Importance of maternal DHA in very early pregnancy

Professor Barbara Meyer
School of Medicine
Importance of maternal DHA in very early pregnancy


1School of Medicine, University of Wollongong, NSW, Australia
2School of Medicine, University of Glasgow, U.K.
3Institute of Cardiovascular and Medical Sciences, University of Glasgow, U.K.
4Robertson Centre for Biostatistics, University of Glasgow, U.K.
5Institute of Infection, Immunity and Inflammation, University of Glasgow, U.K.
Docosahexaenoic acid (DHA)

Molecular structure of docosahexaenoic acid (DHA)

Brain Lipids
- 10–20% is DHA
- Omega-3, 90% is DHA

DHA promotes neurite outgrowth

Docosahexaenoic acid (DHA) and Arachidonic Acid (AA) are important fatty acids for neurological development.

There is rapid accumulation of DHA and AA in the brain during the last trimester of pregnancy.
DHA is important for neurological development

DHA is important for neurological development

DHA is important for neurological development

Longitudinal study in pregnancy

Maternal plasma fatty acids across trimesters (T1, T2, T3) and post-partum (PP)

Stewart F, ... Freeman DJ, Meyer BJ. et al. Lipids 2007;42(4):335-344
Fig. 1. Biosynthesis of long-chain polyunsaturated fatty acids. Arrows with solid line (→) are found both in mammals and lower eukaryotes, while arrows with dotted line (⋯→) are exclusively for lower eukaryotes. Fatty acids in □ indicate the pathway is exclusively in mammals.
Fig. 4. Concentrations of $^{13}$C-labelled $\alpha$-linolenic acid (ALNA, ■), eicosapentaenoic acid (EPA, □), docosapentaenoic acid (DPA, △) and docosahexaenoic acid (DHA, ○) in erythrocyte phosphatidylcholine. Results are mean $^{13}$C fatty acid concentrations/ml whole blood, with standard errors of the mean represented by vertical bars.  

Burdge GC et al, Br J Nutr 2002;88:355
The critical requirement for DHA for fetal brain development, and the poor efficiency of its synthesis in humans, is therefore a metabolic problem to be overcome in pregnancy.
Day 15 of gestation - the first primitive brain cells are formed
Day 28 of gestation - neural tube closure occurs
Early gestational weeks – the head of the embryo grows fastest

Our hypothesis - changes in maternal plasma PUFA concentration in very early gestation are vitally important.
Very Early Pregnancy Study

Aim:
To characterise the earliest fatty acid changes in pregnancy

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
Study Design

- Population of women undergoing assisted conception to achieve accurately timed peri-conceptual sampling
  - October 2007 – April 2010
- Undergoing frozen embryo transfer
- Women with a regular natural menstrual cycle to avoid the effects of exogenous hormones

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
Study Design

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
196 women with a natural menstrual cycle received frozen embryo transfer (FET)

35 withdrew
- 3 withdrew consent
- 25 cycles cancelled
- 7 lost to follow up

161 IVF cycles

123 failed pregnancies

38 successful pregnancies

27 pregnancies with full fatty acid data

36 successful pregnancies

2 excluded due to the FET not being their first attempt in this study

9 excluded due to lack of fatty acid data
## Demographics

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women (n=27)</th>
<th>Not successful in getting pregnant (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at FET (years)</td>
<td>34.7 (3.9)</td>
<td>33.5 (5.2)</td>
<td>0.33</td>
</tr>
<tr>
<td>Smokers (number [%])</td>
<td>3 (11)</td>
<td>8 (23)</td>
<td>0.25</td>
</tr>
<tr>
<td>SIMD quintiles (number [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affluent; Q1 &amp; Q2</td>
<td>10 (37)</td>
<td>18 (52)</td>
<td>0.32</td>
</tr>
<tr>
<td>Intermediate; Q3</td>
<td>6 (22)</td>
<td>5 (14)</td>
<td></td>
</tr>
<tr>
<td>Deprived; Q4 &amp; Q5</td>
<td>11 (41)</td>
<td>12 (34)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 (3.8)</td>
<td>26.4 (5.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>116 (15)</td>
<td>117 (12)</td>
<td>0.66</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>65 (7)</td>
<td>67 (9)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* Missing data points

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
## Demographics (ctd)

<table>
<thead>
<tr>
<th></th>
<th>Pregnant women (n=27)</th>
<th>Not successful in getting pregnant (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of embryos transferred</strong></td>
<td>1, 2, 3</td>
<td>4 (12), 30 (88), 0 (0)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (15), 22 (81), 1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Twin pregnancy according to no. embryos transferred (number (%))</strong></td>
<td>1, 2, 3</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0), 7 (32), 0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Missing data points

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Maternal plasma n-6 polyunsaturated fatty acids (PUFA) (nmol/ml)

<table>
<thead>
<tr>
<th>N-6 PUFA</th>
<th>Pre-LHS</th>
<th>18 days post LHS</th>
<th>29 days post LHS</th>
<th>45 days post LHS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid (18:2)</td>
<td>2569 (384)</td>
<td>2297 (422)</td>
<td>2353 (382)</td>
<td>2334 (420)</td>
<td>0.056</td>
</tr>
<tr>
<td>γ-linolenic acid (18:3)</td>
<td>45a (18)</td>
<td>32b (13)</td>
<td>32b (16)</td>
<td>34a,b (12)</td>
<td>0.0067</td>
</tr>
<tr>
<td>Dihomoγ-linolenic (20:3)</td>
<td>137a (31)</td>
<td>130a (29)</td>
<td>142a,b (36)</td>
<td>163b (46)</td>
<td>0.008</td>
</tr>
<tr>
<td>Arachidonic (20:4)</td>
<td>572 (108)</td>
<td>546 (103)</td>
<td>573 (134)</td>
<td>627 (154)</td>
<td>0.16</td>
</tr>
<tr>
<td>Adrenic (22:4)</td>
<td>12 (5)</td>
<td>11 (4)</td>
<td>12 (5)</td>
<td>15 (5)</td>
<td>0.016</td>
</tr>
<tr>
<td>Docosapentanoic (22:5)</td>
<td>7a (4)</td>
<td>7a (4)</td>
<td>9a,b (4)</td>
<td>12b (5)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Values are mean (SD)

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
Maternal plasma n-3 polyunsaturated fatty acids (PUFA) (nmol/ml)

<table>
<thead>
<tr>
<th>N-3 PUFA</th>
<th>Pre-LHS</th>
<th>18 days post LHS</th>
<th>29 days post LHS</th>
<th>45 days post LHS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-linolenic (18:3)</td>
<td>68 (27)</td>
<td>57 (24)</td>
<td>57 (21)</td>
<td>68 (31)</td>
<td>0.26</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5)</td>
<td>89a (47)</td>
<td>69a (31)</td>
<td>64a (27)</td>
<td>63a (30)</td>
<td>0.042</td>
</tr>
<tr>
<td>Docosapentaenoic (22:5)</td>
<td>49 (15)</td>
<td>43 (13)</td>
<td>44 (17)</td>
<td>46 (19)</td>
<td>0.53</td>
</tr>
<tr>
<td>Docosahexaenoic (22:6)</td>
<td>145a (47)</td>
<td>146a (45)</td>
<td>163a,b (48)</td>
<td>190b (56)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are mean (SD)

No maternal plasma fatty acid changes in the non-pregnant women between pre-LHS and 18 days post LHS

Maternal plasma DHA from pre-pregnancy to 45 days post-LH surge

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
Rate of change
- The rate of change of FAs were calculated as the difference between the FA on day 18 post-LH surge and pre-LH surge and then divided by the difference in time (days) between day 18 & pre-LH surge
- It was repeated for all other time points

Incremental Area Under the Curve
- the amount of fatty acid released into the plasma over 45 days

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Rate of change
- The rate of change of FAs were calculated as the difference between the FA on day 18 post-LH surge and pre-LH surge and then divided by the difference in time (days) between day 18 & pre-LH surge

- It was repeated for all other time points

Incremental Area Under the Curve
- The amount of fatty acid released into the plasma over 45 days

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Polyunsaturated Fatty Acid Synthesis Pathways

(Mammalian)

**Omega-6 Pathway**

18:2n-6 (linoleic acid)

↓ 18:3n-6

**Delta 6 desaturase**  
(FADS2)

↓ 18:4n-3

**Long chain fatty acid elongase**  
(ELOVL5)

↑ 20:3n-6

20:4n-6 (arachidonic acid)

↓ 20:5n-3 (eicosapentaenoic acid)

**Delta 5 desaturase**  
(FADS1)

Very long chain FA elongase  
(ELOVL2)

22:4n-6

↓ 22:5n-3 (docosapentaenoic acid)

24:4n-6

↓ 24:5n-3

**Delta 6 desaturase**  
(FADS2)

24:5n-6

β-oxidation

↑ 22:5n-6

**Omega-3 Pathway**

18:3n-3 (α-linolenic acid)

18:4n-3

**Delta 6 desaturase**  
(FADS2)

20:4n-3

**Long chain fatty acid elongase**  
(ELOVL5)

↑ 20:3n-6

20:5n-3 (eicosapentaenoic acid)

**Delta 5 desaturase**  
(FADS1)

↓ 22:4n-6 (arachidonic acid)

↓ 22:5n-3 (docosapentaenoic acid)

Very long chain FA elongase  
(ELOVL2)

24:4n-6

↓ 24:5n-3

↑ 22:6n-3 (docosahexaenoic acid)

↓ increased conversion of 18:3n-6 to 20:3n-6

↓ reduced levels of 18:3n-6

↓ reduced conversion from 18:2n-6 to 18:3n-6

↑ increased levels of 20:3n-6, 22:5n-6 and 22:6n-3
Maternal plasma linoleic acid concentrations (nmol/mL plasma) in Not pregnant vs Pregnant Women

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Rate of change of DHA concentration (day 18-29) correlates with delta-6 desaturase activity ($R^2$ adjusted = 41%, $p=0.0002$)

Delta-6 desaturase activity index (at day 29 post-LH surge)
(The ratio of dihomo-gamma-linoleic acid 20:3n-6 to LA 18:2n-6 ratio)

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Maternal plasma n-6 DPA and DHA in Singleton (n=20) vs Twin Pregnancies (n=7)

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753  doi: 0.1210/jc.2015-3089
Cell membrane
- phospholipid bilayer including DHA

Importance of n-6 DPA when DHA is low

A good example of scientific evidence that supports this membrane fluidity theory is rhodopsin.

Usually when light hits rhodopsin in the eye, elongation of rhodopsin occurs which is necessary for good vision [Alves et al 2005] and when DHA is replaced with docosapentaenoic acid (22:5n-6) in the eye conformational changes occur that affect the rhodopsin photocycle [Niu et al 2001].


Importance of n-6 DPA when DHA is low

Human studies support this in that supplementation with DHA in infants results in increased visual acuity, especially in girl infants [Innis et al. 2008].

In DHA deficiency, n-6 DPA is used in place of DHA in the brain which seems to be a survival mechanism [Kim HY 2007].

Our observation of elevated n-6 DPA, especially in twin pregnancy, may suggest a secondary mechanism to provide an alternative PUFA if there is insufficient DHA for neurological development at the critical time of neural tube closure.


## Models of Day 18 Post-LH Surge Variables and Pregnancy Outcome at Day 45 Post-LH Surge

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

Meyer BJ ... Freeman DJ. 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. Journal of Clinical Endocrinology and Metabolism 2016;101:1745-1753 doi: 0.1210/jc.2015-3089
Summary of the pregnancy timeline

Implantation

hCG in plasma from placenta

Positive fetal heartbeat

LH surge
Day 0

Day 7

Day 10

Day 18

Day 29

Day 45

Baseline

Gestation (Days)

Summary of the pregnancy timeline

DHA mobilisation

Implantation

hCG in plasma from placenta

Positive fetal heartbeat

Gestation (Days)

LH surge
Day 0

Day 7

Day 10

Day 18

Day 29

Day 45

Baseline

Sources of DHA mobilisation

- Increased dietary DHA
- Increased synthesis from alpha-linolenic acid
- Increased release from maternal adipose tissue and other maternal stores

DHA mobilisation

- Positive fetal heartbeat
- Implantation
- hCG in plasma from placenta

Gestation (Days)

- Day 0: LH surge
- Day 7: Baseline
- Day 10: Day 18
- Day 29: Day 45