B.S. Muhlhausler, R.A. Gibson, L.N. Yelland, M. Makrides
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Prostaglandins, leukotrienes, and essential fatty acids, 2014; 91(4):135-140

Heterogeneity in cord blood DHA concentration: towards an explanation

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Running title: Heterogeneity in cord blood DHA

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Sources of Support:

This study was supported by a Project Grant from the National Health and Medical Research Council of Australia (NHMRC) MM and RG are supported by Senior Research Fellowships from the NHMRC. LY and BM are supported by NHMRC Postdoctoral Fellowships.

SUMMARY

This paper aimed to identify the dietary and non-dietary determinants of docosahexaenoic acid (DHA) levels in umbilical cord blood at delivery. DHA was measured in cord blood plasma phospholipids of 1571 participants from the DOMInO (DHA to Optimize Mother Infant Outcome) randomized controlled trial. Socioeconomic, lifestyle and clinical data relating to the mother and current pregnancy were obtained from all women and their relationships with cord blood DHA assessed. DHA concentrations in the cord plasma phospholipids at delivery covered a 3-4 fold range in both control and DHA groups. The total number of DHA-rich intervention supplement capsules consumed over the course of pregnancy and gestational age at delivery individually explained 21% and 16% respectively of the variation in DHA abundance in the cord blood plasma phospholipids at delivery, but no other clinical or life-style factors explored in this study could account for >2% of the variation. Indeed, more than 65% of the variation remained unaccounted for even when all factors were included in the analysis. These data suggest that factors other than maternal DHA intake have an important role in determining cord blood DHA concentrations at delivery, and may at least partially explain the variation in the response of infants to maternal DHA supplementation reported in published trials.

Key words: DHA, omega-3 fatty acids, pregnancy, cord blood, supplementation

INTRODUCTION

The omega-3 long chain polyunsaturated fatty acid (n-3 LCPUFA), docosahexanoic acid (DHA, 22:6n-3) plays a critical role in development, and an adequate supply of this fatty acid during the period of rapid growth in late fetal and early postnatal life is essential for optimal development of a number of key organ systems [1, 2]. The accumulation of DHA in fetal tissues occurs predominately during the last trimester of pregnancy and continues through the first year of life. Studies that have determined fatty acid abundance in the umbilical cord blood at delivery have shown that levels of n-3 LCPUFA, in particular DHA, are higher in the umbilical cord blood (a marker of fetal supply) compared to the levels of the equivalent fatty acids in the maternal circulation [1, 3]. This apparent biomagnification is reported to be a result of both preferential transfer of DHA by the placenta and fetal uptake/metabolism to ensure that the fetal supply of DHA is maintained even when maternal dietary intake is low [3].

While the fetus has the capacity for DHA synthesis from the 18-carbon precursor, alpha-linoleic acid (ALA) and DHA metabolism, the prevailing view is that the principal determinant of DHA supply to the developing fetus in healthy pregnancies is the DHA intake of the mother[4-6]. However, studies that have measured cord blood fatty acids have also consistently demonstrated a high degree of heterogeneity in the DHA content of cord blood phospholipids between individual women, even when DHA intake is similar [5, 7, 8]. This has led to the suggestion that factors other than nutritional intake also play an important role in determining cord blood DHA content, and therefore the delivery of DHA to the developing fetus. Previous studies, albeit most with small sample sizes, have reported that cord blood DHA levels are related to several non-dietary factors, including parity [9], maternal and fetal genotype in relation to the desaturase enzymes (FADS1 and

2) [10] and maternal smoking and alcohol intake [11]. However, these factors only accounted for a small proportion of the variation in cord blood DHA, and no studies have been able to adequately account for the variability in cord blood DHA concentrations. Given the importance of DHA for fetal development, understanding the determinants of fetal DHA status has clear clinical relevance.

The DOMInO trial is the largest randomized controlled trial to date to investigate the effect of maternal DHA-supplementation during the second half of pregnancy on neurodevelopmental outcomes in the children [12] and cord blood samples were obtained from 1571 of these women at delivery. Detailed sociodemographic, pregnancy and neonatal health information was collected from all DOMInO participants, including maternal smoking, maternal BMI at study entry, intake of (non-DHA containing) dietary supplements, maternal education, parity, pregnancy complications (pre-eclampsia, gestational diabetes mellitus (GDM), induction), caesarean delivery, gestational age at delivery, birth weight and infant sex. In this paper, we have used the large and well-characterised DOMInO study population to report the effect of maternal DHA supplementation on the fatty acid composition of the cord blood and to explore the relationships between the factors listed above and cord blood DHA levels in an attempt to explain differences in DHA abundance in the umbilical cord blood at delivery.

METHODS

Participants

Women with singleton pregnancies at less than 21 weeks' gestation from 5 perinatal centres in Australia were enrolled in a double-blinded multi-centre randomized controlled trial [12]. Women were excluded if they were already taking a prenatal supplement containing DHA, their fetus had a

known major abnormality, they had a bleeding disorder in which tuna oil was contraindicated, were taking anticoagulant therapy, had a documented history of drug or alcohol abuse, were participating in another fatty acid trial, were unable to give written informed consent, or if English was not the main language spoken at home. Approval was granted by the local institutional review boards (human research ethics committees) of each centre and written informed consent was obtained from each participant.

Experimental design

Women were randomly assigned a unique study number corresponding to either the DHA or Control group through a computer-driven telephone randomization service according to an independently generated randomization schedule, with balanced variable-sized blocks. Stratification was by centre and parity (first birth vs subsequent birth). Baseline characteristics, including maternal age, weight, highest level of education, occupation, smoking status, and intake of (non-DHA containing) dietary supplements were recorded. Mothers were classified as smokers if they smoked at trial entry or leading up to pregnancy and non-smokers otherwise.

Women allocated to the DHA group were asked to consume three 500 mg/d capsules of DHA-rich fish oil concentrate, providing 800 mg/d of DHA and 100 mg/d of eicosapentaenoic acid (EPA, 20:5n-3; Incromega 500 TG, Croda Chemicals, East Yorkshire, England); and women in the control group were asked to take three 500 mg/d vegetable oil capsules without DHA which contained a blend of oils designed to match the polyunsaturated, monounsaturated and saturated fatty acid profile of the typical Australian diet [13]. All capsules were similar in size, shape, and colour and were donated by Efamol, Surrey, England. All participants as well as medical, nursing, clinical and laboratory staff were unaware of the treatment group allocation for the entire duration of the trial.

Women were asked to take their assigned capsules daily from study entry until birth, and to return any unused capsules, which enabled the total number of DHA-rich intervention supplement capsules consumed over the course of pregnancy and compliance to be assessed. Information on pregnancy and neonatal outcomes was collected from medical records. These outcomes included: gestational age at delivery, caesarean section vs vaginal delivery, clinical diagnosis of pre-eclampsia, clinical diagnosis of gestational diabetes (GDM), induction, birth weight and infant sex.

At the time of delivery, samples of cord blood were collected from 1571 women (798 in the DHA group and 773 in the control group) for the measurement of the fatty acid composition of plasma phospholipids. The baseline characteristics, pregnancy and neonatal outcomes for the women from whom cord blood was collected are presented in **Table 1**.

Fatty acid analyses

Whole blood was collected into lithium heparin tubes and erythrocyte and plasma fractions separated by centrifugation. Plasma was stored at -20°C at each separate site and all plasma samples were then transported to Adelaide on dry ice for analysis. Cord plasma phospholipids were analysed according to previously established methods [14]. Briefly, plasma lipids were extracted in chloroform:methanol, the chloroform layer removed and evaporated under nitrogen, and phospholipids separated by thin-layer chromatography. Phospholipids were methylated in 1% H_2SO_4 in methanol at 70°C for 3h. The resulting methyl esters were extracted into n-heptane and dehydrated in anhydrous Na_2SO_4. Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 5880 gas chromatograph equipped with a 50m capillary column coated with BPX-70 (0.25micron film thickness, SGE PtyLtd., Victoria, Australia) using conditions described in

detail previously [14] and identified based on retention time to authentic lipid standards obtained from Nucheck Prep Inc (Elysian, MN). For statistical purposes, fatty acids at concentrations lower than the limit of detection (0.05%) were allocated a set value of 0.025% (half the limit of detection). The abundance of all fatty acids was expressed as a percentage of total fatty acids in the sample.

Statistical Analyses

Cord plasma phospholipid fatty acid concentrations were compared between treatment groups using t-tests. Concentrations were log transformed prior to analysis to achieve normality, with the exception of total saturates, arachidonic acid (AA, 20:4 n-6) and total n-6 PUFA, which did not require any transformation.

Log transformed cord plasma phospholipid DHA abundance values were analyzed using linear regression models and effects were exponentiated to give geometric mean cord plasma phospholipid DHA abundance. The predictor variables considered in the regression models were: total number of DHA-rich intervention supplement capsules consumed over the course of pregnancy (0 for women in the control group), maternal smoking status, maternal BMI at study entry, parity, maternal intake of (non-DHA containing) dietary supplements during pregnancy, maternal education, gestational age at delivery, caesarean section vs vaginal delivery, clinical diagnosis of pre-eclampsia, clinical diagnosis of GDM, induction, birthweight z-score and infant sex. In additional analyses, the levels of other quantitatively important fatty acids in the cord blood (saturated fatty acids, monounsatured fatty acids, LA and AA) were also considered as predictor variables. The relationship of LA and AA with gestational age was also examined using linear

158 regression models. Both univariable and multivariable analyses were performed. All tests were two-
159 sided and statistical significance was assessed at the 0.05 level.
160
161 **RESULTS**
162 *Effect of maternal n-3 LCPUFA supplementation on fatty acid composition of cord blood*
163 *phospholipids*
164 The fatty acid composition of the cord blood plasma phospholipids for the DHA and control groups
165 is shown in **Table 2**. Independent of treatment group, AA and DHA were the most abundant n-6
166 and n-3 LCPUFA in the cord blood phospholipids. Cord blood from DHA-supplemented mothers
167 had significantly higher proportions of the three principal n-3 LCPUFA, EPA, docosapentaenoic
168 acid (DPA, 22:5 n-3) and DHA) and a significantly lower proportion of AA compared with cord
169 blood from mothers in the control group **(Table 2)**. The cord blood from the mothers in the DHA
170 group also contained a slightly (40%) higher proportion of LA compared with the control group.
171 There was no evidence of a difference in the proportion of total saturated or total monounsaturated
172 fatty acids in the cord blood plasma phospholipids between the DHA and control groups.
173
174 *Determinants of cord blood DHA concentrations*
175 Cord blood phospholipid DHA abundance ranged from 3.4% to 11.8% of total fatty acids in the
176 control group (median 6.1%), and 3.1% to 13.7% of total fatty acids in the DHA group (median
177 7.5%). The strongest predictors of cord-blood DHA abundance in univariable analyses were total
178 number of DHA-rich intervention capsules consumed during pregnancy (R^2=0.2138, P<0.0001) and
179 gestational age at delivery (R^2=0.1612, P<0.0001). The importance of DHA supplement intake and
180 gestational age was confirmed by the multivariable analysis **(Table 3).** These predictors remained

highly significant in the multivariable model ($P<0.0001$ in both cases) and the R^2 of 0.35 from the model containing all predictors (**Table 3**) was comparable to the R^2 of 0.33 from the model containing only intake of DHA-rich supplement capsules and gestational age at delivery (data not shown).

The proportion of DHA in cord blood phospholipids increased with increasing gestational age ($P<0.0001$), such that for every one week increase in gestation, there was an estimated 7.48% (95% CI 6.61% to 8.36%) relative increase in the mean abundance of DHA in the cord blood phospholipids **(Table 3)**. However, there was considerable variability in cord blood DHA abundance in both the DHA and Control groups at any given gestational age (**Figure 1**).

Maternal factors significantly related to cord blood DHA abundance in univariable analyses included parity ($R^2=0.0074$), maternal intake of (non-DHA containing) dietary supplements ($R^2=0.0044$), maternal BMI at study entry ($R^2=0.0028$), Caesarean section ($R^2=0.0034$), clinical diagnosis of GDM ($R^2=0.0161$) and induction ($R^2=0.0104$). Infant sex was also a significant predictor of cord blood DHA ($R^2=0.0031$). However, each of these variables explained less than 2% of the variation in DHA concentrations (**Table 3**). There was no significant relationship between cord blood DHA and maternal smoking status, maternal education, birth weight z-score or clinical diagnosis of pre-eclampsia (**Table 3**).

Association of cord blood DHA with other cord blood fatty acids

The levels of DHA in the cord blood were positively related to total saturated fatty acids ($R^2=0.00470$, $P=0.0065$), and inversely related to LA ($R^2=0.0885$, $P<0.0001$) and AA ($R^2=0.0152$,

204 P<0.0001) content in the cord blood at delivery. Monounsaturated fatty acid content was also
205 inversely related to DHA content, and accounted for ~26% of the variability in DHA levels
206 (R^2=0.2650, P<0.0001). The proportion of LA (P<0.001) and AA (P=0.01) in cord blood
207 phospholipids decreased with increasing gestational age in both the DHA and control groups; for
208 every one week increase in gestation, there was an estimated relative decrease of 2.48% (95% CI
209 1.71% to 3.24%) for LA concentration and an estimated absolute decrease of 0.12% (95% CI 0.03%
210 to 0.20%) for AA concentration (data not shown).

211

212 **DISCUSSION**

213 The major finding of this paper was that despite the availability of a detailed set of maternal and
214 infant data from over 1500 pregnancies, and inclusion of a large number of clinical and lifestyle
215 variables in a multivariate analysis, the resulting model explained less than 35% of the variation in
216 cord blood DHA abundance. The only factors which explained a substantial proportion of the
217 variation in cord blood DHA were the number of DHA-rich intervention supplements consumed
218 over the course of pregnancy and gestational age at delivery, which together accounted for ~35% of
219 the variation. In contrast, all other variables which were significantly related to cord blood DHA,
220 i.e. maternal BMI, parity and intake of other dietary supplements, maternal diagnosis of GDM,
221 caesarean section, pregnancy induction and infant sex, each accounted for less than 2% of the
222 observed variation in cord blood DHA levels. These data suggest more than 65% of the variation in
223 cord blood DHA is explained by other, as yet unexplored factors, and highlights the need for studies
224 specifically aimed at elucidating other maternal, placental and fetal determinants of fetal DHA
225 supply.

226

As expected, supplementing pregnant women with ~800mg of DHA and 100mg EPA per day in the second half of pregnancy resulted in a significant increase in the n-3 LCPUFA abundance in cord blood phospholipids at the time of delivery. The relative increase in cord-blood DHA abundance in the supplemented group was ~23%, which is comparable with the results of other studies of maternal DHA supplementation using similar doses of DHA [7, 15]. The increase in n-3 LCPUFA levels in the cord blood of supplemented women was associated with a corresponding decrease in the n-6 PUFA content. The decrease in total n-6 PUFA concentrations was almost entirely accounted for by a decrease in the abundance of AA in the supplemented group, the magnitude of which was approximately equal to the increase in total n-3 LPCUFA (EPA, DPA, DHA) abundance. This is not unexpected given the competition which exists between the n-3 LCPUFA and AA for incorporation into plasma phospholipids, such that relative increases in the availability of n-3 LCPUFA reduce the incorporation of AA into this lipid fraction [16, 17].

While DHA abundance in the cord blood was increased by supplementation, there was a wide range (~3-4 fold) in cord blood DHA within each treatment group, and a substantial overlap of cord blood DHA abundance between control and supplemented women. In addition, while cord blood DHA levels were positively predicted by the total number of n-3 LCPUFA intervention capsules consumed during pregnancy, this only accounted for ~21% of the variation in cord blood DHA. It is possible that the n-3 LCPUFA content of the background diet and n-3 LCPUFA status of the mother before and during pregnancy, which was not assessed in the DOMInO women, may account for some of the remaining variation. However, since none of the women were taking n-3 LCPUFA supplements at the time of trial entry, and the average dietary intake of DHA in Australian women is <200mg/day [13], the increase in n-3 LCPUFA intake as a result of the 900mg/day n-3 LCPUFA

supplementation in DOMInO would be expected to substantially exceed the n-3 LCPUFA content of the background diet for the vast majority of the women. The background dietary intake of n-3 LCPUFA is therefore unlikely to have contributed significantly to this variability. Furthermore, our finding that the intake of n-3 LCPUFA in the form of supplements cannot fully account for cord blood DHA abundance confirms the findings of a number of smaller studies [5, 7, 8], and reinforces the suggestion that factors beyond maternal dietary DHA intake play an important role in determining fetal DHA levels.

In addition to maternal n-3 LCPUFA, maternal intakes of other fatty acids in the maternal diet, in particular n-6 PUFA, may also have contributed to variations in n-3 LCPUFA delivery to the fetus in late gestation. Higher maternal intakes of n-6 PUFA could have reduced n-3 LCPUFA status in the mother by increasing competition for cellular incorporation and therefore reduced the availability of n-3 LCPUFA for placental transfer, which is supported the inverse relationship between LA and DHA levels in the cord blood observed in the DOMInO women. The fatty acid composition of the maternal diet preceding and in the early stages of pregnancy also has the potential to contribute to variations in cord blood DHA content, since these fatty acids would be deposited in maternal adipose tissue stores, and could be released as rates of lipid turnover increase in late pregnancy [18]. Detailed assessments of the fatty acid composition of the maternal diet and/or maternal blood at different stages of pregnancy in future studies may provide additional insights into their contribution to the variations in fetal DHA supply.

The only identified variable other than n-3 LCPUFA supplement intake to explain an appreciable amount of the variation in cord blood DHA was gestational age at delivery, and this accounted for a

similar proportion of the variation (~16%) as supplement intake. DHA in the cord blood increased with advancing gestation, and this occurred at the same rate in both the control and supplemented groups. Increases in DHA abundance in cord blood phospholipids with advancing gestation has also been reported by others [19, 20]. A number of physiological changes which occur during pregnancy, including remodelling of the placenta, result in increased efficiency of placental DHA transport [3], which likely contributes to increases in cord blood DHA across gestation. While levels of DHA increased across gestation, the cord blood content of n-6 PUFA, in particular AA, decreased across this same period, and this may also have contributed to the proportional increase in cord blood DHA content.

There was still considerable heterogeneity, however, in cord blood DHA at any given gestational age that was not fully accounted for by any of the clinical and lifestyle variables which were assessed, suggesting that other factors which were not measured in DOMInO also make an important contribution to this variation. The results of this study raise the possibility that considerable inter-individual differences could exist in maternal, placental and/or fetal fatty acid metabolism, which could have significant implications for the relationship between maternal DHA supplementation and fetal DHA supply.

While the fundamental aspects of placenta fatty acid metabolism and transport have been described, there are few studies that have examined the extent to which are modified by other dietary/genetic/environmental factors. Variations in the efficiency of placental DHA transfer would be expected to modulate the relationship between maternal and cord blood DHA levels, and could potentially account for the greater degree of heterogeneity in DHA concentrations observed in cord blood as compared with those in the circulation of children and adults. There is evidence of aberrant

expression of fatty acid binding proteins in pregnancies complicated by GDM and intrauterine growth restriction [21, 22], but it is unclear to what extent these vary in healthy pregnancies. While the DOMInO study included largely healthy pregnancies, women with GDM had lower DHA levels in the cord blood, indicative of impaired placental transfer capacity, which is consistent with previous studies [23]. Interestingly, pre-eclampsia, which has been associated with altered maternal lipid metabolism in previous studies, was not a significant predictor of cord blood DHA in this study, however this may have been due to the relatively small number of pre-eclamptic women (~3%) in our sample.

It is important to note that since all fatty acids in the present study were expressed as a percentage of total lipids, rather than absolute amounts, the proportion of different fatty acids are not independent of each other, and we cannot entirely exclude the possibility that alterations to cord blood DHA were secondary to increases or decreases in the levels of other fatty acids. Thus, the inverse relationship between the level of DHA and monounsaturates in the cord blood, may indicate that decreases in monounsaturated fatty acid transfer/synthesis contribute to higher DHA content in cord blood. The positive relationship between DHA and saturated fatty acids, however, appears more likely to be indicative of an overall increase in the efficiency of placental transfer, since saturated fatty acids in the fetal circulation are largely derived from de novo synthesis [24].

Although the fetus can undoubtedly make use of maternal LCPUFA supplies, it also has the capacity for synthesising LCPUFA from 18 carbon precursors [25]. The inflow of LCPUFA (dietary and endogenous synthesis) together with possible active uptake of DHA by the placenta and fetal synthesis could all contribute to the higher DHA in cord vs maternal blood. Thus,

variations in any of these physiological/biochemical processes could contribute to the large variations in cord blood seen in this study.

Another factor which is increasingly being associated with fatty acid concentrations in both adults and infants is the genotype of individuals in relation to the genes encoding the two key desaturase enzymes in the PUFA metabolic pathway, FADS1 and FADS2. In a study of over 2000 mother-infant pairs from the ALSPAC study, both maternal and infant FADS genotype were related to DHA abundance in the umbilical venous cord blood at delivery [10]. However, while it is possible that the FADS genotype of the mother and child, which were not assessed in DOMInO, could have contributed to differences in cord blood fatty acid composition on, this is unlikely to be a major factor, given that previous studies have suggested that maternal/child FADS genotype accounts for only ~1% of the variation in cord blood n-3 LCPUFA concentrations [10].

Conclusion

In conclusion, we have reported cord blood DHA abundance for >1500 women from the n-3 LCPUFA supplementation DOMInO trial, and demonstrated a 3 to 4-fold variation in cord blood DHA concentration within each treatment group. In spite of the inclusion of detailed clinical and lifestyle data from the DOMInO women in our analyses, we were able to explain less than 35% of this variation and, therefore, the factors which explain the remaining ~65% variation in cord blood DHA remain to be defined. The findings of this study have relevance to current dietary recommendations in relation to DHA intakes in pregnant women, since they suggest that the same level of maternal DHA intake can translate into markedly different cord blood levels of this fatty acid. In addition, these data may help explain previous reports of variability in fetal and postnatal

342 outcomes following maternal n-3 LCPUFA supplementation. A more complete understanding of
343 the factors which determine the fatty acid transfer capacity of the placenta, the extent to which this
344 varies in healthy pregnancies, and the potential impact of maternal/fetal genotype on the response to
345 maternal n-3 LCPUFA will be valuable in assisting us in understanding the most important
346 determinants of fetal n-3 LCPUFA supply and, ultimately, designing personalised supplementation
347 regimens.
348
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Conflict of interest statement

The authors have no conflicts to declare

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425 **Table 1.** Characteristics of women with cord blood samples by treatment group

Characteristic	**DHA (n=798)**	**Control (n=773)**	**Total (n=1571)**
Intake of DHA Supplements: Median (IQ range)	360.0 (303.0-402.0)	0.0 (0.0-0.0)	0.0 (0.0-360.0)
Smoker: N (%)	232 (29.1)	236 (30.5)	468 (29.8)
BMI at Study Entry: Median (IQ range)	26.4 (23.5-30.8)	26.3 (23.0-30.6)	26.4 (23.3-30.7)
Nulliparous: N (%)	311 (39.0)	314 (40.6)	625 (39.8)
Consumed non-DHA Dietary Supplements: N (%)	437 (54.8)	421 (54.5)	858 (54.6)
Completed Secondary Education: N (%)	505 (63.3)	507 (65.6)	1012 (64.4)
Completed Further Education: N (%)	547 (68.5)	546 (70.6)	1093 (69.6)
Gestational Age at Birth: Median (IQ range)	39.9 (39.0-40.7)	39.6 (38.7-40.4)	39.7 (38.9-40.6)
Caesarean Section: N (%)	210 (26.3)	218 (28.2)	428 (27.2)
Clinical Diagnosis of Pre-eclampsia: N (%)	23 (2.9)	22 (2.8)	45 (2.9)
Clinical Diagnosis of GDM: N (%)	58 (7.3)	49 (6.3)	107 (6.8)
Induction: N (%)	264 (33.1)	212 (27.4)	476 (30.3)
Birthweight Z-Score: Mean (SD)	0.3 (1.0)	0.3 (1.0)	0.3 (1.0)
Infant Male Sex: N (%)	402 (50.4)	373 (48.3)	775 (49.3)

426

427 **Table 2.** Comparison of fatty acid composition of cord blood phospholipids in the DHA and
428 placebo groups[#]
429

Fatty Acid (% total fatty acids)	DHA (n=798)	Control (n=773)	P-value
16:0	29.9 (29.1-30.7)	29.9 (29.1-30.6)	0.54
18:0	15.2 (14.3-15.9)	15.1 (14.2-16.0)	0.58
Total Saturates	48.3 (47.5-49.4)	48.3 (47.6-49.7)	0.29
18:1n-9	7.7 (7.0-8.5)	7.6 (6.9-8.3)	0.11
Total Monounsaturates	12.5 (11.5-13.6)	12.5 (11.4-13.6)	0.96
LA, 18:2n-6	7.4 (6.5-8.4)	7.1 (6.2-8.2)	0.0042
AA, 20:4n-6	14.8 (13.8-16.2)	16.6 (15.4-17.7)	<.0001
Total n-6 PUFA	29.3 (27.7-30.6)	30.7 (29.6-31.9)	<.0001
EPA, 20:5n-3	0.5 (0.3-0.7)	0.3 (0.2-0.3)	<.0001
DPA, 22:5n-3	0.5 (0.4-0.6)	0.5 (0.3-0.6)	<.0001
DHA, 22:6n-3	7.5 (6.3-8.9)	6.1 (5.2-7.2)	<.0001
Total n-3 PUFA	8.8 (7.4-10.3)	7.1 (6.1-8.3)	<.0001

430 *#all values expressed as median (interquartile range) % total fatty acids*

431 **Table 3.** Effect of potential predictors on cord blood DHA content

Predictor	Comparison	Univariable Analyses			Multivariable Analysis	
		Effect (95% CI)[+]	P-value	R^2	Effect (95% CI)*	P-value
Maternal Factors						
Intake of DHA Supplements	Increase of 1 week's intake^	1.0134 (1.0121, 1.0147)	<.0001	0.2138	1.0122 (1.0109, 1.0134)	<.0001
Smoking status pre-pregnancy	Smoker vs non-smoker	0.9787 (0.9527, 1.0055)	0.1179	0.0016	1.0034 (0.9796, 1.0276)	0.7834
BMI at study entry	Increase of 1kg/m^2	0.9978 (0.9958, 0.9999)	0.0376	0.0028	1.0000 (0.9982, 1.0019)	0.9690
Parity	≥1 vs 0	0.9572 (0.9335, 0.9816)	0.0007	0.0074	0.9812(0.9597, 1.0032)	0.0929
Intake of (non-DHA) dietary Supplements	Supplements vs none	1.0339 (1.0086, 1.0598)	0.0083	0.0044	1.0141 (0.9927, 1.0359)	0.1976
Completion of Secondary Education	Completed vs not completed	1.0235 (0.9975, 1.0502)	0.0771	0.0020	1.0239 (1.0005, 1.0478)	0.0451
Completion of Further Education	Completed vs not completed	1.0098 (0.9830, 1.0372)	0.4780	0.0003	1.0043 (0.9814, 1.0278)	0.7144
Pregnancy Factors						
Gestational Age at birth	Increase of 1 week	1.0748 (1.0661, 1.0836)	<.0001	0.1612	1.0612 (1.0528, 1.0697)	<.0001
Caesarean Section	Caesarean vs no caesarean	0.9680 (0.9416, 0.9952)	0.0213	0.0034	0.9994 (0.9760, 1.0234)	0.9625
Clinical Diagnosis of pre-eclampsia	Pre-eclampsia vs no pre-eclampsia	0.9843 (0.9141, 1.0600)	0.6758	0.0001	1.0357 (0.9709, 1.1049)	0.2868
Clinical Diagnosis of GDM	Diabetes vs no diabetes	0.8818 (0.8400, 0.9258)	<.0001	0.0161	0.9326 (0.8941, 0.9727)	0.0012
Induction	Induced vs not induced	1.0568 (1.0290, 1.0855)	<.0001	0.0104	1.0042 (0.9804, 1.0285)	0.7342
Infant Factors						
Birth weight Z-Score	Increase of 1 SD	0.9906 (0.9784, 1.0030)	0.1367	0.0014	0.9856 (0.9750, 0.9962)	0.0082
Infant Sex	Female vs male	0.9728 (0.9491, 0.9971)	0.0284	0.0031	0.9753 (0.9553, 0.9956)	0.0175

432 * R^2=0.3467 for multivariable model

433 ^ Equates to 21 treatment capsules each containing 800mg DHA and 100mg EPA

434 [+] Effects have been back-transformed to give ratios of geometric means on the original scale

435

436 **FIGURE LEGENDS**

437 **Figure 1.** The relationship between DHA content in the cord blood phospholipids (expressed as a percentage of total fatty acids) and
438 gestational age at delivery in the Control (open circles) and DHA (closed circles) groups. Lines are estimated geometric mean cord blood
439 DHA concentrations in the DHA group (solid line) and control group (dashed line).