis in humans, is therefore a metabolic problem to be overcome in pregnancy. Furthermore, as the first primitive brain cells are formed by day 15 of gestation, neural tube closure occurs by 28 days' gestation and the head of the embryo grows

fastest in the early gestational weeks, we hypothesized that changes in maternal plasma LC PUFA concentration prior to 13 weeks' gestation are vitally important. Unfortunately the direct assessment of maternal fatty acid changes from prepregnancy to the first weeks of gestation is difficult in a free living population, and there are no data on these earliest changes. Our aim therefore was to assess plasma fatty acid changes in very early pregnancy in a population of women undergoing natural cycle-frozen embryo transfer as a means of achieving accurately timed peri-conceptual sampling.

Materials and Methods

Study design, setting and participants

This prospective, observational study was conducted at Glasgow Royal Infirmary and was approved by the Local Research Ethics Committee (07/S0704/49). Women undergoing frozen embryo transfer (FET) treatment for infertility were recruited from the Assisted Conception Unit (ACU) between October 2007 and April 2010. Women were eligible for the study if they had a regular menstrual cycle. Patients that had ovulation stimulation or induction were excluded. No progesterone supplementation or other hormonal supplements were given. All women were recommended to take 400 μ g per day of folic acid in line with World Health Organisation guidelines. Women were informed of the study when notifying the clinic nurse of their last menstrual period (LMP) date with a view to booking FET treatment. At day 10 after LMP (preluteinising hormone (LH) surge), the women attended the ACU to commence daily hormonal sampling to detect the LH surge and time embryo replacement. At this point women provided written informed consent and a basal blood sample. Embryo transfer was performed on day 3 post-LH surge. Information on patient demographics and fertility history was collected from patient notes. Patient height and weight data were collected at the pre-LH surge visit. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Scottish Index of Multiple Deprivation (SIMD) quintile (10) was derived from patient postcode.

Blood sampling

Fasting blood samples were collected approximately day 10 after LMP (pre-LH surge) and on days 18, 29 and 45 post-LH surge. Plasma was collected by low speed centrifugation and frozen at -80°C within 2 hours. At day 18 post-LH surge the women who were not successful in getting pregnant withdrew from the study and therefore did not provide any blood samples at days 29 and 45 post-LH surge.

Plasma analysis

Fatty acids were extracted from plasma and analyzed as described in detail in the Supplemental Methods.

Statistical analyses

Data were tested for normal distribution using a Ryan Joiner test and log or square root transformed when necessary. Values for continuous biochemical variables are given as mean \pm stan-

standard deviation (SD) or number (%) for categorical variables. The rate of change of fatty acids between time points was calculated as the difference between the fatty acid concentration at the second time point and at the first time point divided by the difference in time (days) between the two time points. The results are expressed as nmol/mL plasma per day. Incremental area under the concentration (pre-LH surge to 45 days post-LH surge) x time curve (iAUC) were calculated using the trapezium method with correction for pre-LH surge parameter level (11). The results are expressed as nmol/mL x day. Differences in concentrations of fatty acids over time were assessed by one way ANOVA for repeated measures, with between group comparison using post hoc Tukey-Kramer HSD. Simultaneous difference testing between twin and singleton pregnancies across time points was carried out using a univariate split-plot approach repeated measures analysis with post hoc ANOVA for specific time/twin singleton pregnancy differences. Associations between variables were examined using univariate regression analysis. Prediction of successful pregnancy outcome at day 45 post-LH surge using 18 day post-LH surge data was assessed by univariate and multivariable logistic regression. Stepwise logistic regression was used to select variables to include in multivariable models with the significant levels to enter and stay in model set at $P < .05$. Results are reported as odds ratio (OR) [95% confidence interval (CI)], associated P-value and the C-statistic for the area under the curve (AUC). Odds ratios represent an increase of 1 U unless

otherwise stated for continuous variables. Statistical analyses were performed using the JMP statistical analysis program (Version 9.02, SAS Institute 2010, Cary, NC, USA) or SAS (Version 9.2, SAS Institute, Cary, NC, USA) and statistical significance was set at $\alpha \leq 0.05$ for all analyses.

Results

Study population

A total of 196 FET cycles were started in the study, of which 161 were completed, from which there were 38 pregnancies: two were excluded as being a repeat attempt at FET treatment within the study and nine were excluded as they did not have complete data for all visits; therefore 27 pregnant women were included. From the 161 FET cycles completed, there were 123 failed pregnancies, of which 35 had full fatty acid data (Supplemental Figure 2).

Table 1 shows the demographic characteristics of the 27 pregnant women and 35 women not successful in getting pregnant and there were no significant differences between the two groups of women, except that use of ICSI

Table 1. Demographic characteristics of study subjects. Values are mean and standard deviation (sd) for continuous variables or number (%) for categorical variables.

	Pregnant women (n = 27)	Not successful in getting pregnant (n = 35)	P
Age at FET (years)	34.7 (3.9)	33.5 (5.2)	0.33
Smokers (number [%])	3 (11)	8 (23)	0.25
SIMD quintiles (number [%])			
Affluent; Q1 & Q2	10 (37)	18 (52)	0.32
Intermediate; Q3	6 (22)	5 (14)	
Deprived; Q4 & Q5	11 (41)	12 (34)	
Number of previous pregnancies of ≥ 24 weeks' gestation (number [%])			
0, 1, 2	20 (74), 4 (15), 3 (11)	24 (71), 8 (24), 2 (6)*	0.57
Number of previous pregnancies of <24 weeks' gestation (number [%])			
0, 1, 2	26 (96), 1 (4), 0 (0)	33 (97), 0 (0), 1 (3)*	0.25
BMI (kg/m ²)	26.2 (3.8)	26.4 (5.1)	0.88
Systolic Blood Pressure (mmHg)	116 (15)	117 (12)	0.66
Diastolic Blood Pressure (mmHg)	65 (7)	67 (9)	0.38
Reason for Treatment			
Chem; Endo; Gen; Male;	0 (0); 3 (11); 0 (0); 15 (56);	1 (3); 2 (6); 1 (3); 14 (40); 0 (0)	
PA; PGD; Tubal; Unexplained	1 (4); 1 (4); 3 (11); 3 (11)*	0 (0); 13 (37); 4 (11)	0.11
Treatment			
IVF; ICSI	8 (30); 19 (70)	19 (58); 14 (42)*	0.03
Number of embryos transferred			
1, 2, 3	4 (15), 22 (81), 1 (4)	4 (12), 30 (88), 0 (0)*	0.40
Twin pregnancy according to no. embryos transferred (number (%))			
1, 2, 3	0 (0), 7 (32), 0 (0)	N/A	

BMI – Body Mass Index; Chem - Chemotherapy; Endo - Endometriosis; FET - frozen embryo transfer; Gen - Genetic; ICSI intra-cytoplasmic sperm injection; IVF – in vitro fertilisation; Male - Male infertility; N/A – not applicable; PA - Pelvic adhesion; PGD – preimplantation genetic diagnosis; Q – quintile; SIMD - Scottish Index of Multiple Deprivation.

* missing data points

was significantly more prevalent in the women who became pregnant.

Plasma fatty acid concentrations over the first 45 days post-LH surge

The baseline prepregnancy sample was taken at the time of sampling to identify the LH surge prior to the women receiving FET. The mean time of this sample was 3 days prior to the LH-surge with a standard deviation of 3 and a range of minus 12 to plus 1 day relative to the LH surge. Plasma fatty acid concentrations over time are shown in Table 2. Palmitic, stearic and oleic acids concentrations were significantly reduced by 12%–15% by day 18 post-LH surge but their concentrations returned back to pre-LH surge levels by day 45 post-LH surge. Lignoceric acid concentration was significantly decreased by 10% by day 29 and was sustained at day 45 post-LH surge. There was no change in AA but there was a trend for a 10% reduction in LA concentration by day 18 post-LH surge which was maintained at days 29 and 45 post-LH surge. γ -linolenic acid concentration was significantly decreased by 29% by day 18 post-LH surge and remained at that concentration at day 29 post-LH surge. Dihomo- γ -linolenic acid, adrenic acid and omega-6 docosapentaenoic acid (DPA) concentrations were significantly increased by 19%, 36% and 71% respectively. There were no changes in plasma ALA and omega-3 DPA with time, but EPA concentration decreased by 29% by day 45 post-LH surge, although the post hoc analysis did not result in significance. DHA concentration was significantly increased by 31% by day 45 post-LH surge.

The ratio of dihomom- γ -linolenic acid to LA is indicative of δ -6 desaturase activity. The ratio of plasma dihomom- γ -linolenic acid to LA concentration (mean \pm SD) was 0.054 (0.012), 0.058 (0.011), 0.060 (0.0113) and 0.070 (0.016) for pre-LH surge and days 18, 29 and 45 post-LH surge respectively. The increase in δ -6 desaturase was significant by day 45 post-LH surge ($P = .0002$). In the women who were not successful in getting pregnant, there were no significant changes in plasma fatty acids from pre-LH surge to day 18 post-LH surge.

Rate of change of plasma fatty acid concentrations and incremental AUC over the first 45 days post-LH surge

The only plasma fatty acid with a continual increasing rate of change in concentration (Figure 1A) and a positive iAUC (Figure 1B) was DHA. The greatest rate of change in DHA was from day 18–29 post-LH surge, when the rate of change of DHA concentration correlated significantly with an index of δ -6 desaturase activity; R-squared adjusted = 41%, $P = .0002$ (Supplemental Figure 3). Outside this time window, no such correlations were present (pre-LH surge to day 18; R-squared adjusted = -4%, $P = .29$; day 29 to 45 post-LH surge; R-squared adjusted = 0.1%, $P = .32$). The other omega-3 PUFA all had stable or decelerated rates of change in plasma concentration. ALA had a negative iAUC (Figure 1B) which could result from increased removal from the plasma compartment by being converted to EPA, DPA and DHA. There was an initial decrease in the rate of change of plasma AA concentrations from pre-LH surge to day 18 post-LH surge which then

Table 2. Plasma fatty acid concentrations from pre-LH surge to 45 days post-LH surge in pregnant women (Preg, $n = 27$) and women who were unsuccessful in getting pregnant (NP, $n = 35$). Means (standard deviation) are shown. Differences between concentrations were tested using one way Analysis of Variance for repeated measures, across sampling from pre-LH surge (pre-pregnancy) to 18, 29 and 45 days post-LH surge and P values are given. NP women withdrew from the study after urine pregnancy testing at day 18 post-LH surge and therefore 29 and 45 days post-LH surge samples are not available (ns) for these women. Different superscript letters indicating differences between individual groups using *post hoc* Tukey-Kramer at significance level $P < 0.05$.

Fatty Acid* (nmol/mL)	Pre-LHS Preg	18 day post-LHS Preg	29 day post-LHS Preg	45 day post-LHS Preg	P Preg	Pre-LHS NP	18 day post-LHS NP	P NP		
SAFA	16:0 palmitic	2092 (346) ^{a,b}	1833 (333) ^a	1952 (394) ^{a,b}	2201 (453) ^b	0.004	1997 (437)	1998 (474)	0.92	
	18:0 stearic	620 (77) ^a	545 (87) ^b	552 (88) ^b	589 (99) ^{a,b}	0.0064	584 (89)	593 (102)	0.72	
	24:0 lignoceric	40 (6) ^a	38 (5) ^{a,b}	36 (4) ^b	36 (6) ^b	0.005	42 (7)	40 (6)	0.19	
MUFA	16:1n-7 palmitoleic	202 (79)	181 (86)	183 (69)	191 (76)	0.78	200 (104)	187 (89)	0.77	
	18:1n-9 oleic	1770 (338) ^a	1512 (377) ^b	1623 (411) ^{a,b}	1843 (467) ^a	0.010	1707 (460)	1787 (470)	0.48	
	24:1n-9 nervonic	88 (11)	88 (12)	85 (16)	92 (18)	0.26	93 (15)	94 (20)	0.89	
PUFA	18:2n-6 linoleic	2569 (384)	2297 (422)	2353 (382)	2334 (420)	0.056	2533 (353)	2495 (367)	0.60	
	n-6	18:3n-6 γ -linolenic	45 (18) ^a	32 (13) ^b	32 (16) ^b	34 (12) ^{a,b}	0.0067	42 (21)	42 (23)	0.98
		20:3n-6 dihomom- γ -linolenic	137 (31) ^a	130 (29) ^a	142 (36) ^{a,b}	163 (46) ^b	0.008	137 (33)	140 (33)	0.70
	20:4n-6 arachidonic	572 (108)	546 (103)	573 (134)	627 (154)	0.16	549 (106)	535 (118)	0.53	
	22:4 n-6 adrenic	12 (5) ^{a,b}	11 (4) ^b	12 (5) ^{a,b}	15 (5) ^a	0.016	12 (4)	11 (5)	0.67	
	22:5n-6 docosapentaenoic	7 (4) ^a	7 (4) ^a	9 (4) ^{a,b}	12 (5) ^b	0.0005	6 (4)	7 (5)	0.60	
PUFA	18:3n-3 α -linolenic	68 (27)	57 (24)	57 (21)	68 (31)	0.26	66 (27)	70 (27)	0.52	
	n-3	20:5n-3 eicosapentaenoic	89 (47) ^a	69 (31) ^a	64 (27) ^a	63 (30) ^a	0.042	77 (43)	68 (31)	0.49
	22:5n-3 docosapentaenoic	49 (15)	43 (13)	44 (17)	46 (19)	0.53	48 (15)	48 (14)	0.97	
22:6n-3 docosahexaenoic	145 (47) ^a	146 (45) ^a	163 (48) ^{a,b}	190 (56) ^b	0.003	135 (55)	131 (49)	0.75		

LHS – luteinising hormone surge; MUFA - monounsaturated fatty acids; NP – not successful in becoming pregnant; Preg – successful pregnancy; PUFA - polyunsaturated fatty acids; SAFA - saturated fatty acids. * Analysis was conducted on untransformed data except for the following: Log transformed – 14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 24:1n-9, 18:2n-6, 20:3n-6, 20:5n-3, 22:5n-3, 22:6n-3. Square root transformed – 22:0, 24:0, 20:4n-6, 18:3n-3, 18:3n-6.

switched to increased rates of change of plasma AA between day 18 and 45 post-LH surge (Figure 1A). However, overall there was no net change in AA concentration; the iAUC was zero (Figure 1B). Other major plasma fatty acids also initially showed declining rates of increasing plasma concentration from pre-LH surge to day 18 post-LH surge which then switched to higher rates of increasing plasma concentration between day 18–45 post-LH surge (Figure 2A), however their iAUC were all negative (Figure 2B) suggesting a loss of these fatty acids from the plasma compartment over 45 days. Of note is the rapid decline in plasma concentration and a corresponding large negative iAUC of LA, as a decrease in LA concentration may allow δ -6 desaturase to favor DHA synthesis from ALA (Supplemental Figure 1).

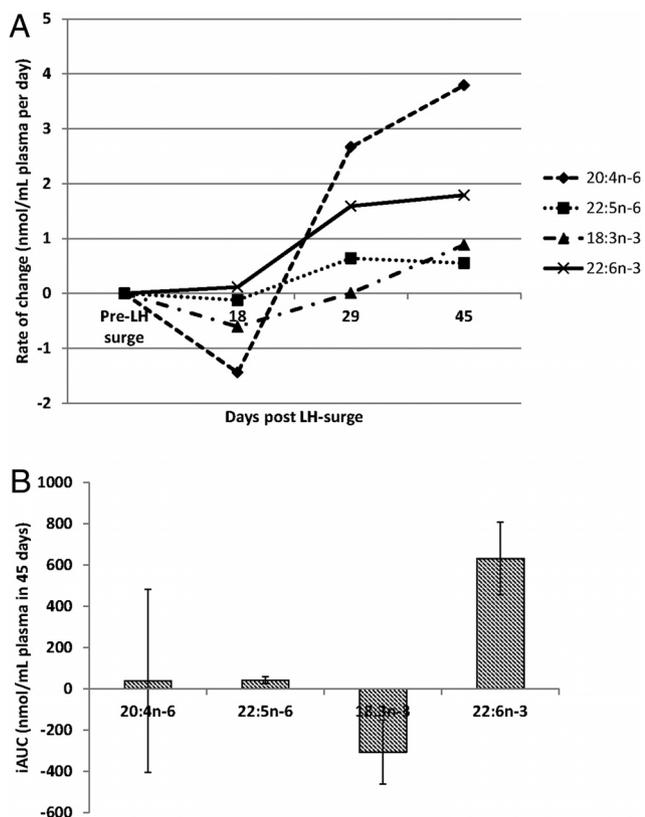


Figure 1. A Rates of change (nmol/mL plasma per day) of maternal plasma polyunsaturated fatty acids. 20:4n-6 = —◆—; 22:5n-6 = ...■...; 18:3n-3 = -▲-; 22:6n-3 = ×. The rate of change of fatty acids were calculated as the difference between the fatty acid on day 18 post-LH surge and pre-LH surge and then divided by the difference in time (days) between day 18 post-LH surge and pre-LH surge. This was then repeated for all other time points, ie, day 29 post-LH surge and day 18 post-LH surge and then day 45 post LH-surge and day 29 post-LH surge. The results are expressed as nmol/mL plasma per day. **B** Incremental Area Under the Curve (nmol/mL plasma x day) of maternal plasma polyunsaturated fatty acids. Incremental areas under the time (pre-LH surge to 45 days post LH surge) x concentration curve were calculated using the trapezium method with correction for prepregnancy parameter level (11).

Plasma DHA and omega-6 DPA concentrations over the first 45 days of gestation in twin pregnancies

Increases in both DHA and omega-6 DPA concentrations were approximately two-fold higher in twin compared to singleton pregnancies (Table 3). By day 45 post-LH surge DHA plasma concentration was significantly raised both in singleton and twin pregnancies and the increase from pre-LH surge levels was greater in twin (74 nmol/mL) than singleton (36 nmol/mL) pregnancies. This two fold higher change in DHA concentration was also observed earlier at day 29 post-LH surge: twin (161–131 = 30 nmol/mL) vs singleton (163–149 = 14 nmol/mL). The iAUC for DHA in twin pregnancies (1341 ± 936 nmol/mL over 45 days) was significantly higher ($P=0.013$) than singleton pregnancies (384 ± 781 nmol/mL over 45 days). At day 29 post-LH surge omega-6 DPA change in concentration from pre-LH surge was three-fold higher in

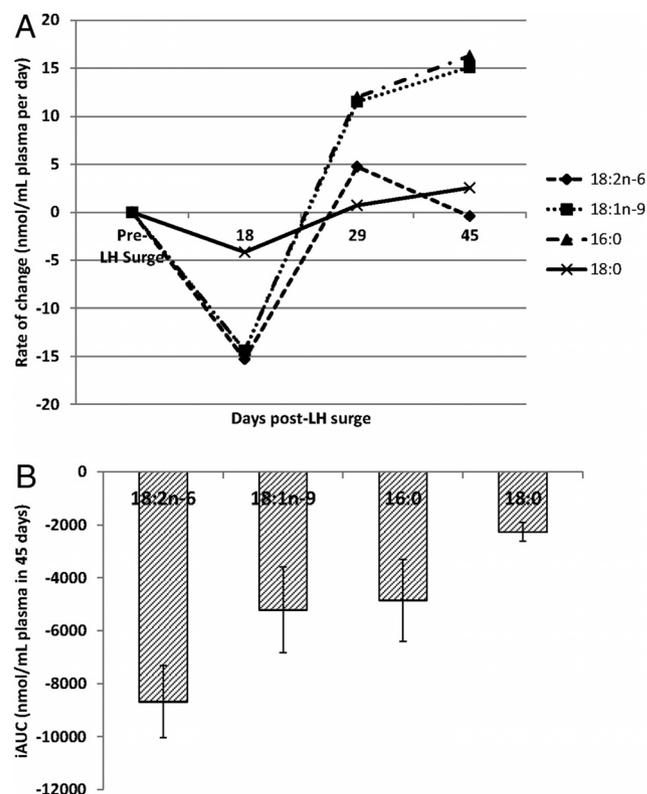


Figure 2. A Rates of change (nmol/mL plasma per day) of the maternal major plasma fatty acids. 18:2n-6 = —◆—; 18:1n-9 = ...■...; 16:0 = -▲-; 18:0 = ×. The rate of change of fatty acids were calculated as the difference between the fatty acid on day 18 post-LH surge and pre-LH surge and then divided by the difference in time (days) between day 18 post-LH surge and pre-LH surge. This was then repeated for all other time points, ie, day 29 post-LH surge and day 18 post-LH surge and then day 45 post LH-surge and day 29 post-LH surge. The results are expressed as nmol/mL plasma per day. **B** Incremental Area Under the Curve (nmol/mL plasma x day) of various maternal plasma fatty acids. Incremental areas under the time (pre-LH surge to 45 days post-LH surge) x concentration curve were calculated using the trapezium method with correction for prepregnancy parameter level (11).

Table 3. Comparison of singleton vs. twin pregnancy plasma 22:5n-6 and 22:6n-3 concentrations from pre-LH surge to 45 days' post-LH surge. Mean (standard deviation) is shown. Differences between concentrations were tested using one way Analysis of Variance for repeated measures, across sampling gestations pre-LH surge and 18, 29 and 45 days post-LH surge and *P* values are given. Different superscript letters indicate differences between individual groups using *post hoc* Tukey-Kramer at significance level $P \leq 0.05$. * analysis carried out on log transformed data.

Fatty Acid (nmol/mL)	pre-LH surge (pre-pregnancy)	18 days post-LH surge	29 days post-LH surge	45 days post-LH surge	<i>P</i>
Singleton (n = 20)/ Twin pregnancy (n = 7)					
22:5n-6 (S)	6.9 (4.9) ^{a,b}	5.9 (4.6) ^a	7.7 (4.1) ^{a,b}	10 (4.8) ^b	0.037
22:5n-6 (T)	8.6 (2.2) ^a	8.5 (2.3) ^a	11 (2.8) ^a	16 (4.0) ^b	0.0002
22:6n-3* (S)	149 (52) ^a	145 (48) ^a	163 (55) ^a	185 (61) ^a	0.093
22:6n-3* (T)	131 (28) ^a	151 (37) ^a	161 (25) ^{a,b}	205 (38) ^b	0.0023

LH – luteinising hormone; S, singleton pregnancy; T, twin pregnancy.

twin (2.4 nmol/mL) vs singleton (0.8 nmol/mL) pregnancy and two-fold higher at day 45 post-LH surge (7.4 nmol/mL) vs (3.1 nmol/mL) respectively. The iAUC for omega-6 DPA tended also to be higher in twin pregnancies (96 ± 49 nmol/mL over 45 days) than in singleton pregnancies (23 ± 95 nmol/mL over 45 days) but this did not reach significance ($P=0.060$). In a multivariate mixed model, plasma DHA levels were higher as gestation progressed ($P < .0001$) but did not differ between twin and singleton pregnancy overall ($P=0.97$). A borderline significant interaction between days of gestation and twin pregnancy ($P=0.06$) suggests that DHA plasma concentration increases are higher in twins compared to singleton pregnancies in response to pregnancy (R-squared adjusted 83%). In a multivariate mixed model, plasma omega-6 DPA levels were associated with day of gestation ($P < .0001$) and were higher overall in twin pregnancy ($P=0.04$). However, the nonsignificant interaction between days of gestation and twin pregnancy (R-squared adjusted 64%, $P = .10$) provides less evidence that plasma omega-6 DPA concentration is increased to a greater extent in twin pregnancy.

Linoleic acid and pregnancy outcome

There was a significant 10% reduction in plasma LA concentration between pre-LH surge and day 18 post-LH surge in pregnant women ($P = .017$) but not in women who were not successful in getting pregnant ($P = .63$). Multivariable stepwise logistic regression analysis considering all day 18 post-LH surge variables (including erythrocyte fatty acid composition and insulin data not shown here) measured in women who became pregnant and those women who did not showed that at high insulin levels, low LA levels and high erythrocyte saturated fatty acid levels were associated with successful pregnancy at day 45 post-LH surge (C-statistic = 0.78) (Table 4).

Discussion and conclusion

This study observed, for the first time, that uniquely among the fatty acids measured, DHA showed a net increase in concentration over the first 45 days of pregnancy with the highest rate of increase being observed after day 18 post-LH surge. This early pressure to increase plasma DHA concentration was further emphasized in twin pregnancies where the increase in concentration was double

Table 4. Univariate and multivariable logistic regression models of day 18 post-LH surge variables and pregnancy outcome at day 45 post-LH surge. Outcome was pregnant or not pregnant at day 45 post-LH surge. Covariates were selected using stepwise logistic regression models run for day 18 post-LH surge covariates individually significant (at $P < 0.05$). Data shown are the univariate and multivariable odds ratio (for the given unit of change) and the 95% confidence interval, with the associated *P*-value, and the C-statistic (for the Area under the Curve). The multivariable model is a stepwise model with *P*-to-enter and *P*-to-stay each set at 0.05.

Covariate	Univariate odds ratio (95% confidence interval)	<i>P</i>	C-statistic (Area Under Curve)	Multivariable odds ratio (95% confidence interval)	<i>P</i>	C-statistic (Area under curve)
Day 18 post-LH surge						0.78
Log insulin (mU/liter)	2.05 (1.07, 3.92)	0.03	0.62	2.23 (1.01, 4.93)	0.048	
Plasma 18:2n-6 (500 nmol/mL)	0.44 (0.24, 0.82)	0.010	0.69	0.48 (0.24, 0.99)	0.046	
Erythrocyte saturated fatty acid (%)	3.37 (1.60, 7.11)	0.0014	0.70	3.12 (1.41, 6.92)	0.0050	

Outcome was pregnant or not pregnant at day 45 post-LH surge. Covariates were selected using stepwise logistic regression models run for day 18 covariates individually significant (at $P < 0.05$). Data shown are the univariate and multivariable odds ratio (for the given unit of change) and the 95% confidence interval, with the associated *P*-value, and the C-statistic (for the Area under the Curve). The multivariable model is a stepwise model with *P*-to-enter and *P*-to-stay each set at 0.05.

that in singleton pregnancies. A 14% increase in plasma volume at day 70 of gestation (12) suggests a change in plasma volume of 6% at day 18 post-LH surge and slightly higher (7%) in twin pregnancy (13). This increase in plasma volume cannot account for the differences in DHA concentrations observed here. The plasma fatty acids measured in this study reflect a complex mixture of fatty acids bound to albumin plus those fatty acids contained within lipoprotein particles, the transport of which is very complex requiring detailed isotope tracer kinetic studies which are beyond the scope of this study.

The rapid and early increase in maternal plasma DHA concentration is consistent with our hypothesis that this fatty acid is of critical importance early in pregnancy. DHA, a major structural lipid in the brain, interacts with the plasma membrane protein syntaxin-3 which is required for the membrane fusion necessary for neurite outgrowth in developing neurons (14). Previous data has shown its importance during the latter stages of pregnancy when the brain accrues its tissue mass (15). Our data shows that DHA is also important during the very early stages of pregnancy when embryological nervous tissue is beginning to form. The timing of the increase in plasma DHA concentration is coincident with the time at which the very first primitive nerve cells appear and, by the time of neural tube closure at around 28 days' gestation, the plasma concentration of DHA has increased significantly by 31% and a steady rate of increasing plasma concentration has been reached.

The early rise in maternal plasma DHA concentration could be achieved either through its release from body stores or via increased synthesis. Diet is unlikely to be substantially changed over such a short period of time, but cannot be discounted. Humans do not store substantial amounts of DHA in adipose tissue, yet there is sufficient DHA content (16) and total adipose tissue mass in pregnant women that its release from adipose tissue could contribute to the increase in plasma concentration observed here. Another likely source is maternal de novo synthesis in the liver. The fractional conversion rates of ALA to DHA in men is only 0.04% (17) with women being higher at 9% (8). Our observations that 1) the initial plasma concentrations of ALA and EPA decrease before rebounding between day 18–45; 2) the significant 10% reduction in plasma LA in pregnant women and 3) the accelerated increase in plasma concentration of DHA between day 18–29 post-LH surge was correlated with an index of δ -6 desaturase activity are all consistent with increased synthesis of DHA from its precursors. This agrees with observations in pregnant rats where plasma and liver DHA concentration as well as liver *FADS2* (δ -6 desaturase) mRNA expression were increased by gestation day 20

(18). The increase in liver *FADS2* mRNA expression was positively correlated with plasma concentrations of pregnancy hormones; estradiol and progesterone (18), suggesting that these hormones are responsible for up-regulating DHA synthesis. The ability of women to convert more ALA to DHA has also been attributed to the presence of estrogen (8) and postmenopausal women receiving estrogen therapy have increased conversion (19). Low levels of LA have previously been shown to be associated with increased DHA levels in mothers in the third trimester and in their newborns (20), and this low placental transport of maternal LA allowed δ -6 desaturase activity to be directed more towards converting ALA to DHA (20). Furthermore, the current study shows that low levels of LA is a predictor of successful pregnancy as indicated by positive fetal heartbeat at day 45 post-LH surge.

The substantial decrease in maternal plasma γ -linolenic acid and the resultant increases in dihomogamma-linolenic acid and omega-6 DPA indicate that the synthesis of omega-6 DPA is important, as it can be used as a substitute when DHA supply is limited (21). In the eye, omega-6 DPA is incorporated into rhodopsin-containing membranes when DHA concentration is low, albeit with a resultant reduction in visual acuity (22) and supplementation with DHA in infants may increase visual acuity (23). In DHA deficiency, omega-6 DPA is used in place of DHA in brain which appears to be a survival mechanism (24–26). Our observation of elevated omega-6 DPA, especially in twin pregnancy, may suggest a secondary mechanism to provide an alternative LC PUFA should there be insufficient DHA for neurological development at this critical time.

Fatty acids in oocytes are primarily saturated fatty acids, oleic acid and virtually no omega-3 PUFA (27). Once an oocyte has been released from the ovary, the oocyte and any resultant embryo needs to rely on its own energy source and oocytes express the relevant lipases to release fatty acids from triglyceride (28). Interestingly, inhibition of oocyte fatty acid oxidation in vitro reduces blastocyst formation and reduces the number of cells within each blastocyst (29). Thus, saturated fatty acids are key to successful embryo formation and implantation (30). Sperm, on the other hand, contain predominately DHA (31), which upon fertilization of the oocyte, delivers at least some of the DHA needed for very early neurological development. After implantation, the embryo must rely on fuels transported directly to the embryo from the mother until the fetal/maternal circulation is established at 9–11 weeks of gestation. Our data show increased plasma concentrations of palmitic acid and oleic acid between day 18 and 45 post-LH surge, consistent with these fatty acids being used to meet the energy requirements by the embryo. Exactly how the embryo obtains fatty acids from the ma-

ternal circulation is yet to be elucidated, but there is evidence for their simple diffusion across lipid vesicles (32).

The strengths of this study were the prospective study design, with repeated within-individual sampling up to day 45 post-LH surge and the comprehensive analysis of fatty acid profiles measuring absolute concentrations. The main limitation of this study was that women undergoing in vitro fertilisation are not a “normal” pregnancy population and it should be noted that over 40% of the women were undergoing assisted conception due to problems with female fertility. However, the women had a natural menstrual cycle meaning there was no interference of exogenous hormones used in cycle reconstruction on their metabolic adaptation to pregnancy. Frozen rather than fresh embryos were transferred, but this should not impact on the maternal metabolic response to implantation of that embryo. The major advantage of the population used was the ability to get accurately timed peri-conceptual and early pregnancy blood samples that would be extremely difficult to achieve from a free-living population. There was no dietary intake data, no information on morning sickness and due to being unable to collect relevant tissues, no direct measure of delta 6 desaturase activity and no assessment of embryo/fetal tissue accrual of fatty acids. Inferences on changes in fatty acid metabolism were made using serial steady state plasma fatty acid concentrations. While we can observe increases and decreases in plasma concentration, it does not fully establish whether these changes in concentration were due to changes in rates of entry into or removal from plasma compartments as would be described by kinetic tracer studies which are clearly extremely difficult to perform in pregnant women.

In conclusion, increases in maternal plasma DHA concentration occur very early in pregnancy highlighting the importance of DHA at this critical time when neural tube closure takes place.

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B.J.M. and D.J.F. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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