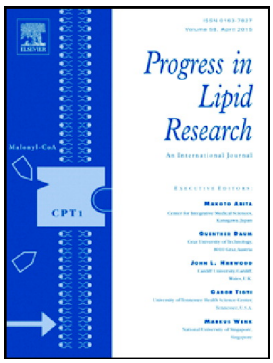

The imperative of arachidonic acid in human reproduction

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PII: S0163-7827(23)00012-7

DOI: https://doi.org/10.1016/j.plipres.2023.101222

Reference: JPLR 101222

To appear in: *Progress in Lipid Research*

Received date: 17 November 2022

Accepted date: 26 January 2023

Please cite this article as: M.A. Crawford, A.J. Sinclair, B. Hall, et al., The imperative of arachidonic acid in human reproduction, *Progress in Lipid Research* (2023), https://doi.org/10.1016/j.plipres.2023.101222

17/11/2022

THE IMPERATIVE OF ARACHIDONIC ACID IN HUMAN REPRODUCTION

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Abstract

We are presenting new evidence on essential fatty acids (EFA) in prenatal human development.
We have demonstrated, for the first time, the detailed process of active selection of some fatty acids by the placenta (biomagnification) and rejection of others (bioreduction) and how this strategy is of supreme importance for understanding of the biology of human reproduction.
The biomagnification process by the placenta is dominated by arachidonic acid (ArA) and its allies: di-homo-gamma-linolenic acid (DGLA), adrenic acid and ω6 docosapentaenoic acid. Stearic acid is similarly bio-magnified which is likely to provide for the sn-1 position in membrane synthesis. In contrast there is a bioreduction of oleic, linoleic and all ω3 precursors for docosahexaenoic acid (DHA), including eicosapentaenoic acid (EPA). Although DHA is biomagnified, the amplification from mother to fetus is small compared to ArA.

We report on the dominant compartmentalisation of ArA from mother to fetal plasma, cell membranes of red cells, mono-nuclear cells, endothelium and the placenta. We conclude that ArA and its allies, play a paramount role in the development of the products of conception. It is plausible that inadequate provision of ArA may be relevant to the neuro-vascular complications of prematurity and neurodevelopmental disorders associated with premature birth. We present evidence of ArA's universal role from an identical arachidonic acid-based strategy observed in contrasting cultures. The dominance of ArA in the prenatal and in post-natal nutritional provision by human milk makes a compelling case for re-evaluation of its role, especially in reproductive biology.
(245 words).

Key words: Arachidonic acid (ArA), di-homo-gamma-linolenic acid (DGLA), adrenic acid, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), stearic acid, linoleic acid, preterm birth, placenta, fetus. biomagnification, bioreduction, complications of prematurity, neurodevelopmental disorder.

1 Introduction.

1.1. Biochemical sex difference prenatally

The brain is a membrane and hence lipid rich organ. The majority of brain cells divide before birth. DHA and ArA are the principal, highly conserved polyunsaturated fatty acid (PUFA) components of brain lipids (1). Our recent studies using magnetic resonance imaging (MRI) of the new-born brain, revealed a striking difference between the prenatal response of the boys and girls to a maternal DHA (omega 3) rich supplement from the first booking for pregnancy care. Several regions of the boys' brains were enhanced but there was no detectable effect on the girls.

We have now analysed the relationship between maternal lipid status and brain scans in girls and boys whose mothers were not receiving any supplement. It turns out that there were significant relationships with various regions of the brain in the girls and maternal lipid status linked to ArA but not with omega 3 fatty acids. There was little or no connection with ArA in the boys, implying a powerful, biochemically driven sex difference operating prenatally on neurodevelopment. Interestingly we published a striking sex difference to essential fatty acid deficiency in a Nature paper in 1974 (2). The survival of the boys prenatally was specifically affected. Whilst there are known sex differences in certain pathologies such as autism and cardiovascular disease, the difference prenatally has received scant attention other than for gross, anatomical differences of prenatal brain development.

There is presently a strong focus favouring omega 3 in nutrition and health, immune, cardiovascular and many other health issues. ArA, however, is generally seen as pro-inflammatory and deleterious to health. This is despite the 1982 Nobel Prize for Bergstrom, Samuelson and Vane highlighting the properties of ArA's oxidative derivatives functioning in the regulation of blood pressure, blood flow, inflammation and the resolution of injury (3, 4). Reviewing our data on reproduction illustrates a dominance of ArA and its long chain, ω 6 allies in the vascular, immune systems and placental structures and their biomagnification to feed the fetus. These are the principal engines of organogenesis and fetal growth and development. The data suggests there is an important role for ArA and its allies which needs re-assessment. Our findings do not in any way diminish the indispensable role of DHA in human biology but add ArA as a vital and equal partner.

1.2 Optimum Physics Hypothesis

The term "OPH" (Optimum Physics Hypothesis) was coined by the late Myer Bloom, professor of physics at the University of British Columbia, to describe the conditions of the cell membrane pertinent to its optimal function. (5) Different cell types and intracellular organelles, would have different optimal compositions depending on their functions. This is a complex issue, but the principle is made clear by studies of the cell membranes of different cell types and the subcellular organelles, with their different fatty acid compositions and different functions. In this paper, we wish to share data from our studies in pregnancy in which the placenta provides an example of Nature providing the OPH for fetal development and extended to human milk. This conclusion challenges the common view on fatty acids and biological health.

In 1976 a report in the Lancet (6), we described the biomagnification of ArA and DHA by the placenta for the human fetal circulation. Since then, several reports have confirmed this principle. The brain is the priority of human fetal development, consuming some 70% of the energy devoted to fetal growth. The original assumption was that DHA would be the primary focus of the transfer from mother to fetus. This was not the case. It was noted that contrary to the expectation, there was powerful biomagnification of ArA, substantially greater than for DHA.

The biomagnification process left the fetus to biomagnify the DHA proportion, from crossing from the umbilical vein into the liver, assembly into high density lipoproteins and then crossing the blood brain barrier to the high levels consistently reported in the brain (see Figures 1 and 5). That is, the proportion of DHA in the maternal circulation is magnified by several transfers across membranes. ArA by contrast is highly conserved by being transported in one bulk from mother to fetus by the placenta Tables 1,2, Figure 6. The plasma choline phosphoglyceride data is particularly relevant to fetal utilisation and more revealing than the RBC data (Table 7).

Moreover, the ArA precursor, linoleic acid, is processed in the reverse manner with its proportion in the fetal plasma choline phosphoglycerides being half that on the maternal side (Table 7). It is treated like a waste product, rejected by the fetus and sent in the umbilical arterial circulation to the mother by bioreduction. The same is true of all DHA precursors, with the small proportions of α-linolenic and EPA also rejected by the fetus. At the same time our data will show that the saturated fatty acids are bio-magnified and the monounsaturated are bioreduced. This represents a formidable, selective action that has evolved in mammalian evolution for prioritising nutrients for fetal nourishment: the OPH operating on a grand scale. The reason for the biomagnification of stearic and other saturated fatty acids is likely to satisfy the occupation of the SN1 position in the lipids of the growing, cell membranes.

By contrast, a primary interest in the essential fatty acid (EFA) field has recently been on EPA. Studies on the prevention of coronary thrombosis, (7) mental ill health in children (8), bi-polar disorder (9), pregnancy outcome, (10, 11) and benefits to lifelong health being attributed to EPA and DHA and often more to EPA (12) Yet it is rejected by the fetus..

At the same time ArA has carried the blame for inflammation and its common, chronic disorders such as rheumatoid arthritis (RA). However, drugs designed to supress cyclo-oxygenase activity on ArA, allegedly responsible for RA, resulted in increased mortality and had to be withdrawn (14). It is likely that the COX2 inhibitors were also inhibiting prostacyclin synthesis. Prostacyclin is synthesised from ArA via COX 2 by activation of phospholipase, aided by the continuous contraction and relaxation of the arterial endothelium. Prostacyclin maintains good blood flow and prevents spontaneous, platelet aggregation (15). Moreover, ArA oxidative products enhance immune function in the response to injury, help seal ruptured blood vessels, assist in the resolution of inflammation and tissue injury (16). For the most part, the focus has been on its role as a precursor of inflammatory mediators.

The message from biomagnification and bioreduction is a statement of approval for ArA in these circumstances. We wish to present the analysis of maternal/fetal, lipid transfers, demonstrating the high selectivity and specificity for fatty acids to serve reproduction.

2. Data validating the Optimum Physics Hypothesis (OPH).

2.1 Red blood cell (RBC) fatty acid composition at recruitment, delivery and cord blood

The fatty acid analysis of the red cell is essentially that of its plasma membrane. The lifespan of the red cell being 120 days, it integrates nutrition and other influences over this time. Hence, the lipid composition of RBC membrane is the result of the maternal diet, behaviour (e.g. exercise, smoking, alcohol, stress), competition between dietary fatty acids, absorption efficiency, metabolomics including oxidation to CO2, fatty acid desaturase (FADS), compartmentalisation into neutral, polar/non-polar lipids with their various partitions and selectivity for membrane synthesis and maintenance with genomic variations in all compartments.

This highly complex, biological integration of impact from ingestion to expression in the cell membrane composition, provides a useful profile of the “maternal lipid status”. Although diet and genomics play basic roles both with regard to the amount of the EFAs and the competing/enhancing

dietary factors, it is the end product in the membrane that ultimately matters: "*the tissue is the issue*".[a]

TABLE 1 Recruitment, delivery and cord blood RBCs showing the principal bidirectional selectivity by the Placenta. FOSS1 (Fish Oils Supplement Study 1) data.

Table 1 presents compositional data of principal RBC fatty acids determined in healthy women from FOSS1[b]. The change from recruitment to delivery identified a notable increase in oleic acid and all monounsaturated fatty acids (MUFA). There was a reduction in ArA, DGLA and adrenic acids but no change in DHA. We found that RBC membrane oleic acid was a powerful predictor of preterm birth (13). Membrane oleic acid is known to increase in response to a fall in long chain PUFA, hence the two opposite movements from recruitment to delivery are consistent. The biomagnification process for ArA and its allies, from mother to fetus, are also consistent with a drain by the needs of the fetal cardiovascular, immune system, muscle growth, placental growth and biomagnification. By contrast linoleic, oleic acids and all DHA precursors were bioreduced.

It is interesting that the proportion of ArA is about twice that of DHA in the mother and nearly three times in the fetus. This contrasts with the biomagnification of DHA which is minimal compared to that of ArA. Distinct from the bioreduction of DHA precursors, all the long chain ω6 fatty acids, C20:3ω6, C20:4ω6, C22:4ω6 and C22.5ω6 were biomagnified. Biomagnification of DHA that we reported in 1976 (6) was for plasma choline phosphoglycerides. In the total RBC fatty acids, it was not significant, but then the turnover of fatty acids in RBCs is long-term compared to the plasma CPG. It is interesting that nervonic acid (C24:1ω9) like oleic acid, was bioreduced whilst lignoceric acid (C24:0) like stearic acid, was biomagnified, consistent with all the other saturated fatty acids except for the small amount of myristic acid (C14:0) which seldom features in cell membranes but is a useful energy source.

The composition of the plasma is quite different from that of the RBC. Table 2 presents data comparing total fatty acids in maternal and cord plasma for comparison to the red cell in Table 1.

TABLE 2. Selectivity for OPH Fatty acid composition (%) of plasma from healthy mothers at delivery and cord plasma

[a] A comment coined by Bill Lands for which we are everlastingly grateful.

[b] FOSS1 was a study of 300 pregnant women at Chelsea and Westminster Hospital: ISRCTN 240687; on line ahead of print DOI: https://doi.org/10.1016/j.plefa.2018.09.001

As regards to the MUFA, there is again selectivity being expressed. Unlike the strong bioreduction of oleic acid, nervonic acid is biomagnified in plasma but not in RBC. Plasma is a pot shot at the time of birth whereas the RBC has historical value.

TABLE 3 Comparison of RBC and plasma

In Table 3 we compare red cell and maternal plasma from a different set of mothers at term, but at the same catchment area in the Chelsea and Westminster Hospital. Every fatty acid is either increased or reduced in the RBC to a highly significant degree ($p < 0.005$ or better) except for C20:2ω6 and C22:2ω6. Stearic acid was strongly selected for the RBC (RBC 14.1% and plasma 5.52% $p<0.001$) with a striking selection for lignoceric in RBC (14-fold). Oleic acid was deselected (RBC 12.4% plasma 23.4% $p<0.001$) but nervonic acid was selected (7-fold). There was also a strong selection for ArA and DHA in the RBC, both being similarly about 2.6 times at a higher proportion in the RBC versus the plasma reflecting the CPH. Whilst there is selection for RBC DHA, there is none for EPA and indeed both RBC and plasma contain relatively little. Opposite to ArA there was a striking bio-reduction of linoleic acid. (8.31% in RBC but 24.4% in plasma $p<0.001$).

TABLE 4 Correlations BWT Spearman's correlation coefficient (r_s) and P values for correlations between proportions of fatty acids (%) and gestational age at delivery and Aberdeen birth centiles, in the placentae

In addition, in this study an analysis was conducted looking for any correlations with gestational age and placental fatty acid status. Table 4 summarises the correlations for placental fatty acid composition with subsequent gestational age, and Aberdeen birthweight centiles which were determined using Nomograms developed according to data from the Aberdeen Maternity and Neonatal Databank. The nomograms allow the determination of a specific centile as opposed to a centile group and takes into account ethnicity, parity, gestational age at delivery, and gender. Placental adrenic acid (C22:4ω6) and its precursor ArA were negatively correlated with gestational age at delivery (r_s= -0.29, p =0.01; -0.30, p=0.01, respectively). In addition, adrenic acid and ω-6 DPA were negatively correlated with birthweight centiles (r_s= -0.36, p =0.01; -0.46, $p<0.0001$, respectively). DHA, as has been reported before (17), was associated with gestational age. There was no independent relation with birthweight in our study other than that for adrenic acid, 22:5ω6

and the 22:5/22:4ω6 ratio. The latter is an indicator of an insufficiency of DHA (18). These ratios are often more sensitive than the individual fatty acids (19).

TABLE 5 Plasma and RBC fatty acids

2.2 Plasma Choline Phosphoglycerides (CPG) Maternal-Fetal. Hackney

To provide more specific evidence regarding the C20 and C22 long chain polyenoic fatty acids we report data taken from a different set of mothers in the East End of London in Table 5, where we have data on the maternal and fetal, cord plasma choline phosphoglycerides (CPG), RBC choline and ethanolamine phosphoglycerides. The differences with the saturated and monounsaturated are the same as above for plasma versus RBC but are not shown to simplify the presentation to focus on ArA and its allies.

There are significant differences across the board, and we again see the biomagnification of ArA across the placenta in the plasma CPG (maternal 8.35%± 1.93, cord (fetal) 17.6% ± 1.9, p<0.0001). DGLA, adrenic and the ω6 docosapentaenoic acids were all significantly biomagnified for the fetus, in all fractions.

The CPG are the principal source of the ω6 and ω3 fatty acids in the outer leaflet of the cell membrane. In the plasma CPG of fetal cord blood, the proportions of the long chain ω6 fatty acids are three times that of the corresponding long chain ω3 with neither ethanolamine phosphoglyceride EPA nor the ω3 docosapentaenoic acid being biomagnified and if anything, reduced or unchanged. DHA was significantly biomagnified, but this was only seen in the maternal-fetal plasma CPG (4.14± 1.51 compared with 7.16± 2.13 p<0.0001).

TABLES 6 and 7 Plasma CPG and RBC from Vietnam and Thailand.

To assess if the evidence presented above on arachidonic acid biomagnification and its dominance is not the consequence of a skewed UK diet or genomic status, we take the opportunity to present data obtained from Vietnam (RBC EPG compared to CPG). In Table 6 we show data of plasma CPG from the Vietnamese mothers to illustrate the similarity with our local data in Table 1. The biomagnifications are close to identical with similar proportions.

The mothers in the refugee camp, in Karen, Thailand had come from Vietnam and the study was done as part of an investigation into visual problems in the children in the Karen refugee camp (20) We have summarised the principal data for the polyenoic fatty acids and included stearic acid, in Table 7 which summarises key data comparing the mothers from Hackney in the East-end of London with those from Thailand and Vietnam. All sets of data confirm the dominance and fetal biomagnification of stearic acid, ArA and its allies and bioreduction of linoleic acid and DHA precursors. Despite the differences in diet and living conditions, the transfer of EFAs to feed the fetus are remarkably similar in all three locations.

TABLE 8 Placental fatty acids

2.3 Placenta

Early and Term Placentae Compared.

The placental tissue data in Table 8 summarises the consistent and strong presence of ArA and its allies (C20:3ω6, C22:4ω6, C22:5ω6) in the principal phosphoglycerides in early preterm and term placentas. The data here again, largely represent the placental tissue membranes. The placenta develops ahead of the fetal brain thrust of the last trimester. Our starting point is the placenta itself. These data are from mothers with no adverse health issue, the early data being obtained from elective abortions before 22 but mostly around 14 weeks and less, after conception. The mothers were from the Newham General Hospital[c] in the East end of London. Our data show that the human placenta captures remarkably high proportions of ArA and its allies from its early inception. It is consistent with several lines of evidence supporting an important role for di-homo-gamma-linolenic, ArA and adrenic acid and their metabolites in the cell regulation (21) and in the present context in the mediation of metabolic and endocrine function of ovarian and placental cell membranes and in the establishment and maintenance of pregnancy. Experimental studies have demonstrated that ArA and its eicosanoid metabolites are involved in follicular development and ovulation and corpus luteum function. (22, 23).

Placental Choline Phosphoglycerides.

The data from the placental choline phosphoglycerides for the early and term placenta makes the same point as seen in red cells versus plasma and maternal versus cord bloods. We saw a dominance of ArA and its allies in the placental cell membranes. There was remarkably little DHA,

[c] The hospital is a part of the St Bartholomew's NHS, Trust.

EPA or ω3 docosapentaenoic acid (ω3DPA). Moreover, compared to the early placentas there was a reduction in ArA (18.9±3.12 vs 17.2±2.44 p<0.01), adrenic, the ω6 docosapentaenoic acid, but an increase in DGLA (2.41±0.57 vs 4.00±0.71 p<0.0001) in the term placentas.

Placental Ethanolamine Phosphoglyceride (EPG).

The differences between the early and term placental EPG were minimal. The EPG is the main home for the long-chain PUFA and is in the inner leaflet of the bi-layer. There was a reduction in ArA, adrenic acids and ω3DPA at term. In contrast, there was significantly more DGLA in the term placentas (1.92±0.50 vs 3.47±0.56 p<0.0001) (see comment above). DHA was present but at less than a third of the proportion of ArA and was no different in the early compared to the term placental EPG. It was better represented than in plasma and RBC's (8.74±1.8 vs 8.87±1.99 ns) although in the early placenta, all C20 and C22 ω6 were present at nearly 4 times the total C 20 and C22 ω3 fatty acids.

Placental Serine Phosphoglycerides (SPG).

The serine phosphoglycerides might be expected to be rich in DHA in both early and term placentas, but this was not the case. However, contrary to expectations from general membrane data, ArA and its acolytes remained dominant. In the early placenta SPG the ArA proportion was 11.2% compared to 3.88% for DHA which is usually found to dominate SPG (especially in neural tissue). The major difference was in DGLA (3.96%±0.91 compared to 9.15%±1.44 P<0.000). There was no detectable difference between early and term ArA although there was a reduction in its elongation products. The expression of SPG in the placental membranes regulates the formation of the syncytiotrophoblast, and ArA represents a large proportion of the syncytiotrophoblast lipids. DGLA is seldom discussed but here it is present in significantly increased proportions at term which needs to be noted and explained.

Placental Inositol Phosphoglycerides (IPG)

The inositol phosphoglycerides in contrast to the SPG above, are expected to be rich in ArA. Indeed, ArA and all long chain ω6 LCPUFA account for 42% of the fatty acids whereas the LCPUFA ω3 amount to 3.7% so the ω6 LCPUFA is 12 times the proportion of the ω3LCPUFA in the IPG. Note again the higher proportion of DGLA at term with the lower ArA and adrenic acids. These differences seem common to all the phosphoglycerides and are unrelated to prematurity as the mothers were all in good health. As stated, the early placentas were from elective abortions of 14 weeks or shorter gestation and from women in good health.

The results falsified the idea that the level of ArA would increment with time to a critical point for eicosanoid participation in delivery. The early placenta ArA was, if anything, marginally higher than term. Perfusion of the isolated lobe of the human placenta with ArA did not elicit any eicosanoid production. However, when squeezed with alternating air pressure, substantial prostanoid production was observed suggesting the prostaglandin response to naturally induced contractions would provide a failsafe mechanism. Once started, delivery would be assured (24).

The inositol phosphoglycerides are of special interest. On phosphorylation they give rise to a family of seven phosphoinositides which have a wide array of cell regulation functions including control of cell proliferation, migration, survival and differentiation, all pivotal in developing the products of conception, adding to the plethora of biological control functions attributable to ArA and its various, cell regulatory products (25).

The data in Table 8 imply that the changes in placental composition from early appearance of the placenta to delivery at term are minimal but none-the-less significant. If anything, ArA in the choline, ethanolamine and inositol phosphoglycerides is at a higher percentage in the early placentas (EPG 28.5 cfd 24.7, and in the IPG 35.9 cfd 31.9 $p<0.001$). There is also a significant difference in the CPG for the ArA precursor being greater at term (20:3ω6, 2.41vs 4.00 $p<0.001$) whereas ArA was less (18.9% cfd 17.2% $p< 0,005$).

The astonishing accumulation of DGLA reported from the early to the term placentae in all phosphoglyceride fractions requires comment. In all the phosphoglycerides fractions reported, ArA, adrenic and the ω6 docosapentaenoic acid were less at term and DGLA was greater. A plausible implication is that the velocity of maternal ArA synthesis is stretched with the supply of ArA and its products, being challenged by the demands of placental and fetal growth. The placenta processes great lakes of blood through its extensive endothelial system supporting the placental terminal villi, which are the functional unit of maternal-fetal oxygen exchange and nutrient transport. It is plausible that the increment in DGLA is also contributing to the eicosanoid protection of blood flow through prostaglandin E1 (PGE1). The change in ArA in the choline and serine phosphoglycerides are trivial yet the reduction in the inositol and ethanolamine phosphoglycerides were significant. This theoretically could have influenced prostacyclin synthesis. A deficit could likely have been made good by PGE1. The consistent increase in DGLA, suggests the velocity of placental growth is tending to outstrip the metabolic conversions (see later Tables 13 and 14).

An open mind needs to be held regarding cause and effect. A report in the preterm placenta suggested that chorioamnionitis was the cause of elevated ArA in the preterm placenta (26). Demlis reported 25.9% total phospholipid ArA in preterm placenta with chorioamnionitis and 20% in preterm without the presence of chorioamnionitis. (27). An increase in ArA is usually associated with a reduction in DHA. Whilst we have focussed on ArA, DHA still remains as an important ally in the developmental process.

Powell et al (1999) analysed the fatty acids in the total phospholipid of the polar brush border or microvillous membrane (BBM or MVM) and basal membrane (BM) of syncytiotrophoblasts. Their study suggested that the ArA content is relevant to the syncytiotrophoblast membranes that comprise the epithelial barrier to transport across the human placenta (28).

2.4 Immunity: Cord Blood Mononuclear Cells (CBMC)

Survival of the fetus and indeed the demand for the immune system during pregnancy and at birth requires a competent system to maintain the pregnancy and then meet the real-world challenge of moving from a sterile environment, with the cutting of the umbilical cord, the assault from maternal body fluids and the open-air environment. Here again we find the biological emphasis is on ArA seen in the CPG and EPG, the two principal phosphoglycerides in Tables 9 and 10. Moreover, in ex vivo experiments, ArA has been found to support mono-nuclear cell function (29) .

TABLES 9 and 10 IMMUNE CELLS CPG and EPG

The mono-nuclear cells isolated from cord blood at birth, can be expected to represent the prenatal state. As with the placenta, ArA and its allies dominated. Even in the EPG fraction which has the highest proportion of DHA at 6.61% in term cord blood mono-nuclear cells (CBMCs), the proportion of ArA is 6-times greater than DHA which is similar to the ratio in CBMC CPG at 9-times.

Notably there were significant differences between term and preterm CBMCs fatty acids. ArA in the EPG at term was 42% compared to 39% ($p<0.05$) in the preterm. DHA was 6.1% compared to 3.47% ($p<0.001$). The difference was also reflected in the CBMC CPG in which ArA was 16.3% compared to 13.3% in the preterm group ($p<0.05$). DHA was similarly lower (1.83% cfd 1.09% $P<0.01$). The ex-vivo studies suggested the preterm cells were less competent[24]. Preterm infants are

known to be more susceptible to infection and disorders in which membrane integrity is likely to be relevant (30,19,38)/

2.5 Endothelium

The vascular endothelium has the highest membrane to cytoplasm ratio of any other single cell type. Indeed, in the human, there are a trillion or more endothelial cells lining the blood vessels and it becomes the largest single cell type mass weighing an aggregate of at least 1 Kg. (31). Laid lengthways, cell to cell, they would stretch for 60 miles. Whist these are data for adults, the velocity of prenatal endothelial development must be high and as mentioned earlier will be achieved before, and assumedly in preparation for the fetal brain growth thrust of the last trimester.

At the same time, the cell's plasma membrane is the first point of contact with the external environment. Membrane proteins account for about one third of all the proteins for which there is currently a detailed coding. The proteins are compositionally and precisely determined by the genome. By contrast, the composition of the lipid component is susceptible to nutritional and other external influences. Moreover, the composition of the lipid affects membrane protein function and signalling (32). A major variable in membrane composition is diet especially as regards the EFAs and their membrane derivatives. This places the membrane lipid at the forefront of the environmental and nutritional interactions. Whilst there is a paucity of data on the endothelium prenatally, a small study of differences in birthweight reported data from cord endothelium in which the membrane phosphoglycerides were dominated by ArA and its allies. Hugh Sinclair proposed that atherosclerosis was an EFA deficiency (33). A principal sign of EFA deficiency is water loss through the skin, basically due to leaky cell membranes. Leaking vascular endothelial cell membranes is consistent with what is known about the pathogenesis of atherosclerosis. Endothelial dysfunction is characterized by reduced vasodilation (34) likely caused by reduced prostacyclin. Any rupture of the endothelium will result in exposure to collagen, release of phospholipase A2, free ArA with its eicosanoids and the response to injury. The breakdown of unstable endothelium in the brain would be expected to lead to ischemia and reperfusion injury.

2.6 Astrocytes and ArA

The fatty acid data on astrocyte phospholipids, presented by Christine Bénistant et al (35), showed that the sum of DGLA, ArA and adrenic acids was about 4 times greater than all long chain ω3 with the ArA/DHA ratio being 4.8 (see Figure 1). There are more astrocytes in the brain than neurons. They play an important role in the brain including glutamate, ion (i.e., Ca2+, K+) and water

homeostasis, defence against peroxidation and myelin formation and maintenance. They can also be thought of as the brain's immune system (36). Whilst the neurons and synapses may have more DHA than ArA, the quantitative dominance of the astrocytes and their pivotal role in brain health, should be a matter for consideration.

2.7 Fatty acids as markers of prematurity.

In Figure 1 we illustrate the polyenoic acid content of human aortic endothelium to illustrate again the dominance of ArA. The trace of a capillary gas-liquid chromatogram of the endothelial cells scraped from the cord of very low birthweight, preterm infant is presented in Figure 2. The image shows the high proportion of ArA. It importantly shows a large peak for the Mead Acid (C20:3ω9), the characteristic biochemical indicator of essential fatty acid deficiency (37). It also shows a similarly large peak of the Mead Acid elongation product C22:3ω9 (19). which has hardly ever been reported. This surely signifies a significant deficit of EFA and likely their long chain derivatives.

Mead Acid accounted for 6.4% and its elongation product (C22:3ω9) 3.6%, that is 10% of the total fatty acids in this very preterm infant's endothelium. These are extraordinarily high proportions and raise the question as to the role of EFAs in fetal nutrition and the stability of the endothelium referred to above. There were also significant levels of both these fatty acids in normal term births, in the order of 2% and 1%, respectively. The triene/tetraene ratio for the ratio of Mead acid to ArA has been used as a quantitative assessment of the degree of EFA deficiency. Although a small study of 14 births of different birthweights there were none-the-less strong correlations with birth weight and head circumference (e.g. endothelial EPG and birthweight 20:3ω9/20:4ω6 with a Pearson coefficient at −0.87 t value 6.46 n= 14 p <0.001 and head circumference −0.83 t= 5.14 n=12 <0.001) (38).

The umbilical endothelium is a piece of fetal tissue and although it is a throw-away tissue, it seems to us as a highly significant message regarding prematurity. If it reflects the state of the endothelium in the developing brain, then that is a matter of serious concern. Further research is needed to properly assess the significance of the Mead acid in the umbilical endothelium of preterm infants in relation to the high prevalence of neurodevelopmental disorders and cerebral palsy in prematurity.

Interestingly, oleic acid is the precursor of the Mead acid and is a marker of preterm birth, it also is a biomarker for low levels of long chain PUFA (13, 38). It is interesting that in the comparisons in all the Tables, oleic acid and most MUFA are raised when ArA is lowered and vice versa.

The primary focus on the vascular system serves organogenesis and eventually the prodigious energy needs of the late fetal brain growth thrust. The complications of prematurity including peri-ventricular leukomalacia (PVL) intraventricular haemorrhage (IVH), hypoxic ischemic encephalopathy (HIE), retinopathy prematurity (ROP), broncho-pulmonary dysplasia (BPD) and necrotising enterocolitis (NEC), all have a background of failure of vascular integrity and immune activity. ArA is almost certainly involved in the earliest cardio-vascular development and angiogenesis delivering the nutrients for organogenesis and then in promoting and maintaining rapidly expanding vascular networks with the needed regulation of function through prostacyclin possibly assisted by DHLA and PGE1. In an MRI study of intrauterine growth restriction (IUGR) evidence was presented implicating substandard blood flow (39).

ArA has had a poor press in the well-known claim of PGE2 being pro-inflammatory and prothrombotic. However, within the immune system PGE2 is immune regulatory and has anti-inflammatory effects from decreasing Th1 lymphocyte cytokines to inducing tolerogenic effects on dendritic cells to reducing inflammatory cytokines eg TNF to inducing suppressor T-reg cells (40, 41). The thrombotic role of thromboxane derived from ArA is a response to injury, which is vital for survival. At the same time there is also a claim that ArA derivatives may be beneficial (42) and have resolving-like activity to clean up after injury in which inflammation and cell migration to the site of the injury is an essential component (43). These mediators, eg eicosanoids and cytokines, act differently in these phases of maintenance, the response to injury (whether physiological or traumatic) and its resolution. Moreover, COX2 inhibitors which were originally developed to arrest the synthesis of immune triggering eicosanoids and treat the inflammation in rheumatoid arthritis had to be withdrawn because of increased cardiovascular events and mortality. The likely reason being the suppression of prostacyclin synthesis which happily most of the time maintains vascular tone and prevents platelet adhesion (44, 45).

2.8 ArA and DHA compared in an ex-vivo vascular relaxation study.

To test the idea that ArA has a role in arterial relaxation, small mesenteric arteries *ex vivo* were used in the myograph technique developed by Dr Priscilla Poston of St Thomas's Hospital Medical

College, London, now part of King's University (46). Dr Ivan Golfetto isolated mesenteric arteries which were perfused with individual fatty acids bound to the albumin. This procedure allowed the arteries to be enriched with specific fatty acids. The presence of albumin mimics the release of free fatty acids in a normal physiological state. After 30 min, the albumin and fatty acids were removed, and the arteries washed with PSS (physiologic salt solution) The arteries were then challenged with vasoconstrictor and vasodilator agonists to get the dose-response curve for each one of the fatty acids.

In Figure 3 we show the response to pre-incubation with ArA. After contracting the arteries with noradrenaline (NA) progressive amounts of acetylcholine were added from 10^{-9} to 10^{-5} mol/L. Compared with controls, there is a progressive relaxation of the ArA treated arteries starting very early (10^{-9}) and continues until (10^{-6}). That is, ArA improved relaxation capability. Figure 4 shows the effect seen of pre-incubation of the mesenteric arteries with DHA, where no such relaxation was seen, making a distinction between physiological effects of ArA and DHA.

2.9 Different response of males and females prenatally to an essential fatty acid supplement.

We studied the impact of an EFA supplement (DHA 300mg, 8.4 mg ArA /day) on the Magnetic Resonance Imaging (MRI) of the brain of the infants. The mothers were given the supplement shortly after booking for pregnancy care (ca. 12+ weeks after conception), in a randomised trial with a placebo of olive oil (13). Magnetic Resonance Imaging of the infant brain was carried out shortly after birth.

Sex Difference: Girls

The proportionate reduction of maternal RBC ArA (due to loss by biomagnification) between recruitment and term (LA/ArA ratio) with the increase in linoleic acid (bioreduction) is a measure of the efficiency of fetal nourishment for ArA. Significant Pearson correlations were observed with the cortex (0.748 p< 0. 004), deep grey matter (0.659 p< 0.014), whole grey matter (0.753 p< 0.003), hippocampus (0.611 p< 0.03), lentiform (0.774 p< 0.002), thalami (0.654 p< 0.015), corpus callosum (0.640 p< 0.018), brain (0.685 p< 0.0098), brain with CSF (0.774 p< 0.0019 n=12). There was only one correlation in the data on the boys. This was the difference between ArA at term and recruitment (0.500 P<0.035, N=18). (Table 11).

Sex difference: Boys

By contrast to the girls, there was raft of significant correlations in the data on the boys emerging from the correlation of volume with both cord/maternal DHA as for the cortex (0.852 p<0.001),

Deep grey matter (0,729 p<0.01), Whole grey matter (0,850 p<0.001), hippocampus (0.855 p<0.001), lentiform (0.681 p<0.021), thalami (0.846 p< 0.001), corpus callosum (0.834 p< 0.001), brain without CSF (0.815 p< 0.002), brain size with CSF (0.888 p< 0.000) n=11. The cord/maternal DHA ratio for DHA is analogous to the LA/ArA ratio as it denotes the better the delivery of DHA in the cord the better the DHA status of the fetus.

The cord/maternal DHA ratio is analogous to the ArA/LA ratio of the girls. Both signify the separate provision for ArA and DHA for fetal nourishment. If we had seen perhaps 2, 3 or 4 correlations they might have been dismissed as type 1 errors. However, the consistency and range of these correlations is not chance but powerful evidence for the robustness of the data contrasting male and female prenatal biology.

2.10 Fatty Acid Composition of Human Milk

In Table 12 we present data from 9 previous studies on the composition of human milk. These include some 2,000 samples in a study for WHO (48) comparing the effect of steroidal contraceptives on the composition of human milk done in Hungary and Thailand in which milk samples were collected over a period of 6 months (49). We also include detail from Vietnamese mothers' milk for comparison in Table 12. In all cases, DHGLA, ArA and adrenic acids were present at twice the proportion of EPA and DHA.

Thomas Brenna has more recently reviewed the composition of human milk globally with the same results (50) . Brenna's Figure 1 plots the proportions with time postnatally with the sum of ArA and adrenic acid displayed as the most prominent of the long chain PUFA that notably are used in vascular, immune competence and brain growth postnatally. He commented on the misinformed recommendation by the EFSA to omit ArA from infant formula (51).

3. DISCUSSION

The data in this paper describe an overwhelming presence of ArA and its ω6 allies in the reproductive process. Regardless as to whether one looks at the data as total fatty acids or their composition in the principal polar phosphoglycerides of red cells, endothelium, placenta and mononuclear cells, it is the same ArA-rich story including its high biomagnification across the placenta to nourish the fetus. (Tables 1 to 10, Figures 6, 7 and 8). With the high density of ArA and adrenic acids in the endothelium they will play a principal role in cardio development and vascularization: a pre-requisite for organogenesis and the prenatal brain growth spurt. The evolution of the mammalian system appears to have rejected the precursors of DHA including EPA. By contrast, the DGLA, precursor of ArA, is biomagnified, although its precursor (linoleic acid) is rejected and bioreduced, plausibly protecting the utilisation of ArA and DHA.

The increase in linoleic acid from recruitment to delivery and the fall in ArA and adrenic acid in maternal RBC, is consistent with the formidable extraction of these two fatty acids by the placenta and rejection of linoleic acid by the fetus (Table 1). The same fall in ArA and DHA during pregnancy was reported previously (52). The extraction of ArA and DHA is likely from across different maternal lipid pools and suggests that maternal levels act as reservoirs for fetal growth (53)

The idea of maternal reserves being called on during pregnancy requires further investigation. Bear in mind the data is derived from the RBC, that is from an endothelial plasma membrane. To what extent is this compositional depression also happening in other endothelial cells as for example, the lining of the arterial system? The extraordinary level of the ω9 Mead acid and its elongation product seen in the fetal cord artery of a very preterm delivery (Fig 2) adds to notion of an EFA deficiency and likely, an ArA inadequacy. It also raises the question: does this mean that present maternal dietary intake is physiologically insufficient and would have a deleterious effect on the mother? The possibility of inadequacy is supported by the reduction of ArA and adrenic acids in the phosphoglyceride compartment, except for the serine phosphoglycerides, in the comparison of early and term placenta. (Table 8). Is the depletion of these fatty acids involved in the reports of shrinking of the brain in pregnancy? (54).

The ω6 docosapentaenoic acid is a bit of a maverick as it is synthesised, especially in neural tissue, when there is a deficiency of dietary alpha-linolenic acid and when tissue DHA decreases, assumedly to support and maintain membrane unsaturation and OPH (18, 55).

It is interesting that DGLA stood out in the early placenta compared to term, particularly in the serine phosphoglycerides (early 3.96% to 9.15% at term, table 8). Prior to the discovery of prostacyclin (PGI2 from ArA) (15), PGE1 formed from DGLA was the most powerful anti-thrombotic agent known, suggesting a significant role in placental and fetal cardio-vascular health (56) . PGE1 and prostacyclin both favour the maintenance of blood flow which is vital during this critical period of fetal growth.

Even the biomagnification of DHA is at a low level by comparison with ArA and its allies. The original publication on biomagnification implied that it was left to the fetus to further amplify DHA at each step on its way to the brain's signalling (see Figure 5). There is no evidence of biosynthesis in the placenta (57). The reality is that there is little if any ω3 precursor available for DHA synthesis to explain the high proportions reached in the neural signalling systems (Figures 1 and 5) and in any case, in all the relevant data reported here, the DHA precursors are rejected by the fetus. There has to be a high selectivity specifically for DHA used for membrane incorporation as seen in *in vitro* studies of synaptosomes and their subcellular particles (58).

It is important to recall that data from Martinez's group showed that the proportions of ArA and adrenic acids in developing human forebrain EPG and ethanolamine plasmalogens of infants aged 24-42 weeks significantly exceeded that of DHA. With time, the proportion of DHA exceeded ArA in the EPG fraction (59). The implication is that ArA and adrenic acids have a prominent role in early development and is likely to include glial cell development and connectivity.

During early development, differentiation and cell division requires the nourishment, including lipids, for building plasma membranes, mitochondria, nuclear envelope, reticulo-endothelial systems, lysosomes, Golgi apparatus and other structural organisations of the cells. Importantly they will be proceeding at a fast pace from a zygote weighing less than a milligram, turning into a 3.5 Kg new-born in a matter of months. There will also be a high demand for membrane liquidity and elasticity during the extremes of curvature achieved during cell division. Melting point has usually been taken as an index of liquidity. PUBCHEM gives the melting point of ArA as -49.3^{o}C and -44 ^{o}C for DHA.

ArA has been blamed for much and this is despite the 1982 Nobel Prize for Sune Bergstrom, Bengt Samuelsson and John Vane which included products of ArA acting in the control of blood pressure and maintenance of blood flow and in the resolution of injury. Attempts to supress ArA might cause harm (60, 61). The possibility that reduced ArA may be responsible for the reduction in benefit on cognitive outcomes seen at high EPA+DHA level supplements needs consideration. The DHA/ArA balance has been suggested as an important variable in the contribution of long chain PUFAs to cognitive and behavioural development in infancy (62). It may be a time to re-think the role of ArA and its allies.

3.1 Males vs females

Males are known to have a greater sensitivity for EFA status compared to females which has also been seen to operate prenatally (2). We reported on an MRI study of the brain after birth following an ω3 rich fatty acid supplement containing a small amount of ArA, compared to olive oil placebo which started at first booking for pregnancy care. Quantification of regional volumetrics provided evidence of the supplement enhancing several regions of brain growth prenatally but in the boys only, not the girls (47). Subsequently, we wanted to test if there were any fatty acid differences between boys and girls without supplement. Examination of the fatty acid profiles in the placebo boys and girls uncovered a striking sex difference. There was basically no correlation with the fatty acid status of the boys and regional brain enhancement without supplement. On the other hand, several regional volumetric correlations were evident in the girls (Table 11).

Sex differences

The reason for the ArA tilt in the girls is likely due to the priority investment in ArA-rich growth requirements during reproduction which involve expansion of blood volume, uterine muscle, rapid placental growth, immune, and cardio-vascular systems on which organogenesis, including that of the brain (63, 64, 65). The brain growth spurt after all, occurs in the last trimester. Its success will depend on preceding events which make provision for its high specific requirements. Conversely, the male sperm is rich in and dependent on DHA for function (66).

These data suggest that the female physiological use made of the long chain PUFA is significantly different from the boys and includes a greater investment in ArA and its allies thus explaining the lack of response to the supplement. The suggestion from these data is a requirement for specific fatty acids to meet the optimum growth and physical demands for placental and fetal membrane growth and function, which is of course a major property of female physiology. That explanation

covers the requirement for ArA by the girls. The lack of impact of the DHA rich supplement could be due to the relatively small number studied. The female brains, as well as their bodies, are smaller at birth and it is plausible that there is smaller requirement for DHA for the last trimester brain growth spurt. A larger study is needed to explain the difference in requirement.

3.2 Complications of prematurity

During embryogenesis the endothelial and haemopoietic cells are the first to differentiate, then follows the cardio-vascular system as the first system to become fully functional to provide for organogenesis (67). For any organ to develop, a good flow of blood is necessary. In humans the fetal brain growth thrust is in the last trimester and uses some 70% of the energy delivered to the fetus from the mother. The requirements for this prodigious growth are provided by the endothelia of the placenta processing great lakes of maternal and fetal blood. Again, the placenta is a rapidly growing, ArA-rich organ (Table 8).

It is plausible that inadequate provision of the long chain ω6 profile in preterm infants may contribute to the complication of prematurity. i.e. intraventricular haemorrhage, periventricular leukomalacia, hypoxic ischemic encephalopathy, retinopathy of prematurity, broncho-pulmonary dysplasia and necrotising entero-colitis. These involve the vascular and immune system, all being ArA-rich. Figures 3 and 4 also illustrate the function of ArA in arterial relaxation but not DHA.

Deficiency of ArA and its allies such as seen in a very preterm infant (Figure 2) would be expected to undermine the integrity of the vascular endothelium which could rupture in response to waves of high blood pressure or be challenged by infection. Either would elicit an immune response. In Figure 2 the extraordinary presence of the Mead acid (C20:3ω9) and its elongation product (C22:3ω9) is shown in the umbilical endothelium of a very preterm infant (need to note that Mead acid is formed when there is a deficiency of linoleic acid, rather than a deficiency of ArA). Although the ArA peak seems large, the triene/tetraene ratio (C20:3ω9/C20:4ω6) has long been used as a quantitative assessment of essential fatty acid deficiency. In the preterm infants studied the implication is there is not enough ArA and other LCPUFAs to satisfy OPH (22).

Interestingly there is a report on glial cell lipid composition describing the total ω6LCPUFA at 30.3% compared to 23.7% ω3LCPUFA in the ethanolamine phosphoglycerides of rat brains (68) . The glial cells are in some respects the brain's immune system but carry the responsibility of nourishing and maintaining neurons and myelin, and a variety of house-keeping operations in the

brain (69). Like the early development of the vascular system following embryogenesis, the glial cells will be required first to nourish neurogenesis, dendrite, synaptic and myelin formation.

3.3 The importance of the ω6 to ω3 ratio.

Regional analysis of the baboon brain described certain regions DHA rich and others ArA rich e.g. frontal, temporal, cingulate and hippocampus, and why is the amygdala rich in ArA and DHA poor and the opposite with the inferior colliculus (70). More so, the data presented in this paper makes it clear that linoleic and ArA are being treated in opposite manners by the placenta and fetus, with the same being seen for DHA versus all its precursors, including EPA. However, it does seem likely that there is an optimum balance between ArA and DHA as there is evidence the two are strongly associated in maternal and fetal blood. (71) The clear selection and rejection process is not just happening with the placenta and fetus, but examples of the same selectivity and rejection can be seen in the biosynthesis of membrane lipids in every cell system. Scientific and popular press typically refer to ArA as a precursor of inflammatory cascades, in contrast to DHA which is the precursor of anti-inflammatory and pro-resolving lipid mediators. The explanation lies in the peculiarity of the ω6 and ω3 fatty metabolic pathways which share the same desaturase enzymes. This introduces a competition for the desaturation. To avoid this, the ratio of dietary ω6 to ω3 of around 5:1 and 2:1 has been considered optimal (72, 73). The ratio, thought to be present in the early human diet as 1:1 or 2:1 has now been exceeded to reach up to 20:1. It is this lack of understanding of the difference between the risk posed by excessive exposure (to omega 6 fatty acids in this case) and the exposure required by the biological necessity (natural physiological response of ArA to injury, for example) that has led to the "inflammatory" label attached to ArA.

Moreover, whilst the dietary ratio of ω6 to ω3 might be useful, the data in pregnancy shows that placenta/fetal axis discriminates by amplifying all long chain ω6 and reducing linoleic acid. On both sides of the placenta the total ω6 is the same but the individual data for linoleic and ArA are quite different. The same applies to ω3 although the amount and difference in DHA precursors is small. Hence the total ω6 to ω3 ratio in pregnancy is seriously misleading. At a conference organised by William Lands in Biloxi, 1987, it was suggested that the individual members of the two families should be respected for their individual properties (74). There is much more evidence to support this view today.

3.4 What is known about the role of ArA and its metabolites in the brain

The effect of ω3 PUFA deficiency on neural function has been extensively characterised in rodent and primates (55). There has been far less research on the role of ArA in the brain, mainly because it is difficult to deplete brain ArA levels by dietary deficiency studies. An example of the effect of simultaneous reductions of brain ArA and DHA levels resulting from the use of delta-6 KO (knock-out) mice showed that they had significantly lower performance in behavioural function test. The best performing groups were those fed DHA alone and DHA plus ArA (75). These data support the importance of balanced levels of ArA and DHA in the brain. High doses of fish oil are known to reduce tissue and brain ArA levels. Conversely, in ω3 PUFA deficiency neural long chain 22-carbon ω6 PUFA increase, and DHA levels are reduced, arguing the case for an OPH.

Clearly, brain ArA is important structurally and as a precursor of a wide range of oxygenated PUFA derivatives known as eicosanoids (prostaglandins, thromboxanes, lipoxins, leukotrienes, hydroxyeicosatetraenoic and epoxyeicosatetraenoic acids), which are locally acting hormone-like compounds and which play critical roles in many aspects of neural function including a role in sleep (PGD2) (76) and short- and long-term-memory and spatial learning (in rats) (77, 78). Readers are referred to a publication which reviews the impacts on brain physiology and metabolism in genetically altered mice in whom various ArA metabolising enzymes have been deleted (79).

There has been a sustained interest in lipoxin modulation of the proinflammatory responses in neural tissue with recent research suggesting analogues of lipoxin A4 might be even more potent in terms of inhibiting synthesis of pro-inflammatory cytokines while increasing the anti-inflammatory cytokines (80).

Additional and critical neural derivatives of ArA are the endocannabinoids (2-arachidonoyl-glycerol and anandamide), lipid messengers involved in fine tuning of synaptic function (81) and the release of important signal transduction molecules, arachidonoyl-diglycerides, from phosphoinositides (82).

On the other side of the ledger, neuroinflammation plays an important role in several neurodegenerative disorders and 'hypermetabolism' of ArA has been implicated. This remains a controversial area (83), though there are indications of exaggerated metabolism of neural ArA by COX-2 to PGE2 in mood disorders (84, 85, 86) and also in ω3 deficiency (87). This, and much of the anti-inflammatory role of the long chain ω3 PUFA is different from the role of ArA in organogenesis, neurogenesis, fetal and early post-natal development. Aside from inflammation, sn-1-stearoyl-2-arachidonoyl-glycerol has been shown to be a specific activator of protein Kinase C (88) which would be relevant to fetal growth and likely to its resolvin activity (16).

3.5 Adrenic acid and the brain

Adrenic acid (docosatetraenoic acid, C22:4ω6) is the third most common PUFA in grey matter glycerophospholipids as shown by Svennerholm (89) in 1968, and it is the most prominent PUFA in EPG in cerebral white matter. Like ArA, adrenic acid is incorporated into brain lipids with high efficiency (90). In the brain, adrenic acid proportions decline with age (91). Retroconversion of adrenic acid to ArA has been shown in astrocytes and bovine aortic endothelial cells in vitro (92, 93). While adrenic acid is metabolised by cyclooxygenase, lipoxygenase and cytochrome P450's, little is known of the role of these metabolites in vivo (94).

3.6 Preformed ArA is a far better precursor of tissue ArA than LA.

In Table 13 we present Andrew Sinclair's data obtained by double labelling linoleic and ArA with ^{14}C and tritium, respectively, in rat pups during the period of rapid brain growth. Determination of the isotope ratio in liver and brain ArA gives a quantitative measure of the preferential use of ArA preformed (tritium label in arachidonate) rather than synthesis from linoleic acid (^{14}C in arachidonate). The similar experiments from Ahmed Hassam is shown in Table 14. The data illustrate the superiority of gamma-linolenic acid over linoleic acid as a precursor for liver and brain structural lipids. That result also exemplifies the rate limiting delta-6 desaturase (FADS2) in the metabolic journey from linoleic acid to ArA. The preferential use of ArA has also been shown by a study which showed that about 60% of a bolus dose of linoleic acid was oxidised to CO2 in 24 hours, whereas only about 15% of ArAc was oxidised in the same time. Stearic was similarly conserved (95) analogous to its selection by biomagnification (Tables 1 and 2) for the sn-1 positions of the phosphoglycerides and for the polar membrane lipids accompanying ArA and other LCPUFA as well as its use in the polar membrane lipids (96). It also has to be related to the "affinity" of the special tissues for ArA+ allies (acylation and incorporation into membrane PL). Converse is true , as in the case of the brain, a poor affinity of these special tissues for linoleic acid where we know from the labelling that linoleic acid was extensively degraded to acetyl CoA and then used to make saturated, monounsaturated fatty acids and cholesterol (97). So, two things are happening – ArA plus allies are accepted, and LA is rejected. These papers demonstrate that ArA preformed, is a far better precursor of tissue membrane ArA than LA. This is an important statement. It is seriously misleading to lump all the omega 6 fatty acids together as a group, since there a clearly distinct differences between linoleic acid and ArA plus allies as shown here.

Similarly misleading is to summate all the omega 3 fatty acids. Nature treats precursors and membrane end-products in quite specific and different manner. It does this for prenatal and cardio-

vascular and immune systems, muscle and placental growth and development. The vascular system is the driver of fetal organogenesis and growth.

3.7 Marine mammals provide support for ArA.

A tantalising piece of evidence in support of ArA comes from the most unlikely place. Marine mammals live in what we are told is an ω3-rich environment. How does that fit with the evidence that mammals require ω6 fatty acids for reproduction? Surprisingly, the tissue levels of the Gray Whale (*Eschrichtius robustus*) (98) and Dolphin (*Tursiops truncates*) (99) are ArA-rich. The oil from the Gray Whale, by contrast, is rich in EPA and DHA. Like cod liver oil, it is the left over from the selective efforts of biology in serving OPH for membrane growth and/or the maintenance requirements of all the cells in the body. The body oil is if you like, the junk even though it serves purpose insulating against cold and providing energy and some fat-soluble nutrients.

The dependence on ω6 is likely the explanation for the visits of the Gray Whale to the warm waters of its Baja breeding lagoons. The food web in warm waters contains ArA and other ω6 fatty acids (100). 50 million years of evolution in the ω3-rich sea do not seem to have changed the requirement for ArA for reproduction. Similar findings have been reported in the Harbour Porpoise and Grey Seal and are likely to be common amongst marine mammals.

3.8 The role of ArA in the evolution of mammals.

The ArA evidence raises an interesting thought about the evolution of the mammals. Prior to mammals the primary food for the giant reptiles were the giant trees, ginkgoes, ferns and their allies. These reproduced by spreading spores and by their root systems. The vegetarian dinosaurs would eat leaves and bark and possibly dig for roots. The leaves would be a prime source of nourishment. We do not know the balance of fatty acids in their lipids but the general rule for green leaves is that they are α-linolenic acid rich (101).

A key to mammalian development is its initiation through implantation of the fertilised egg instead of it being ejected inside a hard shell. This had been the dominant process prior to extinction at the Cretaceous-Paleogene boundary. Although both flowering plants and small mammals would have been in existence for some time previously, the extinction of the dinosaurs and with them the giant trees made way for a coincident explosion of the flowers and mammals. So, what was the trigger? Was it the new abundance of the flowering plants with their protected seed, with oil rich in linoleic acid just a coincidence?

Perhaps not: before the mammals, reproduction was achieved by egg laying. It is plausible that the start-up of mammalian reproduction could have resulted from ArA's adhesive eicosanoids resulting in the egg sticking to the uterus. The new wealth of the flowering plants with their linoleic acid rich seed oils could well have been the trigger leading to the emergence of the mammals in abundance. All studies thus far on the composition of the brain in different species demonstrate the presence of ArA and adrenic acids in equal measure to DHA if not a greater sum. Prior to the Cretaceous-Paleogene boundary it is plausible that the egg laying system and paucity of omega 6 would have restricted brain growth, as evidently happened. The emergence of an abundant source of linoleic as the precursor to ArA together with the ArA-rich placenta nourishing the product of conception for a long period of time, would have filled the missing gap to result in a new diverse range of mammals with larger brains than ever seen before.

It was only about 10 million years after the collapse of the dinosaurs and the appearance of the flowering plants and mammals in abundance, when the first mammals migrated to live in the sea. Despite the marine habitat being omega 3 rich, they have held on to their dependence on Ar. Whilst the essentiality for DHA in signalling systems is no longer controvertible, this and the other evidence presented here on the ArA dominance in the endothelium, placenta and prenatal immune cells is a sobering thought.

3.9 Infant nutrition.

If there was any doubt about the impressive significance of ArA and its allies to reproduction, then the composition of human milk from nine countries cited (49) dispels the doubt (Table 12). The composition of the lipids for post-natal development, and continuation of post-natal brain growth contains both ArA and DHA. In all the samples we have analysed, ArA was in a higher proportion than DHA and was accompanied by DGLA and adrenic acid The proportions of C20 and C22ω6 fatty acids is twice the proportion of all long chain ω3 fatty acids. Similar data is available from a much larger study of several thousand human milk samples across the planet, the sum of ArA and its allies is consistently greater than the sum of DHA and its allies (50).

3.10 Lipids and the OPH

There is a problem with the common view on lipids being so frequently just considered as a water barrier between two parts of the cell. Whilst true, the membrane lipids are fundamental to cell function and epigenetics and likely played a major role initiating the specialised structures which

led to the intra-cellular compartments, cell specialisation and the speciation that followed at the beginning of the evolution of air breathing life (102, 100).

None of this story diminishes the importance of DHA for brain development. It has its own trajectory from food to the super-saturation in the photoreceptor (Figure 5). Its functional requirement for the brain is incontrovertible.

Moreover, in the topic of this paper, reproduction, there is also a need to take the health and nutrition of the mother prior and around the time of conception into account, a principle known for some time (103, 104). The implantation of the zygote and its development, take place 7 days after conception into the milieu intérieur of the mother which is determined by the history of the mother. When a woman reports for pregnancy care at 12 weeks post conception, the cells that will form the brain are already migrating to do so. The evidence here is that these early events are ArA dominated. The oleic acid status reflecting the ArA and DHA status of the mother and predicting preterm (13), has potential relevance to preconception care and prevention of low birthweight and prematurity (105, 106) It could be used in preconception and first recruitment clinics for pregnancy care to identify risk, especially when there is a clinical and behavioural assessment of likely risk to prematurity. Because individual fatty acids have different origins in foods the same analysis would provide evidence-based information for targeted interventions.

In addition to the discussion on the LCPUFA, the message from reproductive biology also makes clear the importance of the saturated fatty acids which are biomagnified alongside the LCPUFA, doubtless to fill the sn-1 positions in the company for the sn-2 LCPUFA. Both are needed. Radio-isotope studies showed that stearic acid was conserved whilst oleic, linoleic and alpha-linolenic acid were largely oxidised to CO_2 in 24 hours (95). This coincided with stearate uptake by brain membrane lipids (107).

Our knowledge is scant as regards the specific lipid-protein interactions with the creation of optimal, local lipid domains for optimal membrane/protein function. Then there is the oft remarked concept of liquidity with DHA being cited a highly liquid molecule. Interestingly, ArA has a lower melting point than DHA. Is this the reason for the greater proportion of ArA in the arterial endothelial cell membrane? This membrane must expand and relax with monotonous regularity. Figures 3 and 4 provide evidence for ArA in this role as opposed to DHA.

Indeed, the flexibility of the cell membrane will be a characteristics of different cell types and common to all will be the extremes of curvature required during cell division. The placenta is possibly the most rapidly growing organ. Confined placental mosaicism, characterised by

uniparental disomy, is associated with fetal growth restriction and mortality (108). The uniparental disomy is most likely an example of membrane failure leading to a trisomy which collapses into either a male-male or female-female or normal male-female status. The role of membrane lipids, Myer Bloom's OPH and its nutritional dependence is a topic too little recognised yet could be central to many of the neuro-developmental and health issues of concern today.

3.11. Conclusions and perspective

The topic of this paper is lipids in human reproduction. We have summarised the data identifying ArA and its allies (20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6) as major role players in the growth of cell systems in the reproductive process by a set of pie charts comparing the biomagnification of ArA and allies with DHA, linoleic and stearic acids (Figures 6 and 7). Stearic acid was chosen as a comparator as it usually occupies the sn-1 position alongside ArA and DHA in the sn-2. There was no point including alpha-linolenic, EPA or the DPA as the proportions are so small and our data show that they were rejected by the fetus.

The pie chart of maternal plasma choline phosphoglycerides at delivery compared to cord plasma choline phosphoglycerides is given independently as it is the contents of the plasma lipid which is most readily taken up by the fetus and shows the greatest biomagnifications and bioreductions. Figure 7 summarises the supremacy of ArA and adrenic in the cell types which play a major role in the reproductive process and neurogenesis. Inherent in the data is a need to take the health and nutrition of the mother prior to and around the time of conception into account, a principle known for some time (103, 104). With the oleic acid status inversely reflecting the ArA and DHA status of the mother and predicting preterm birth (13), it will be important to include consideration of the dietary lipids with a balance of ArA and DHA in a proportion targeting the brain (1:1 – 2:1) in preconception and pregnancy care. The dominance of ArA and its allies in vascular and immune system membranes raises the question as to their potential role in the vascular driven complications of prematurity and prenatal neurodevelopmental disorders. The data also demonstrates the selectivity and biomagnification of stearic acid which has received little attention. It would seem time to reconsider the role of these fatty acids in reproduction and nutrition.

4. METHODS USED IN THE ABOVE STUDIES

Data from six studies are included in this paper.

4.1 Term and preterm fatty acid status of mother and newborn.

These studies (Fish Oil Supplement Study – FOSS1) were carried out at the Chelsea and Westminster Hospital, South West London, UK 2010-2016 Tables 1 to 3, and 5

The population for the FOSS1 study consisted of 300 women, recruited for a randomised trial of ω3 and 6 fatty acids, has been described previously. The study group was drawn from the 300 mothers attending the clinic booking for pregnancy care at the Chelsea and Westminster Hospital. Recruitment included women who became diabetic or developed pregnancy-induced hypertension. However all data used in this paper is only from healthy controls with birthweights between 3,200 – 4,500g.

Total fatty acid analysis was carried out on the red blood cells and plasma. Plasma and blood cells were separated by low temperature, centrifugation. The buffy coat was removed by aspiration. Details are given in our previous paper (13).

Sample collection: Two sub-studies, one investigating the biomagnification of fatty acids in 25 matched maternal - cord samples and another one analysing the fatty acid composition of 73 placental tissue samples were conducted between 2016 and 2018. The first pilot study, conducted by Dr. Priya Sivarajasingam and Dr. AnnieBelle Sassine, included 25 matched maternal and cord blood samples from 40 women with singleton pregnancies delivering via elective or emergency Caesarean sections at the NHS labour ward of Chelsea and Westminster Hospital. Maternal blood (20mL) was collected at cannulation by the obstetric anaesthetist and cord blood was collected within 10 minutes of delivery in a 10mL Lithium Heparin blood tube.

The second study involved the collection of biopsies of human amnion and placenta from women who had a preterm (<37 weeks) or term delivery (>37 weeks) via Caesarean section. Samples were either stored at -80°C within half an hour of the Caesarean section or placed in phosphate-buffered saline (PBS; Sigma-Aldrich, Poole, UK). Placental biopsies were sampled from the central region of the maternal side. The acquisition of these samples was led by Miss Natasha Singh (Clinical Research Lead) at Chelsea & Westminster Hospital labour ward as part of a tissue bank. These samples were collected between 2013 and 2015. Dr Natasha Singh, Dr. Bronwen Herbert, Dr. Sivatharjini Priya Sivarajasingam, Dr Maria Francesca Fais, and Gavin Sooranna collected, cut, and

stored the tissues for later analysis. The samples were subdivided by clinical indication for delivery; 13 were term non-labouring samples and 60 were spontaneous and iatrogenic preterm samples divided as preterm non-labour (N=14), preterm chorioamnionitis (N=12), preterm abruption (N=7), preterm twins in early labour (N=9), preterm twins not in labour (N=14), preterm polyhydramnios (N=1), and preterm idiopathic (N=3). Analysis of samples for gene expression levels and fatty acid composition was conducted in 2016-2018 and was carried out by Dr. AnnieBelle Sassine under the supervision of Professor Mark Johnson at the Chelsea-Brompton and Chelsea and Westminster Hospital Campus of Imperial College.

4.2. Early term and term placentae.

These studies were carried out in Newham General Hospital, East London, UK, 2000 to 2009 Table 8

The principal supervisor for the studies was Professor Ovrang Djahanbakhch and the work was done at the Newham General Hospital, a member of the Bart's NHS Trust by Dr. Dimitris Bitsanis. Placentae were obtained from one hundred and three (n=103) healthy pregnant women. The mothers were non-smokers and non-alcoholics, normotensive and free of medical and obstetric complications. Ethical approval was granted by the East London Health Authority and a signed consent was obtained from the mothers. Placentae were collected from legally terminated pregnancies (n=63; 8-14 weeks) by evacuation, from elective preterm abortions and term delivery (n=40; 38-41 weeks of age) from Newham General Hospital, East London, UK. Collection of the early placentae by vacuum aspiration is a preferred process as it captures a substantial mass of (4-10g) intact without, for example activating certain proteases to change tissue consistency and also importantly eliminates contamination with maternal blood. The early termination of the pregnancy was due to socio/psychological reasons. Further details of the methodology for this study were published 2005 (109).

4.3. Immune function in term and preterm infants at birth.

Women delivering at term (37-40+ weeks) and preterm (30-35 weeks) were recruited at Newham University Hospital, London prior to delivery and informed consent was obtained for the collection of cord blood samples. Women were aged between 20-40 years with a parity of 0-3. Prior to delivery women in preterm labour were treated with dexamethasone sodium phosphate (dexamethasone, 12mg); a majority of women (n=14) received a second dose approximately 12 hours after the first dose. Babies born to mothers undergoing treatment associated with infection

and inflammation, or any condition that was not related to her pregnancy were excluded from the study. At birth, all the participating neonates were considered healthy as long as they were not suffering nosocomial infection nor infection acquired in utero or inflammatory reactions. Further details of this study were published 2009 Tables 9,10. These studies were done in collaboration and under the supervision by Professor Ovrang Djahanbakhch.

4.4. Thailand Refugee camp in Karen, and Vietnam:

Karen samples: maternal blood, 8ml, was obtained at delivery time and at 12 weeks post-delivery. Cord blood was also collected at birth from healthy term babies. Vietnam samples: 8ml of blood, from healthy mothers and babies were also collected at delivery. After being spun and separated, the red cells and plasma were frozen, immersed into liquid nitrogen and subsequently transported, to London for analysis. Tables 6-7 - the methodology for breast milk sampling and transportation had previously been validated in a WHO collaborative study on the effect of steroidal contraceptives on the composition of human milk done in Thailand, Hungary and the UK (48).

4.5. Vascular relaxation activity of ArA and DHA.

These studies were done under the supervision of Dr Lucilla Poston at St Thomas's Hospital. South East London, UK Figures 3,4

Female Sprague-Dawley rats (200-250g) were sacrificed by CO2 inhalation and cervical dislocation. Mesenteries were removed and placed in cold phosphate saline solution (PSS). Branches of the mesenteric arteries (150-300 μm) were dissected from these animals and mounted as ring preparations on a small-vessel wire myograph (110). The arteries were stretched to achieve an internal circumference to 90% of that which would be obtained when relaxed in situ under a transmural pressure of 100 mmHg. Arteries that failed to produce tensions equivalent to 100 mmHg in response to 5 μmol/L noradrenaline (NA), in KPSS (potassium phosphate saline solution) (125 mM KCl in substitution of physiological salt solution, PSS) were rejected.

After testing the arteries, they were pre-incubated separately with 40 μM AA, EPA or DHA. Then, they were solubilized in dimethyl sulfoxide (DMSO) (with a final concentration of DMSO 0.4%) and 0.3 % albumin in 10ml physiological salt solution, for 30 min. Only the control group was pre-incubated with PSS, albumin and DMSO without fatty acids, at a temperature of 37ºC, aerated with 95% O2 and 5% CO2. The incubation medium was replaced with PSS. A concentration-response curve was constructed to NA (10^{-7} mol/L – 10^{-5} mol/L) and to the thromboxane mimetic, U46619 (10^{-10} mol/L–$3x10^{-6}$ mol/L). Arteries pre-constricted with NA ($5x10^{-6}$ M) achieved approximately 80% maximum response and then relaxed with an endothelium-dependent dilator, acetylcholine

(Ach) (10^{-9}–10^{-5} M) and an endothelium-independent dilator, spermine NONOate (10^{-9} –10^{-5} M). More details of the methodology were published 2001. (46)

The chromatogram of figure two was taken from a study of 63 preterm births carried out by Dr Alison Leaf with Professor Kate Costeloe at the Special Care Baby Unit of the Homerton Hospital described in 1992 (111, 112)

4.6. Maternal Nutrition and low birthweight

Analysis of 7-day weighed intakes were initially carried out on 533 women delivering at the then Salvation Army Hospital in the East End of London 1982 (113) and after its closure transferred to the Homerton Hospital, Hackney in East London (114), where the follow-up revealed poor maternal nutrition as defined by the failure to meet 6 of the RDAs for pregnancy as a risk factor for low birthweights independent of smoking, socio-economic status and ethnicity (115). This led logically to a randomised clinical trial of a supplement of one third of the WHO recommendation for pregnancy resulting in a better than two-fold reduction in the small for gestational age births (116).

Acknowledgements.

Our data presented here came from studies funded by the Wolfson Foundation, MRC, AFRC, Borne, The Worshipful Company of Innholders, The Mother and Child, Letten and Waterloo Foundations. We are grateful to the maternity staff at Newham General and Chelsea and Westminster Hospitals as well as the Special Care Baby Unit at the Homerton Hospital, under the care of Professor Kate Costeloe at the time. We also wish to mention the support of the midwifery and staff at the Chelsea and Westminster and, Homerton Hospitals who helped organise the work on the preterm infants, and those at the Newham University Hospital who greatly supported the work on the placenta and immune system. In addition, we wish to record our gratitude to all the mothers and their infants, who participated in these studies. Dr Ivan Golfetto wishes to express his thanks to Professor Lucila Poston for allowing him to realize the experimental procedures in her lab at St Thomas' Hospital in order to be able to demonstrate the different effects of, AA, DHA, EPA in the contraction of mesenteric rat artery. We are also indebted to Professor David Edwards and Dr Nora Tusor of King's College, London, for conduction the MRI scans of the newborn infant brains.

Ethical approval for the studies at the Homerton and Newham was granted by the East London Health Authority and a signed consent was obtained from the mothers. Approval was also obtained from the Research Ethics Committees of the Chelsea and Westminster Hospital, Imperial College

and the Chelsea-Brompton Hospital. A signed consent was obtained from the mothers. For the studies in Thailand and Vietnam ethical approval was given by the Karen Refugee Committee and the ethical committee of the Faculty of Tropical Medicine, Mahidol University. The purpose and methods of the survey were explained to all participants, in their own language and they were free to withdraw from the protocol at any time without any consequences. The Medical and Scientific committee at Hung Vuong Hospital gave ethical approval for the Vietnam study.

The authors have no conflicts of interest.

TABLES

Table 1 Recruitment, delivery and cord blood RBC fatty acids with bidirectional selectivity

	Recruit n=46	Delivery n=29	Cord n=22
	Mean±SD	Mean±SD	Mean±SD
14:0	0.79±0.23	0.71±0.20	0.64±0.19^
16:0	23.9±1.39	25.8±2.36***↑	27.1±2.34^^^
18:0	11.6±1.33	11.7±1.18	15.2±1.98^^^###
20:0	0.33±0.07	0.36±0.08	0.54±0.13^^^###
22:0	0.99±0.25	1.11±0.28	1.25±0.24^^^
24:0	2.05±0.50	2.44±0.59*↑	3.51±0.90^^^###
ΣSFA	39.8±2.04	42.3±3.26***↑	48.4±4.29^^^###
16:1ω7	0.71±0.35	0.78±0.26	0.82±0.37
18:1ω9	13.8±1.58	15.2±1.74***↓	10.1±1.66^^^###
18:1ω7	1.12±0.13	1.08±0.13	1.60±0.28^^^###
20:1ω9	0.25±0.06	0.28±0.05	0.20±0.26
22:1ω9	0.05±0.02	0.05±0.02	0.04±0.01
24:1ω9	2.70±0.60	3.07±0.53*↓	2.72±0.50
ΣMUFA	18.6±1.84	20.4±2.19***↓	15.4±2.02^^^###
18:2ω6	13.3±1.67	12.6±1.78↓	4.18±2.01^^^###
18:3ω6	0.06±0.03	0.05±0.03	0.05±0.02
20:2ω6	0.04±0.02	0.06±0.09	0.03±0.04
20:3ω6	1.63±0.27	1.50±0.31↑	2.38±0.60^^^###
20:4ω6	12.8±1.59	10.6±2.34***↑	14.1±3.30###
22:2ω6	0.13±0.05	0.13±0.06 ↓	0.07±0.03^^^###
22:4ω6	1.91±0.38	1.61±0.38* ↑	2.49±0.65^^^###
22:5ω6	0.29±0.13	0.36±0.14↑	0.73±0.27^^^###
∑ω6	29.9±2.56	27.4±3.35	25.4±4.54^^^#
∑ω6LC	16.8±1.87	14.2±3.00***↑	19.9±4.46^^^###
18:3ω3	0.41±0.20	0.27±0.13***↓	0.12±0.18^^^#
20:3ω3	0.02±0.02	0.04±0.02**	0.04±0.02^^^
20:5ω3	0.80±0.45	0.55±0.24*↓	0.38±0.17^^^#
22:5ω3	1.81±0.32	1.40±0.43***↓	0.72±0.29^^^###
22:6ω3	4.92±0.91	4.82±1.54	5.74±1.59
∑ω3	7.96±1.55	7.07±2.08	7.01±1.78
∑ω3LC	7.55±1.48	6.81±2.03	6.89±1.76
20:3ω9	0.22±0.05	0.20±0.07	0.14±0.05^^^###
LA/AA	1.06±0.21	1.26±0.46↓	0.33±0.22 ^^^###
LA/DHA	2.80±0.65	2.98±1.46↓	0.86±0.84^^^###
AA/DHA	2.68±0.51	2.36±0.71	2.54±0.65
22:5ω6/22:4ω6	0.15±0.06	0.23±0.07***↑	0.28±0.08^^^
ω6/ω3	3.92±0.73	4.16±1.46	3.56±0.92
ω6LC/ω3LC	2.30±0.45	2.24±0.66↑	2.96±0.72^^^###
Omega3 Index	5.72±1.26	5.37±1.72	6.13±1.65
16:0/18:0	2.09±0.30	2.22±0.30	1.82±0.26^^^###
S/P	1.05±0.13	1.29±0.36**	1.69±0.83^^

Table 2 Selectivity for OPH Fatty acid composition (%) of plasma from health mothers at delivery and cord plasma showing the same principle of selectivity by the placenta, but with more striking differences than in the RBCs. Percentage of FAs in maternal and fetal plasma pairs. Dr. AnnieBelle Sassine's data (117).

	Plasma (n=24 pairs) Median (Min-Max)[1]	
(% of total FAMEs)[2]	Maternal	Fetal
16:0	27.4 (24.2-32.0)	28.7 (25.8-32.3)**
18:0	5.64 (4.59-7.75)	11.0 (9.49-12.4)***
20:0	0.16 (0.05-0.25)	0.50 (0.34-0.70)***
22:0	0.42 (0.26-0.67)	1.09 (0.81-1.86)***
24:0	0.30 (0.13-0.42)	0.78 (0.52-1.27)***
ΣSFA	33.9 (30.4-37.6)	42.3 (38.8-47.0)***
16:1ω7	2.20 (0.81-4.98)	3.17 (2.61-4.10)**
18:1ω9	23.1 (17.3-30.6)	15.9 (13.9-19.3)***
18:1ω7	1.57 (1.07-2.49)	2.37 (1.91-3.12)***
20:1ω9	0.20 (0.09-0.34)	0.00 (0.00-0.23)***
24:1ω9	0.76 (0.45-1.14)	1.32 (0.50-1.81)***
ΣMUFA	28.3 (22.7-35.5)	23.1 (20.6-27.3)***
18:2ω6	24.1 (19.2-30.3)	10.2 (8.09-12.2)***
20:2ω6	0.16 (0.07-0.29)	0.12 (0.01-0.20)*
20:3ω6	1.40 (0.84-2.24)	2.77 (1.58-3.67)***
20:4ω6	4.94 (3.01-7.58)	13.1 (9.20-15.0)***
22:2ω6	0.13 (0.004-0.23)	0.06 (0.00-0.20)**
22:4ω6	0.10 (0.02-0.48)	0.34 (0.17-0.64)***
22:5ω6	0.17 (0.05-0.63)	0.33 (0.10-1.13)***
∑ω6		
18:3ω3	0.78 (0.14-1.03)	0.12 (0.04-0.51)***
20:5ω3	0.41 (0.14-0.86)	0.24 (0.04-0.75)**
22:5ω3	0.29 (0.16-1.85)	0.22 (0.04-0.43)
22:6ω3	2.75 (1.85-7.35)	4.03 (2.34-6.47)***
∑ω3		
AA/LA	0.21 (0.11-0.35)	1.21 (0.97-1.65)***
22:5/22:4ω6	1.49 (0.64-6.38)	1.09 (0.50-2.23)**
AA/DHA	2.29 (1.06-6.13)	2.90 (1.83-6.05)***
Omega 3 Index[3]	2.70 (1.19-5.37)	4.30 (2.38-6.93)***
AA+DHA/MUFA	0.27 (0.14-0.39)	0.71 (0.55-0.97)***

Table 3 The OPH of selectivity for matched maternal red cell fatty acids compared to plasma at delivery. Dr AnnieBelles Sassine's data (117).

Fatty acid profile (% of FAMEs)[2]	Erythrocyte (n=34) Median (range) [1]	Plasma (n=34) Median (range)	*P*
16:0↓	23.3 (21.9-25.6)	27.0 (23.2-32.0)	<0.0001
18:0↑	14.1 (12.7-15.3)	5.58 (4.59-12.9)	<0.0001
20:0↑	0.27 (0.17-0.45)	0.16 (0.05-0.25)	<0.0001
22:0↑	1.39 (0.92-1.89)	0.42 (0.26-1.14)	<0.0001
24:0↑	4.23 (3.20-5.99)	0.30 (0.13-4.38)	<0.0001
ΣSFA	43.3 (41.2-46.5)	33.5 (29.1-41.9)	<0.0001
16:1ω7↓	0.27 (0.04-0.74)	2.08 (0.37-5.23)	<0.0001
18:1ω9↓	12.5 (11.2-14.5)	23.1 (11.6-30.6)	<0.0001
18:1ω7↓	0.84 (0.63-1.18)	1.54 (0.83-2.49)	<0.0001
20:1ω9	0.21 (0.07-0.38)	0.18 (0.02-0.34)	0.042
22:1ω9	0.05 (0.00-0.12)	0.00 (0.00-0.04)	<0.0001
24:1ω9↑	5.54 (3.82-6.66)	0.78 (0.45-6.19)	<0.0001
ΣMUFA	20.0 (17.6-23.1)	28.2 (20.0-35.5)	<0.0001
18:2ω6↓	8.31 (6.79-10.2)	24.4 (7.28-30.6)	<0.0001
20:2ω6	0.20 (0.11-0.35)	0.17 (0.07-0.29)	0.06
20:3ω6	1.57 (1.05-2.84)	1.42 (0.84-2.24)	0.018
20:4ω6↑	12.6 (10.0-14.7)	4.83 (3.01-7.30)	<0.0001
22:2ω6	0.18 (0.00-0.32)	0.12 (0.00-0.23)	0.13
22:4ω6↑	2.45 (1.38-4.58)	0.10 (0.02-2.01)	<0.0001
22:5ω6↑	0.46 (0.19-1.03)	0.16 (0.04-0.66)	<0.0001
∑ω6↓	26.0 (22.8-31.4)	32.3 (23.6-39.4)	<0.0001
18:3ω3↓	0.15 (0.04-0.22)	0.68 (0.14-1.27)	<0.0001
20:5ω3↑	0.50 (0.16-1.22)	0.35 (0.04-1.15)	0.0005
22:5ω3↑	2.05 (1.16-2.81)	0.23 (0.08-1.85)	<0.0001
22:6ω3↑	7.19 (3.42-8.93)	2.59 (1.15-7.36)	<0.0001
∑ω3↑	9.94 (4.80-11.8)	3.92 (2.16-9.81)	<0.0001
AA/LA↑	1.47 (1.12-2.08)	0.21 (0.11-2.04)	<0.0001
22:5/22:4ω6↓	0.19 (0.12-0.36)	1.48 (0.00-6.38)	<0.0001
AA/DHA↓	1.75 (1.14-4.29)	2.00 (1.02-6.13)	0.0002
Omega 3 Index[3]	7.71 (3.58-9.83)	2.96 (1.19-7.77)	<0.0001
AA+DHA/MUFA↑	0.97 (0.75-1.13)	0.28 (0.14-1.11)	<0.0001

Table 4 Spearman's correlation coefficient (r_s) and P values for correlations between placental proportions of fatty acids (%) and gestational age at delivery and Aberdeen birth centiles from the placental fatty acid composition study (Dr AnnieBelle Sassine's data, 117)

	Gestation age at delivery (n=73)		Aberdeen birth centiles (n=49)[2]	
Placental fatty acid (% of total FAMEs)[3]	r_s	*P*	r_s	*P*
16:0	-0.05	0.66	-0.22	0.14
18:0	-0.17	0.16	-0.11	0.46
ΣSFA	-0.12	0.31	-0.16	0.28
16:1ω7	0.07	0.96	0.03	0.84
18:1ω9	-0.10	0.42	-0.004	0.98
18:1ω7	-0.19	0.11	0.10	0.51
ΣMUFA	-0.13	0.26	0.04	0.81
18:2ω6	0.24	0.04	0.12	0.47
20:4ω6	-0.30	0.01	-0.08	0.59
22:4ω6	-0.29	0.01	-0.36	0.01
22:5ω6	-0.18	0.14	-0.46	<0.001
Σω6	-0.04	0.72	0.002	0.99
20:5ω3	0.01	0.99	0.22	0.12
22:6ω3	0.34	0.003	0.19	0.19
Σω3	0.35	0.002	0.23	0.12
(AA+DHA)/MUFA	0.04	0.75	-0.002	0.99
AA/LA	-0.30	0.01	-0.09	0.53
22:5/22:4ω6	-0.02	0.86	-0.31	0.03
AA/DHA	-0.40	0.000	-0.21	0.15
ω6/ω3	-0.35	0.003	-0.24	0.10
Omega 3 Index[4]	0.34	0.003	0.21	0.16

Table 5 Long chain polyenoic fatty acid composition of plasma and red cell choline and ethanolamine phosphoglycerides of the mothers and their neonates (area % of total fatty acids, mean± SD) (Hackney unpublished data)

	PLASMA CHOLINE PHOSPHOGLYCERIDES			RBC CHOLINE PHOSPHOGLYCERIDES			RBC ETHANOLAMINE PHOSPHOGLYCERIDES		
FAs	Mother N=51	Neonate N=28	*P*	Mother N=54	Neonate N= 38	*P*	Mother N=54	Neonate N=38	*P*
20:3ω6	3.67 ± 0.86	5.26 ± 1.35	<0.0001	2.53 ± 0.75	3.61± 0.90	<0.0001	1.23± 0.45	1.72± 0.49	<0.0001
20:4ω6	8.35± 1.93	17.6± 1.9	<0.0001	11.1± 2.57	14.6± 3.27	<0.0001	15.5± 2.16	19.5± 2.48	<0.0001
22:4ω6	0.29± 0.13	0.66± 0.15	<0.0001	1.26± 0.57	1.97± 0.69	<0.0001	4.69± 1.08	5.81± 1.26	<0.0001
22:5ω6	0.49± 0.30	0.89± 0.43	<0.0001	0.62± 0.27	1.33± 0.50	<0.0001	0.75± 0.32	1.59± 0.47	<0.0001
20:5ω3	0.71± 0.64	0.52± 0.24	NS	0.47± 0.33	0.26± 0.14	<0.0001	0.84± 0.39	0.32± 0.20	<0.0001
22:5ω3	0.57± 0.18	0.58± 0.30	NS	1.47± 0.61	0.62± 0.35	<0.0001	3.21± 0.62	0.95± 0.35	<0.0001
22:6ω3	4.14± 1.51	7.16± 2.13	<0.0001	5.42± 2.33	5.89± 2.41	NS	7.41± 2.18	7.23± 2.12	NS

Table 6 Plasma choline phosphoglycerides fatty acid composition of mothers and neonates (cord) from Vietnam (119) (% Total Fatty Acids, Mean ± SD)[1]

Fatty acids	Mothers (n=44)	Neonates (n=39)	*P*
14:0	0.30 ± 0.11	0.19 ± 0.12	<0.0001
16:0	34.83 ± 3.4	30.61 ± 3.38	<0.0001
18:0	9.80 ± 1.84	14.56 ± 1.94	<0.0001
20:0	0.04 ± 0.01	0.05 ± 0.02	NS
ΣSFA	44.95 ± 3.60	45.37 ± 3.97	NS
16:1ω7	1.18 ± 0.36	1.11 ± 0.38	NS
18:1ω9	12.67 ± 1.69	11.80 ± 1.43	0.013
20:1ω9	0.11 ± 0.03	0.06 ± 0.03	<0.0001
24:1ω9	0.03 ± 0.01	0.10 ± 0.09	0.009
ΣMUFA	14.00 ± 1.94	13.06 ± 1.74	0.024
18:2ω6	18.56 ± 3.05	6.31 ± 1.27	<0.0001
18:3ω6	0.05 ± 0.03	0.07 ± 0.02	0.038
20:2ω6	0.54 ± 0.13	0.30 ± 0.09	<0.0001
20:3ω6	3.57 ± 0.72	5.73 ± 1.16	<0.0001
20:4ω6	8.76 ± 1.49	17.54 ± 3.22	<0.0001
22:4ω6	0.37 ± 0.12	0.70 ± 0.48	<0.0001
22:5ω6	0.55 ± 0.13	0.84 ± 0.31	<0.0001
Σω6	32.41 ± 3.41	31.48 ± 3.57	NS
18:3ω3	0.18 ± 0.12	0.06 ± 0.06	0.05
20:5ω3	0.36 ± 0.12	0.30 ± 0.13	0.027
22:5ω3	0.43 ± 0.14	0.34 ± 0.16	0.005
22:6ω3	4.13 ± 0.98	5.79 ± 1.69	<0.0001
Σω3	5.11 ± 1.13	6.43 ± 1.85	<0.0001
20:3ω3	0.17 ± 0.06	0.70 ± 0.35	<0.0001

Table 7 Summary data comparing selected fatty acids from Hackney, Vietnam and Thailand data (120)

		PLASMA CPG		
SAMPLE	FATTY ACIDS	MATERNAL	CORD	P
HACKNEY n= 54	18:0	1 0.2 ± 1.0	15.2 ± 1.2	<0.0001
VIETNAM n= 44	18:0	9.80 ± 1.84	14.6 ± 1.94	<0.0001
THAILAND n= 22	18:0	9.38 ± 1.63	15.5 ± 1.63	<0.0001
HACKNEY n= 54	18:2ω6	23.7 ± 2.8	8.05± 1.23	<0.0001
VIETNAM n= 44	18:2ω6	18.56 ±3.05	6.31 ± 1.27	<0.0001
THAILAND n= 22	18:2ω6	14.57 ± 3.03	5.36 ± 1.0	<0.0001
HACKNEY n= 54	20:4ω6	8.35 ± 1.93	17.6 ± 1.9	<0.0001
VIETNAM n= 44	20:4ω6	8.76 ± 1.49	17.5 ± 2.22	<0.0001
THAILAND n= 22	20:4ω6	8.55 ± 1.63	17.17 ± 2.58	<0.0001
HACKNEY n= 54	22:6ω3	4.14 ± 1.51	7.16 ± 2.13	<0.0001
VIETNAM n= 44	22:6ω3	4.90 ± 1.81	5.73 ± 1.53	0.017
THAILAND n= 22	22:6ω3	4.30 ± 1.05	6.74 ± 2.09	<0.0001

		RBC CPG		
	FATTY ACIDS	MATERNAL	CORD	P
VIETNAM	C20:4ω6	6.0 ± 3.7	20.0 ± 3.5	<0.0001
HACKNEY	C20:4ω6	15.5 ± 2.16	19.5 ± 2.48	<0.0001
VIETNAM	C22:6ω3	8.28 ± 2.7	8.53 ± 2.8	NS
HACKNEY	C22:6ω3	7.41 ± 2.18	7.23 ± 2.12	NS

		RBC CPG		
	FATTY ACIDS	MATERNAL	CORD	P
HACKNEY	20:3ω6	3.67 ± 0.86	5.26 ± 1.35	<0.0001
VIETNAM	20:3ω6	3.57 ± 0.72	5.73 ± 1.16	<0.0001
HACKNEY	20:4ω6	11.1 ± 2.57	14.6 ± 3.27	<0.0001
VIETNAM	20:4ω6	10.0 ± 3.0	13.9±2.8	<0.0001
HACKNEY	20:5ω3	0.71 ± 0.64	0.52 ±0.24	NS
VIETNAM	20:5ω3	0.36 ± 0.12	0.30 ± 0.13	0.027
HACKNEY	22:6ω3	5.42 ± 2.33	5.89 ± 2.41	NS
VIETNAM	22:6ω3	4.90 ± 1.81	5.73 ± 1.53	0.017

Table 8 Fatty acid composition of phosphoglycerides of early (8-14 wk) and term placentae (109) illustrating the dominance of ArA and its C20 and C22 partners in the membrane bi-layer (NEWHAM DATA)[1].

	CHOLINE PHOSPHOGLYCERIDES			ETHANOLAMINE PHOSPHOGLYCERIDES			SERINE PHOSPHOGLYCERIDES			INOSITOL PHOSPHOGLYCERIDES		
FAs	EARLY	TERM	*P*	EARLY	TERM	*P*	EARLY	TERM	*P*	EARLY	TERM	*P*
20:3ω6	2.41±0.57	4.00±0.71	<0.0001	1.92±0.50	3.47±0.56	<0.0001	3.96±0.91	9.15±1.44	<0.0001	3.71±0.83	6.87±1.03	<0.005
20:4ω6	18.9±3.12	17.2±2.44	<0.0001	28.5±2.12	24.7±1.83	<0.0001	11.2±1.90	10.8±1.52	NS	35.9±2.58	31.9±2.48	<0.005
22:4ω6	0.67±0.20	0.31±0.08	<0.0001	3.90±0.80	2.41±0.45	<0.0001	1.95±0.55	1.67±0.38	<0.005	1.57±0.24	1.11±0.21	<0.005
22:5ω6	0.40±0.16	0.22±0.07	<0.0001	2.37±0.74	1.60±0.56	<0.0001	1.33±0.44	1.16±0.33	<0.05	0.60±0.18	0.61±0.22	NS
20:5ω3	0.16±0.06	0.18±0.06	NS	0.29±0.13	0.27±0.10	NS	0.50±0.18	0.65±0.20	<0.05	0.48±0.17	0.56±0.30	NS
22:5ω3	0.26±0.10	0.22±0.09	<0.05	1.43±0.4	1.68±0.39	<0.0001	3.02±0.69	0.69±0.23	<0.0001	0.64±0.19	0.82±0.24	<0.005
22:6ω3	1.81±0.54	1.64±0.58	NS	8.24±1.8	8.87±1.99	NS	3.08±0.93	3.62±1.10	<0.0001	2.61±0.70	2.22±0.69	<0.05

[1] Data are means followed by standard deviations, difference between means is tested using independent t-test. P>0.05 is considered non-significant. NS=non-significant

Table 9 Comparison of the long chain polyenoic acids in cord blood mononuclear cells[1] choline phosphoglycerides (CPG) from term, preterm and DNI (29)

CPG fraction	Mean fatty acid percent ± SE (Median and interquartile range)			Kruskal-Wallis test for Significance (*P* value)		
	Term (n=9)	Preterm (0=10)	DNI (n=9)	Term& Preterm	Term& DNI	Preterm &DNI
16:0	35.1±0.3 35.3:34.9-35.4	34.6±2.1 36.0:32.9-40.0	36.7±0.9 37.1:35.1-37.5	NS	NS	NS
18:0	11.9±0.5 11.4: 11.0-12.1	13.0±1.4 12.6:11.8-16.0	12.6±14 13.9:12.7-14.1	NS	NS	NS
16:1ω7/11	1.7±0.06 1.7:1.7-1.8	1.6±0.14 1.6:1.25-1.91	1.0±0.3 0.5: 0.4-1.9	NS	NS	NS
18:1ω7/9	14.1±0.5 14.2:13.9-14.7	18.4±1.3 18.0:17.4-21.1	16.4±07 15.8:15.3-16.8	NS	<0.05	NS
18:2ω6	5.6±03 5.4:4.9-6.0	5.7±0.8 5.9:3.69-6.67	4.6±0.5 4.7:3.3-5.3	NS	NS	NS
18:3ω6	0.37±0.03 0.4: 0.3-0.4	0.5±0.08 0.4:0.32-0.62	0.1±0.01 0.1:0.1-0.1	NS	<0.01	<0.001
20:3ω9	0.2±0.03 0.1:0.1-0.2	0.06±0.02 0.07: 0.06-0.08	0.8±0.2 0.8:0.5-1.05	<0.01	<0.01	<0.01
20:3ω6	2.2±0.07 2.2:2.05-2.4	1.9±0.2 2.1:1.8-2.2	1.7±0.14 1.7:1.5-1.7	NS	<0.05	NS
20:4ω6	15.1±0.4 15.2: 14.7-16.1	12.5±1.05 12.9:11.4-14.4	12.9±0.9 13.0:11.5-13.8	<0.05	NS	NS
22:4ω6	1.02±0.06 0.9; 0.9-1.1	0.8±0.08 0.9:0.7-0.94	0.9±0.08 0.96:0.7-1.06	NS	NS	NS
22:5ω6	0.3±0.03 0.3: 0.2-0.3	1.0±0.78 0.2:0.13-0.31	0.27±0.05 0.3:0.18-0.36	NS	NS	NS
18:3ω3	0.05±0.01 0.05: 0.04-0.06	0.04±0.01 0.05:0-0.06	0.06±0.01 0.07:0.05-0.08	NS	NS	NS
20:5ω3	0.28±0.04 0.3:0.2-0.3	0.2±0.04 0.2:0.1-0.3	0.12±0.03 0.08:0.06-0.2	NS	<0.05	NS
22:5ω3	0.3±0.04 0.3: 0.26-0.3	0.06±0.04 0:0-0.12	0.2±0.04 0.2:0.15-0.3	<0.01	N3	<0.05
22:6ω3	1.7±0.16 1.6:1.4-1.7	1.03±0.11 1.12:0.9-1.2	1.3±0.13 1.2:1.09-1.5	<0.01	NS	NS

[1] Data are means followed by standard error and medians with interquartile range. Fatty acid correlations between term, preterm, and DNI groups are tested using the non-parametric Kruskal-Wallis test. EPG=ethanolamine phosphoglycerides; DNI = definitely not infected preterm; NS= non-significant

Table 10 Comparison of the long chain polyunsaturated fatty acid composition of cord blood mononuclear cells[1] ethanolamine phosphoglycerides (EPG) from term, preterm and DNI (119).

EPG fraction	Mean fatty acid percent ± SE (Median and interquartile range)			Kruskal-Wallis test for significance (*P* value)		
	Term (n=9)	Preterm (n=10)	DNI (n=9)	Term& Preterm	Term& DNI	Preterm & DNI
16:0	5.4±0.18 53: 5.1-5.6	6.2±0.6 6.3: 4.5-7.7	4.9±0.3 5.0: 4.1-5.8	NS	NS	NS
18:0	17.1±0.5 17: 16.5-18.1	17.1±1.5 17.5: 14.3-20.2	14.9±0.33 15.1: 14.5-15.5	NS	<0.05	NS
16:1ω7/11	0.5±0.03 0.5: 0.45-0.53	0.4±0.06 0.4: 0.4-0.5	0.8±0.09 0.8: 0.6-0.9	NS	<0.05	<0.05
18:1ω7/9	4.5±0.2 4.4: 4.3-4.5	5.7±0.4 5.3: 5.1-6.6	5.1+0.3 5.05: 4.5-5.8	NS	NS	NS
18:2ω6	1.7±0.1 1.6: 1.5-1.7	1.7±0.1 1.8:1.5-1.9	2.9±0.2 3.0:2.4-3.3	NS	<0.01	<0.01
18:3ω6	0.25±0.03 0.26:0.18-0.27	0.5±0.14 0.3:025-0.67	0.07±0.02 0.05:0.04-0.12	NS	<0.01	<0.01
20:3ω9	0.27±0.03 03:0.2-0.4	0.11±0.06 0.03: 0-0.27	1.05±0.20 1.1:0.6-1.6	NS	<0.05	<0.01
20:3ω6	1.2±0.01 12:12-1.3	1.02±0.05 0.95: 0.92-.21	1.4±0.08 1.4:13-1.6	NS	NS	<0.01
20:4ω6	34.1±0.6 33.7:32.5-35.5	28.8±2.01 28.7:23-33.6	31.3±1.2 31.0:28.3-34.7	<0.05	NS	NS
22:4ω6	5.02±0.25 5.3:44-5.4	4.4±0.23 4.5:3.8-4.9	4.06±0.21 43:3.6-4.5	NS	NS	NS
22:5ω6	1.2±0.10 1.2:1.0-1.5	0.98±0.18 0.73: 0.63-1.46	1.03±0.14 1.0:0.7-1.2	NS	NS	NS
20:5ω3	0.36±0.04 0.4:0.2-0.5	0.15±0.07 0.00 0-03	0.15±0.03 0.1:0.07-0.2	<0.05	<0.05	NS
22:5ω3	1.3±0.1 1.1:1.06-1.5	0.3±0.1 0.4:0-0.6	0.9±0.08 0.9:0.8-1.1	<0.001	NS	<0.01
22:6ω3	4.9±0.3 4.8:4.5-5.5	2.5±0.24 2.6:2.5-2.9	3.8±0.3 4.0: 3.14.5	<0.001	NS	<0.05

[1] Data are means followed by standard error and medians with interquartile range. Fatty acid correlations between term, preterm, and DNI groups are tested using the non-parametric Kruskal-Wallis test. EPG=ethanolamine phosphoglycerides; DNI = definitely not infected preterm; NS= non-significant

Table 11 Volumetric MRI fatty acid correlates of regions of the brain in the placebo group of girls compared to boys from refs (47, 121) but only partially reported.

A. Girls Placebo Group (a. Recruitment; b. Delivery; c. Cord)

RBC Fatty acid /Brain Volumes	Cortex	Deep grey	Whole grey	Hippocampus	Lentiform	Thalami	White matter	Corpus callosum	Whole brain no CSF	Whole brain with CSF
SAT FAs[a], N=13	0.641*	0.597*	0.648*	0.3473	0.586*	0.450	0.140	0.698*	0.736**	0.643*
P	0.018	0.031	0.016	0.245	0.035	0.123	0.647	0.008	0.004	0.018
18:0[a], N=13	0.854**	0.469	0.847**	0.419	0.484	0.424	-0.118	0.699**	0.792**	0.733**
P	0.002	0.106	0.002	0.154	0.094	0.148	0.700	0.008	0.001	0.004
24:1[a], N=13	0.602*	0.241	0.592*	0.260	0.306	0.102	0.035	0.635*	0.602*	0.566*
P	0.029	0.427	0.033	0.390	0.308	0.741	0.908	0.0197	0.0296	0.044
LA/AA[b], N=12	0.748**	0.659*	0.753**	0.611*	0.774**	0.654*	-0.297	0.640*	0.685**	0.774**
P	0.003	0.014	0.003	0.030	0.002	0.015	0.325	0.018	0.009	0.002
20:4ω6[b]-20:4ω6[a], N=12	-0.653*	-0.420	-0.651*	-0.660*	0.422	-0.600*	0.722**	-0.264	-0.407	-0.510
P	0.021	0.173	0.0218	0.0194	0.171	0.0391	0.008	0.408	0.189	0.090
(20:4ω6[b] -20:4ω6[a]) /20:4ω6[a], N=12	-0.663*	-0.405	-0.660*	-0.623*	-0.411	-0.604*	0.732**	-0.293	-0.415	0.529
P	0.018	0.191	0.019	0.030	0.184	0.0375	0.007	0.355	0.179	0.077
(20:4ω6[c]-20:4ω6[b]) /20:4ω6[b], N=9	0.753*	0.352	0.745*	0.277	0.353	0.496	-0.344	0.782*	0.748*	0.805**
P	0.020	0.353	0.021	0.471	0.351	0.174	0.364	0.013	0.020	0.009
22:6ω3[b]-22:6ω3[a], N=12	0.557	-0.678*	0.571	-0.685*	-0.667*	-0.817**	0.410	0.394	-0.452	-0.564
P	0.060	0.0154	0.0527	0.0139	0.0178	0.001	0.180	0.205	0.140	0.050
(22:6ω3[b]-22:6ω3[a]) /22:6ω3[a], N=12	-0.561	-0.704*	-0.577*	-0.694*	-0.655*	-0.867**	0.375	0.413	0.475	-0.581*
P	0.057	0.010	0.049	0.012	0.021	0.003	0.229	0.182	0.119	0.048

B. Boys Placebo Group (a. Recruitment; b. Delivery; c. Cord)

RBC Fatty acid /Brain Volumes	Cortex	Deep grey	Whole grey	Hippocampus	Lentiform	Thalami	White matter	Corpus cal-losum	Whole brain without CSF	Whole brain with CSF
SAT FAs[a], N=21	0.214	0.150	0.211	-0.033	0.082	0.199	0.258	0.033	0.249	0.203
P	0.352	0.517	0.358	0.887	0.723 0	0.387	0.259	0.889	0.276	0.376
18:0[a], N=21	0.113	0.049	0.110	0.095	0.097	0.092	0.296	-0.039	0.188	0.158
P	0.626	0.831	0.636	0.683	0.675	0.693	0.193	0.866	0.414	0.494
24:1[a], N=21	0.241	0.190	0.240	0.043	-0.048	0.354	0.354	0.299	0.325	0.286
P	0.292	0.410	0.295	0.855	0.837	0.116	0.116	0.138	0.150	0.209
LA/AA[b], N=19	-0.145	-0.025	-.0139	-0.185	0.003	-0.094	-0.285	0.033	-0.213	-0.122
P	0.554	0.919	0.571	0.448	0.989	0.701	0.236	0.894	0.382	0.620
20:4ω6[b]-20:4ω6[a], N=18	0.121	0.179	0.126	0.382	0.113	0.216	0.500*	-0.172	0.271	0.240
P	0.634	0.477	0.619	0.118	0.655	0.389	0.035	0.494	0.277	0.338
(20:4ω6[b]-20:4ω6[a]) /20:4ω6[a], N=18	0.099	0.138	0.102	0.379	0.068	0.184	0.477*	-0.178	0.244	0.224
P	0.697	0.586	0.686	0.121	0.790	0.465	0.045	0.481	0.329	0.371
(20:4ω6[c]-20:4ω6[b]) /20:4ω6[b], N=13	-0.408	-0.063	-0.390	0.050	-.036	-0.173	0.209	-.0374	-.0293	-0.334
P	0.167	0.838	0.188	0.872	0.908	0.571	0.494	0.208	0.332	0.265
22:6ω3[b]-22:6ω3[a], N=18	0.127	0.227	0.136	0.318	0.153	0.222	0.430	-0.035	0.252	0.201
P	0.614	0.364	0.592	0.198	0.543	0.377	0.075	0.891	0.314	0.423
(22:6ω3[b]-22:6ω3[a]) /22:6ω3[a], N=18	0.108	0.211	0.116	0.325	0.152	0.193	0.444	-0.07	0.240	0.202
P	0.670	0.400	0.647	0.189	0.548	0.444	0.065	0.782	0.337	0.422

Table 12 Composition of human milk

Table 12a Milk fatty acid composition from 9 countries (49)

SATURATES			OMEGA 6 SERIES		
FAs	MEAN %	S.E.M	FAs	MEAN %	S.E.M
10:0	1.81	0.35	18:2	8.65	0/52
12:0	7.24	0.96	20:2	0.26	0.02
14:0	9.86	0.90	20:3	0.35	0.02
16:0	24.1	0.44	20:4	0.5	0.01
18:0	5.98	0.52	22:4	0.12	0.01
20:0	0.26	0.06	22:5	0.11	0.02
22:0	0.10	0.03			
24:0	0.05	0.02	Sum LCPUFA	1.34	

MONOENES			OMEGA 3 SERIES		
FAs	MEAN %	S.E.M	FAs	MEAN %	S.E.M
16:1	3.36	0.45	18:3	0.61	0.07
18:1	31.7	1.26	20:5	0.11	0.06
20:1	0.56	0.08	22:5	0.18	0.02
22:1	0.22	0.06	22:6	0.37	0.07
24:1	0.11	0.02	Sum LCPUFA	0.66	

Table 12b Mean total lipid concentration (mean values) and fatty acid composition (percentage) of mature milk of refugee Karen mothers (120)

	Camp 1 N=36	Camp 2 N=53
Total lipids (g/100mL)	3.48±1.3	4.78±2.3
Fatty acids		
10:0	0.92±0.43	1.42±0.45
12:0	9.30±3.6	9.17±2.8
14:0	10.4±3.7	10.7±4.0
16:0	26.7±2.7	27.0±2.5
18:0	4.26±0.67	4.39±1.1
20:0	0.15±0.06	0.13±0.03
24:0	0.04±0.03	0.05±0.03
Σ Saturates	51.5±6.0	53.1±5.8
16:1ω7	5.20±1.3	4.51±1.2
18:1ω9	29.0±4.4	28.6±4.7
20:1ω9	0.42±0.09	0.32±0.08
22:1ω9	0.07±0.03	0.06±0.05
24:1ω9	0.05±0.08	0.07±0.08
Σ Monoenes	34.7±4.5	33.7±4.8
18:2ω6	7.84±1.9	7.99±1.7
20:2ω6	0.21±0.06	0.19±0.05
20:3ω6	0.37±0.08	0.38±0.07
20:4ω6	0.49±0.10	0.48±0.07
22:4ω6	0.13±0.03	0.12±0.02
22:5ω6	0.09±0.05	0.09±0.03
Σω6 metabolites	9.29±0.24	1.26±0.42
18:3ω3	0.52±0.29	0.44±0.19
20:5ω3	0.16±0.08	0.18±0.3
22:5ω3	0.17±0.09	0.20±0.06
22:6ω3	0.52±0.14	0.54±0.22
Σω3 metabolites	0.85±0.24	0.92±0.42

Table 13 Incorporation of 14C-labelled linoleic acid or tritium-labelled ArA in the liver and brain of suckling rats. The results are shown as the percentage of radioactivity in ArA relative to total radioactivity in all fatty acids in the tissue lipid fraction 22 hours after oral dose of the labelled fatty acids (122). The two isotopes were given simultaneously to each animal ensuring a direct comparison in each case. Hence the isotope in brain ArA can be identified as either coming from linoleic or ArA and providing a quantitative measure of the preferential use of ArA preformed compared to its synthesis[1,2].

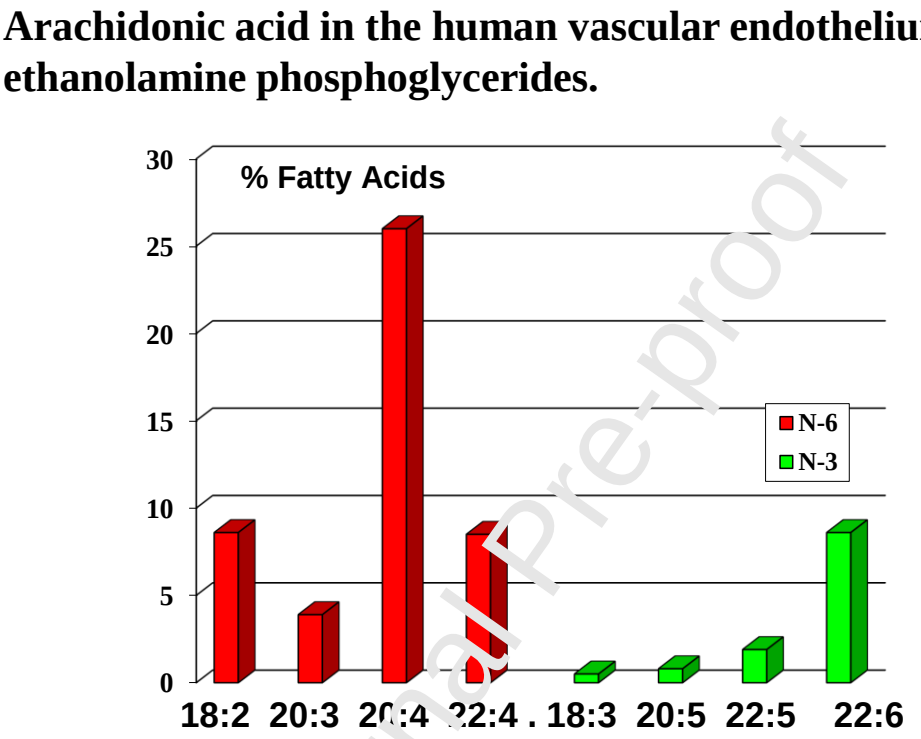
	Liver TG		**Liver PL**		**Brain PL**	
Fatty Acid	**18:2-1-^{14}C**	**20:4-^{3}H**	**18:2-1-^{14}C**	**20:4-^{3}H**	**18:2-1-^{14}C**	**20:4-^{3}H**
18:2ω6	93 ± 1.2	---	83 ± 1.5	---	31 ± 0.8	---
18:3ω6	3.6 ± 1.4	---	2.3 ± 0.5	---	2.0 ± 0.4	---
20:3ω6	1.6 ± 0.3	1.4 ± 0.2	2.1 ± 0.2	---	4.7 ± 0.5	---
20:4ω6	0 ± 0	79 ± 0.7	7.4 ± 1.0	94 ± 1.1	24 ± 0.9	85 ± 1.1

Table14. Incorporation of tritium labelled linoleic acid or 14C-labelled γ-linolenic acid into arachidonic acid in the liver and brain of suckling rats. The results are shown as the percentage of radioactivity in ArA relative to total radioactivity in all fatty acids in the tissue lipid fraction 22 hours after oral dose of the labelled fatty acids (123).

	Liver TG		**Liver PL**		**Brain PL**	
Fatty acid	**18:2-^{3}H**	**γ-18:3-1-14C**	**18:2-3H**	**γ-18:3-1-14C**	**18:2-3H**	**γ-18:3-1-14C**
18:2ω6	67±3.2	-------	67±0.2.4	----------	39±1.7	------
18:3ω6	14±1.0	31±0.4	13±1.1	4.3±065	8.3±0.5	5.6±0.2
20:3ω6	3.5±0.3	24±0.9	2.8±065	12.0±1.0	6.8±02	9.9±01
20:4ω6	3.4±0.5	20.±0.5	6.8±065	56.0±0.3	25.8±0.8	43±1.12

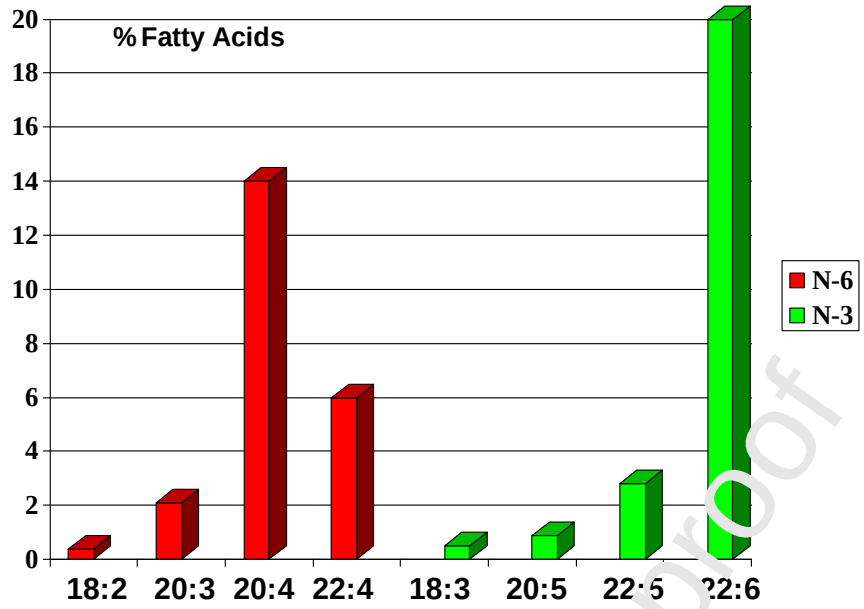
FIGURES

FIGURE 1 Polyenoic acid content of the endothelial ethanolamine phosphoglycerides from a human aorta, brain motor cortex and astrocytes.

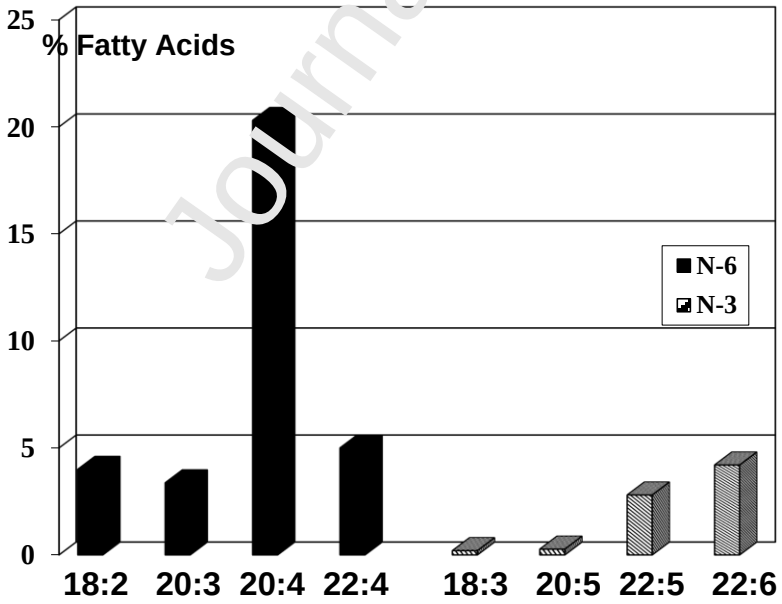


Crawford, M.A., Costeloe K, Ghebremeskel K, Phylactos A., Skirvin L, Stacey F. (1997) Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? Am J. Clin. Nutr. 66: 1032S-1041S.

Brain motor cortex, essential fatty acid composition: Ethanolamine phosphoglycerides. The balance between arachidonic, adrenic and docosahexaenoic acids - 2 or 1 to 1.



ASTROCYTE LCPUFA IN TOTAL PHOSPHOGLYCERIDES



after ref 35. Christine Bénistant C, Dehouck,M-P, Fruchart J-C, Cecchelli R., Lagarde M, (1995)

Figure 2 Endothelial choline phosphoglycerides from the umbilical artery of the 820g male preterm (28 weeks) infant reported earlier (55)[5]

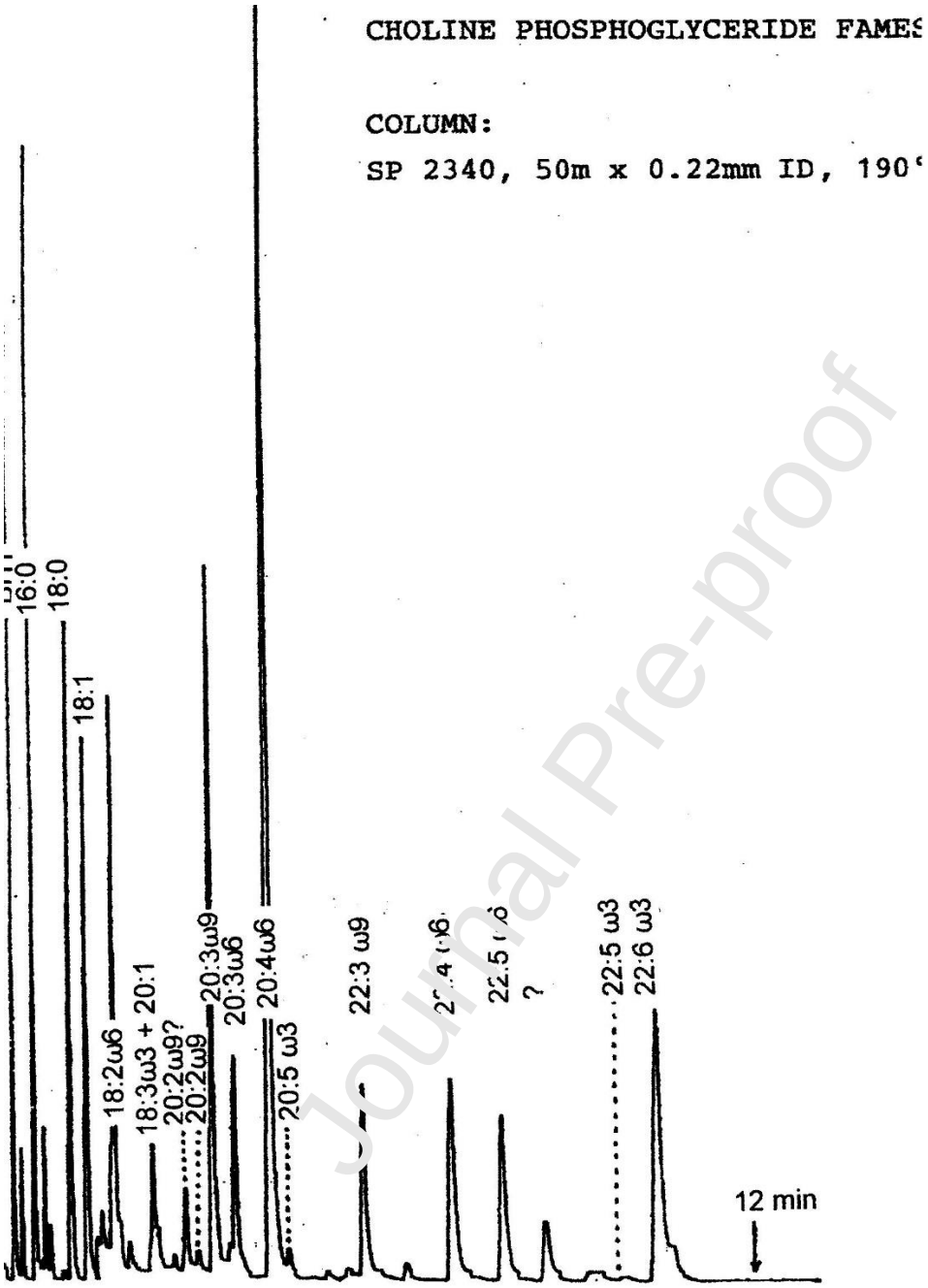


Figure 3 Dose-response curve of arteries with ArA. Pre-incubation of the mesenteric arteries with ArA and relaxed with acetylcholine, improved relaxation capability.

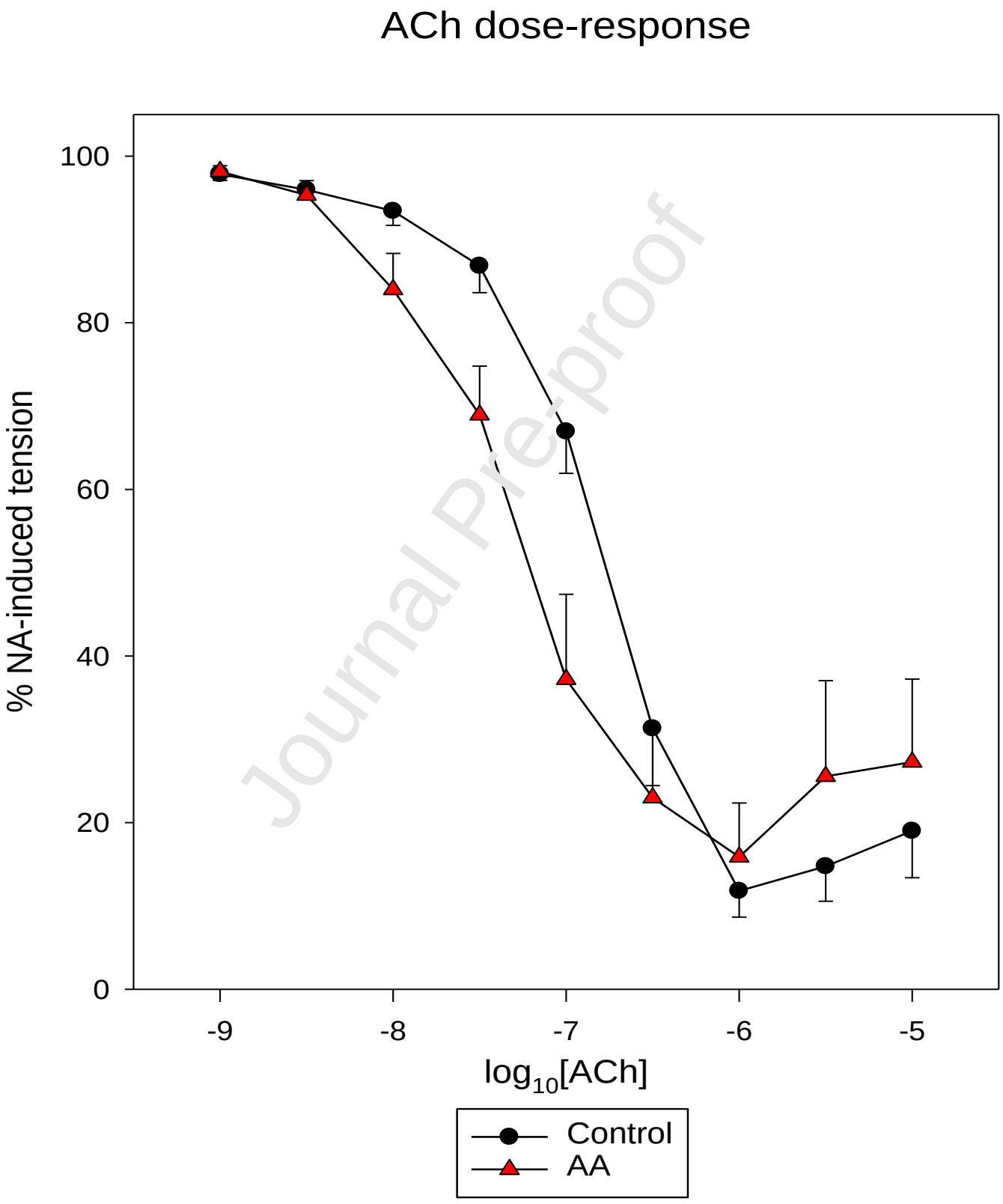


Figure 4 Pre-incubation of the mesenteric arteries with DHA, did not affect relaxation. The DHA dose-response curve to agonists ACh failed to show any statistically significant difference when compared with control arteries, after the arteries were pre-contracted with noradrenaline in contrast to ArA.

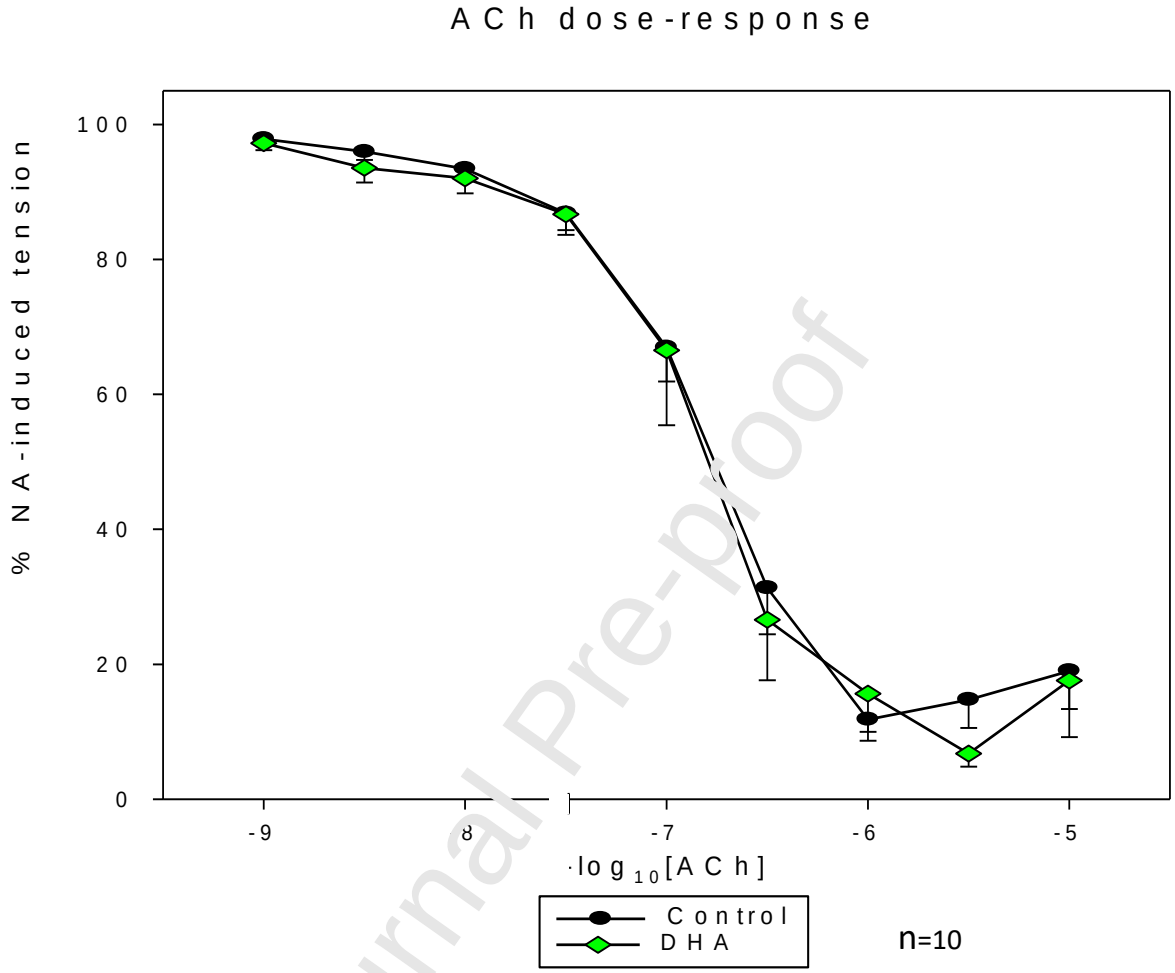


Figure 5 Biomagnification of DHA at every step from food to its super-saturation in the photoreceptor. Figure constructed based on widely accepted data.

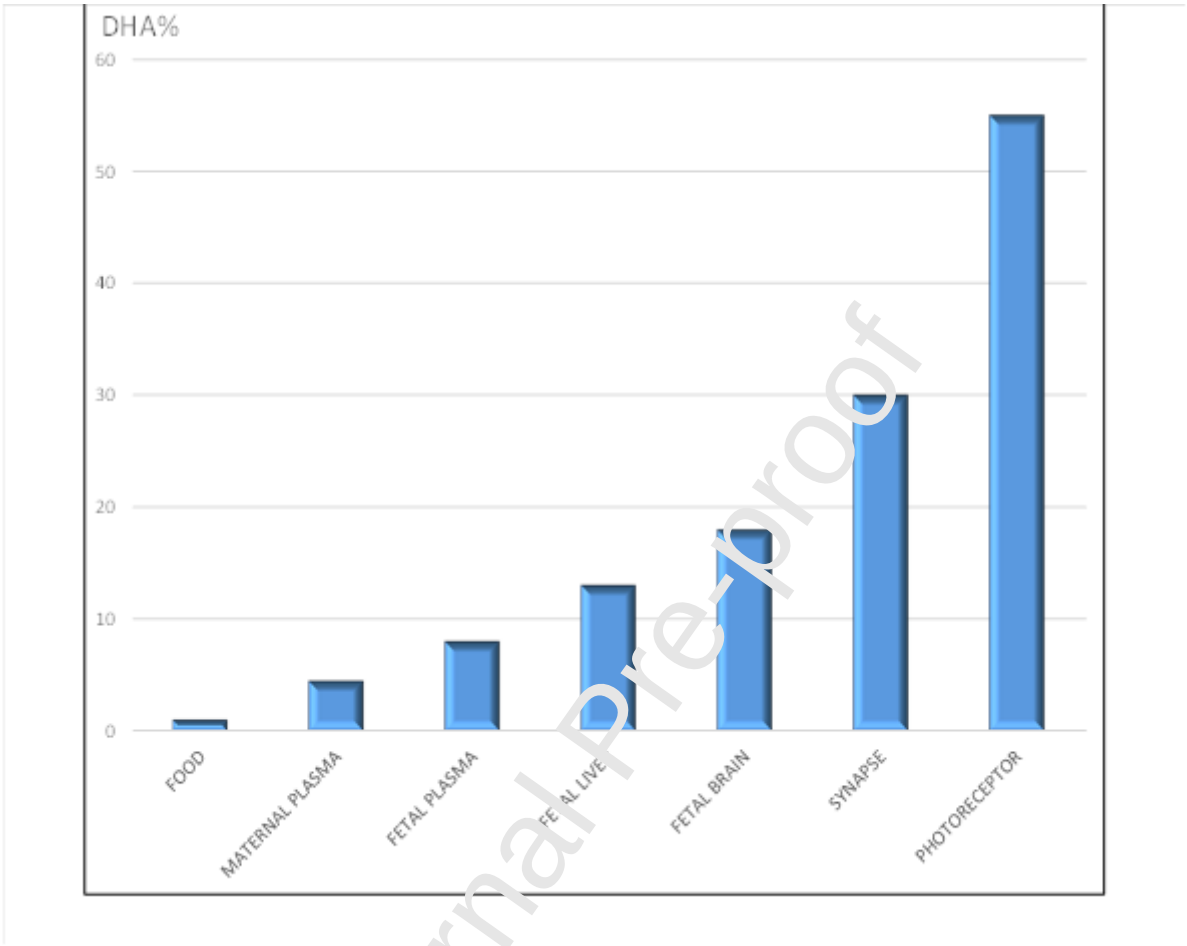
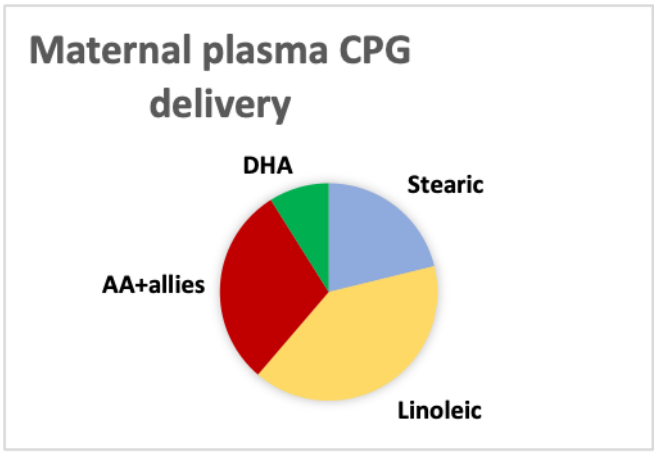


Figure 6. Showing the biomagnification of ArA and allies (20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6) and stearic acid from maternal plasma choline phosphoglyceride at delivery through to cord plasma choline phosphoglyceride. This shows clearly that the fetus is bathed in plasma enriched in ArA and allies and stearic acid.



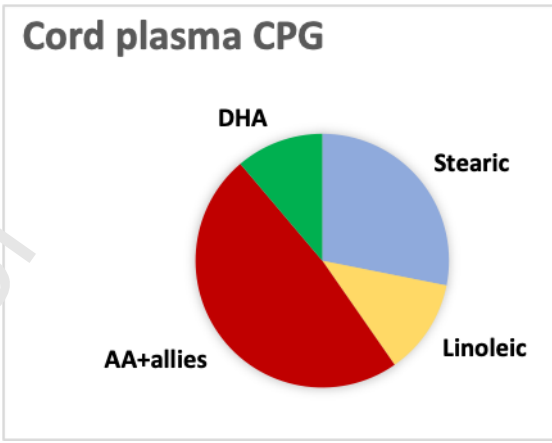


Figure 7 The summary comparison of ArA and stearic compared to DHA as selected by the placenta for the fetus.

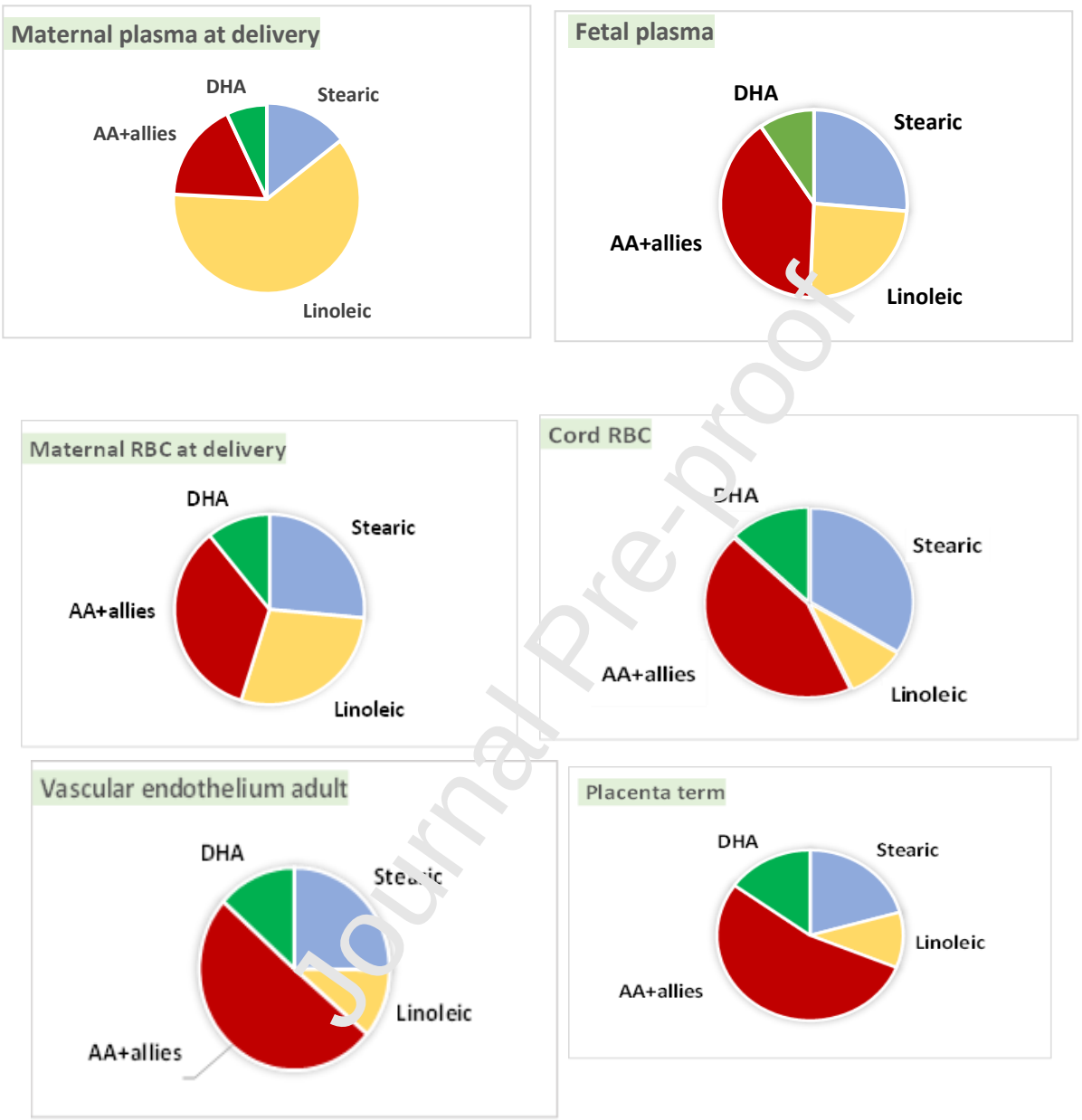
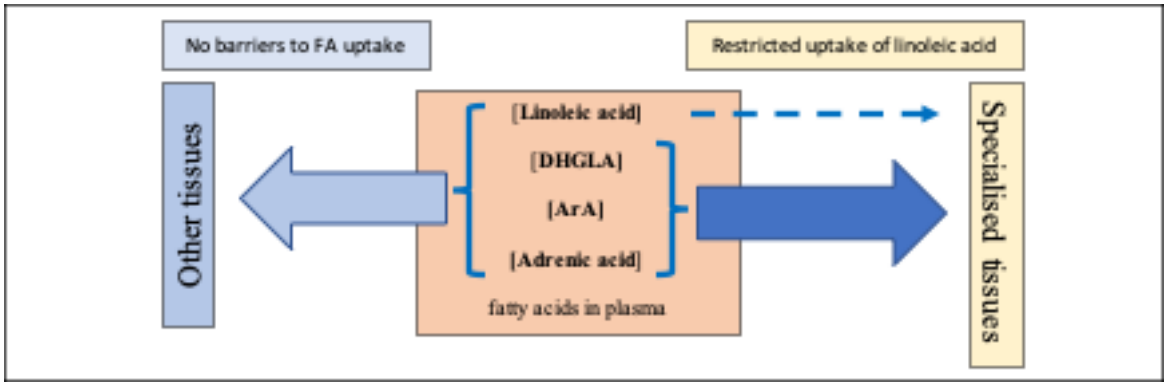


FIGURE 8 A pictorial summary of biomagification and bioreduction.



LEGENDS

Table 1

[1] Data are means followed by standard deviation; differences in means are tested using independent t-tests.

[2] Omega 3 index is the sum of DHA and EPA proportions

[3] S/P represents the ratio of saturated to polyunsaturated fatty acids

M=maternal; ∑=sum; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; LC=long chain; LA= linoleic acid; AA=arachidonic acid; DHA=docosahexaenoic acid

R vs D: *** P<0.005; ** P<0.01; * P<0.05; R vs C: ^^^ P<0.005; ^^ P<0.01; ^ P<0.05; D vs C: ### P<0.005; ## P<0.01; # P<0.05

Legend to Table 2

[1] Data are medians followed by the minimum to maximum range; differences in medians between maternal and fetal plasma lipids are tested using the non-parametric Wilcoxon matched-pairs signed rank test.

[2] Relative proportions of fatty acids are measured, as percentage of total FAMEs=fatty acid methyl esters; ∑=sum; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; LC=long chain; LA= linoleic acid; AA=arachidonic acid; DHA=docosahexaenoic acid

[3] Omega-3 index represents the sum of DHA and eicosapentaenoic acid EPA proportions

*P<0.05, **P<0.01, ***P<0.001

Legend to Table 3

[1] Data are medians followed by the minimum to maximum range; differences in medians between matched maternal erythrocyte and plasma fatty acids (n=34) are tested using the non-parametric Wilcoxon matched-pairs signed rank test

[2] Relative proportions of fatty acids are measured, as percentage of total FAMEs=fatty acid methyl esters; ∑=sum; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; LC=long chain; LA= linoleic acid; AA=arachidonic acid; DHA=docosahexaenoic acid [3] Omega-3 index represents the sum of DHA and eicosapentaenoic acid (EPA) proportions

*P<0.05, **P<0.01, ***P<0.001

Legend to Table 4

[1] Only includes birthweight from singleton pregnancies

[2] Aberdeen birth centiles refer to birth centiles accounted for race, parity, gestational age at delivery, and gender using nanograms developed according to data from the Aberdeen Maternity and Neonatal Databank.

[3] Relative proportions of fatty acids are measured, as percentage of total FAMEs= fatty acid methyl esters; ∑=sum; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; LC=long chain; LA= linoleic acid; AA=arachidonic acid; DHA=docosahexaenoic acid [4] Omega-3 index represents the sum of DHA and eicosapentaenoic acid (EPA) proportions.

Legend to Table 5

[1]Data are means followed by standard deviation, differences in means are tested using independent t-tests.. P>0.05 is considered non-significant. FAs=fatty acids; NS=non-significant.

Note the contrast between the biomagnification of plasma CPG and RBC EPG and CPG which makes the points on specificity of compositional data as per OPH and at the same time the strong biomagnification of arachidonic acid. In particular, the plasma CPG DHA is well biomagnified whereas the RBC was not consistent with concept of OPH for membranes and indeed, Jean-Marie Bourre's "long term parking".

Legend to Table 7

Selected fatty acid data comparing maternal and cord plasma CPG from three countries; and maternal and cord RBC EPG and CPG from two countries.

Legend to Table 6

1 Data are means followed by standard deviations, differences in means are tested using independent t-tests. P>0.05 is considered non-significant. SFA= saturated fatty acids; MUFA=monounsaturated fatty acids; NS=non-significant. This table and table 7 demonstrates the consistency of the biomagnification data regardless of ethnicity or geographic location. Note the bio-reduction consistency in the rejection of EPA and the ω3-DPA by the fetus. This pattern of biomagnification and bioreduction can therefore be considered to be a universal biological norm consistent with OPH..

Legend to Table 8

[1] Data are means followed by standard deviations, difference between means is tested using independent t-test. P>0.05 is considered non-significant. NS=non-significant. The data basically represents the array of membranes in the placenta. The dominance of arachidonic acid throughout is consistent even in the serine phosphoglycerides which are usually expected to be DHA rich.

Legend to Table 9

[1] CPG Data are means followed by standard error and medians with interquartile range. Fatty acid correlations between term, preterm, and DNI groups are tested using the non-parametric Kruskal-Wallis test. CPG=choline phosphoglycerides; DNI = definitely not infected preterm; NS= non-significant. Both arachidonic and DHA were depressed in the preterm infants. There was little or no difference in those definitely not infected and the others in whom there was some evidence of maternal infection during the pregnancy. The dominance of arachidonic acid in the immune cells from normal pregnancies. The higher proportions of arachidonic acid were associated with enhanced cell functionality (29) .

Legend to Table 10

[1] Data are means followed by standard error and medians with interquartile range. Fatty acid correlations between term, preterm, and DNI groups are tested using the non-parametric Kruskal-Wallis test. EPG=ethanolamine phosphoglycerides; DNI = definitely not infected preterm; NS= non-significant. The comment is the same as for table 9 except note that the arachidonic is twice the proportion and adrenic 5 times the proportion of their presence in the CPG.

Legend to Table 11.

Statistically significant data are highlighted to facilitate comparison between boys and girls. The correlations that were most striking in the girls were functions of arachidonic acid.

Table 12 a Countries: Denmark, Hungary, Saudi Arabia, Tanzania, Uganda, Sri Lanka, Thailand, UK, Vietnam. There are 512 samples except for Hungary and Thailand from where we obtained 2,000+ samples each. Data are means followed by standard error of the mean (SEM). LCPUFA= long-chain polyunsaturated fatty acids.

Table 12 b. The data from Vietnam is chosen here because the background diet is not swamped with linoleic acid revealing proportions of DHA similar to those we saw in Hackney in the 1970s. Contemporary data shows a strong increase in linoleic acid with a loss of DHA [50].

Legend to Table 13

Note that the isotope in brain arachidonic is many times greater than its synthesis from linoleic acid.
[1]Percentage of radioactivity in arachidonic acid relative to total radioactivity in all fatty acids in the TG and phospholipid fraction from liver and brain.
[2]Results are shown as mean ± SD from at least 4 animals per isotope.

Legend to Table 14

[1]Percentage of radioactivity in DGLA and arachidonic acid relative to total radioactivity in all fatty acids in the TG and phospholipid fraction from liver and brain. Note the greater incorporation of γ-18:3-1-14C into DGLA and arachidonic acid compared to linoleic acid which is identifying the rate limitation of the first desaturation (FADS2)
[2]Results are shown as mean ± SD from at least 4 animals per isotope.
In both tables 13 and 14 the use of two different isotopes made it possible to determine the extent to which the end product came from one or other or both.

LEGEND TO FIGURES,

Figure 1: ArA is clearly the major acyl component of inner cell membrane lipids of the endothelium which lines the arteries. arterial system. As the heart beats, the pressure waves cause the arteries to expand and contract. This action likely bring the inner membrane into intermittent contact with the lysosomes and contact with phospholipase A2. The subsequent release of ArA would explain the regular synthesis of prostacyclin which maintains good order in the arterial blood flow. The brain by contrast is comminated by DHA which is concentrated in the signalling systems. ArA in the brain is more concentrated in the astrocytes which are responsible for maintenance and myelination.

Figure 2: The striking presence in the cord artery endothelium of the Mead acid (20:3ω9) and its 22-carbon elongation product is a classical sign of essential fatty acid deficiency. It is from a piece of fetal tissue and this demonstration really requires further investigation with regard to the vascular incited pathophysiology's associated with prematurity. Intraventricular haemorrhage, periventricular leukomalacia, retinopathy of prematurity, and broncho-pulmonary dysplasia all have a vascular bed rock. Necrotising enterocolitis likewise is involved in immune dysfunction with vascular and immune system perturbations with multiple organ failure at its worst. The proportion of the ω9 trienes synthesised from oleic acid was approximately 25% of the ω6 tetraenes is astonishingly high. This magnitude woud be considered to be a severe stress. The powerful predictive value of RBC oleic acid for preterm birth, together with the ArA correlations with the volumetrics of the girls amd that of DHA with thei boys, indicates EFA and long chain PUFA needs to be investiaged as a likely aetiological disorders of prematrity which can adversely affect a child for life.

Figures 3 and 4: This study of the relaxation of the the mesenteric arteries by ARA (Figure 3) is in stark contrast to the absence of effect of DHA (Figure 4), implying this is a property of ArA in normal vascular function,

Figure 6: The plasma choline phosphoglycerides illustrate the biomagnification of ArA and bioreduction of linoleic acid are seen more starkly than the same contrast in the red cells. The plasma choline phosphoglycerides are more in contact with metabolic events rather than the red cell lipids which are sequestered in the membrane. Jean-Marie Bourre used to refer to the membrane lipid as the "long term parking".

Suggested illustrations

1. Fatty acid families, presented as vertical columns, down from stearic, oleic, linoleic , α linolenic.
   Just number of C atoms, double bonds, ω and name.

2. Schematic representation of blood flow between mother and fetus: placenta, villi, cord, veins and arteries etc including directions of biomagnification/reduction

3. Schematic representation of phospholipids in membranes

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Highlights

- The placenta regulates flow of fatty acids, selecting some and rejecting others.
- Arachidonic and Docosahexaenoic acids are biomagnified, their precursors are bioreduced.
- Arachidonic acid is dominant in vascular, immune cells, placental and neural neogenesis.
- Arachidonic acid appears to be paramount in prenatal development and growth of the fetus.