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Review

The imperative of arachidonic acid in early human development

Michael A. Crawford [a,b,*], Andrew J. Sinclair [c,d], Barbara Hall [b], Enitan Ogundipe [a], Yiqun Wang [a,b], Dimitrios Bitsanis [b,e], Ovrang B. Djahanbakhch [f], Laurence Harbige [b,g], Kebreab Ghebremeskel [g], Ivan Golfetto [b,h], Therishnee Moodley [b,i], Ahmed Hassam [j], AnnieBelle Sassine [a,b], Mark R. Johnson [a]

[a] Chelsea and Westminster Hospital Campus of Imperial College, London, UK
[b] The Institute of Brain Chemistry and Human Nutrition, SW10 9NH, UK
[c] Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, Victoria, Australia
[d] Faculty of Health, Deakin University, Burwood, Victoria, Australia
[e] EvexiaDiet dietetic practise, Athens, Greece
[f] Reproductive Medicine, Barts Health NHS Trust, London, UK
[g] School of Human Sciences, London Metropolitan University, UK
[h] Department of General Pathology and Physiopathology, Central University of Venezuela, Venezuela
[i] Centre for Reproductive Medicine, St Bartholomew's, Barts Health NHS Trust, London, UK
[j] Quest NutraPharma, Dubai, United Arab Emirates

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ABSTRACT

This review is about the role of arachidonic acid (ArA) in foetal and early growth and development. In 1975 and '76, we reported the preferential incorporation of ArA into the developing brain of rat pups, its conservation as a principal component in the brains of 32 mammalian species and the high proportion delivered by the human placenta for foetal nutrition, compared to its parent linoleic acid (LA). ArA is quantitatively the principal acyl component of membrane lipids from foetal red cells, mononuclear cells, astrocytes, endothelium, and placenta. Functionally, we present evidence that ArA, but not DHA, relaxes the foetal mesenteric arteries. The placenta biomagnifies ArA, doubling the proportion of the maternal level in cord blood. The proportions of ArA and its allies (di-homo-gamma-linolenic acid (DGLA), adrenic acid and ω6 docosapentaenoic acid) are similar or higher than the total of ω3 fatty acids in human milk, maintaining the abundant supply to the developing infant. Despite the evidence of the importance of ArA, the European Food Standard Agency, in 2014 rejected the joint FAO and WHO recommendation on the inclusion of ArA in infant formula, although they recommended DHA. The almost universal dominance of ArA in the membrane phosphoglycerides during human organogenesis and prenatal growth suggests that the importance of ArA and its allies in reproductive biology needs to be re-evaluated urgently.

Abbreviations: Ach, acetylcholine; ALA, alpha-linolenic acid; ArA, arachidonic acid; BPD, broncho-pulmonary dysplasia; CBMC, cord blood mononuclear cells; CO2, carbon dioxide; COX2, cyclooxygenase-2; CPG, choline phosphoglycerides; CSF, cerebrospinal fluid; DGLA, di-hommo-gammalinolenic acid; DHA, docosahexaenoic acid; DNI, definitely not infected; DPA, docosapentaenoic acid; EFA, essential fatty acids; EFSA, European Food Safety Authority; EPA, eicosapetaenoic acid; EPG, ethanolamine phosphoglycerides; FA, fatty acids; FADS, fatty acid desaturase; FAMEs, fatty acid methyl esters; HIE, hypoxic ischemic encephalopathy; IPG, Inositol phosphoglycerides; IUGR, intrauterine growth restriction; IVH, intraventricular haemorrhage; K–Pg, Cretaceous–Paleogene; KO, knock-out; LA, Linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; MRI, Magnetic Resonance Imaging; MUFA, monunsaturated fatty acids; NA, noradrenaline; NEC, necrotising enterocolitis; OPH, Optimum Physics Hypothesis; PGD2, prostaglandin D2; PGE1, prostaglandin E1; PGE2, prostaglandin E2; PGJ2, prostaglandin J2; PL, phospholipids; PUFA, polyunsaturated fatty acids; PVL, peri-ventricular leukomalacia; RBC, red blood cell; ROP, retinopathy prematurity; SAFA, saturated fatty acids; SPG, serine phosphoglycerides; Th1, Type 1 T helper; Th2, Type 2 T helper.

* Corresponding author at: Chelsea and Westminster Hospital Campus of Imperial College, London, UK.
E-mail address: michael.crawford@imperial.ac.uk (M.A. Crawford).

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1. Introduction

Arachidonic acid (ArA) is generally considered to be inflammatory and undesirable on account of its inflammatory derivatives. These have been claimed to have a significant involvement in neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and neuropsychiatric disorders and linked to the pathogenesis of acute stroke, global ischemia, subarachnoid haemorrhage, and anticoagulation-related haemorrhagic transformation [1,2]. However, in human studies, an increased intake of ArA does not increase the concentration of inflammatory markers [3] and more recently,it was conceded that the role of ω6 and ω3 fatty acids in inflammation is complex and not fully understood [4].

That the concern about ArA might be misplaced was suggested by the experience with non-selective COX2 inhibitors. These drugs are designed to supress cyclo-oxygenase activity on ArA, for the treatment of rheumatoid arthritis. At first, they seemed to be beneficial but an excess of cardiac events and mortality prompted their withdrawal [5]. It is likely that the selective COX2 inhibitors reduced prostacyclin synthesis, a critical factor for the maintenance in endothelial health and suppression of platelet activation. 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 blood flow and prevents spontaneous platelet aggregation [6]. Moreover, ArA oxidative products enhance immune function in the response to injury, help seal ruptured blood vessels, assist in the resolution of inflammation, a wide range of adverse conditions [7]. These data show that an imbalance in ArA derivatives can cause disease, but that an adequate sufficient supply of ArA is critically important for normal cardiovascular function.

The material of the brain is 60% lipid. The acyl composition of membrane lipids is rich in both ArA and DHA [8] which must be derived from their parent essential fatty acids, linoleic (LA) and alpha-linolenic acid (ALA) or from the diet. Between 1974 and 1976, we published a series of papers that compared differentially labelled LA, gamma-linolenic acid and ArA in the same animal, to test the efficiency of the biosynthesis from LA or incorporation of ArA during brain growth in rat pups [9–11]. The data revealed the desaturation steps can be rate limiting in a stronger manner than the elongation and the selective incorporation of ArA into developing brain lipids with an order of magnitude higher efficiency than its synthesis from LA (Fig. 1). We found the same regarding the preferential incorporation of preformed DHA [9,10].

At present, there is a strong focus favouring ω 3 fatty acids in nutrition and health, immune system [22], cardiovascular [23], cognition and other functions [24]. 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

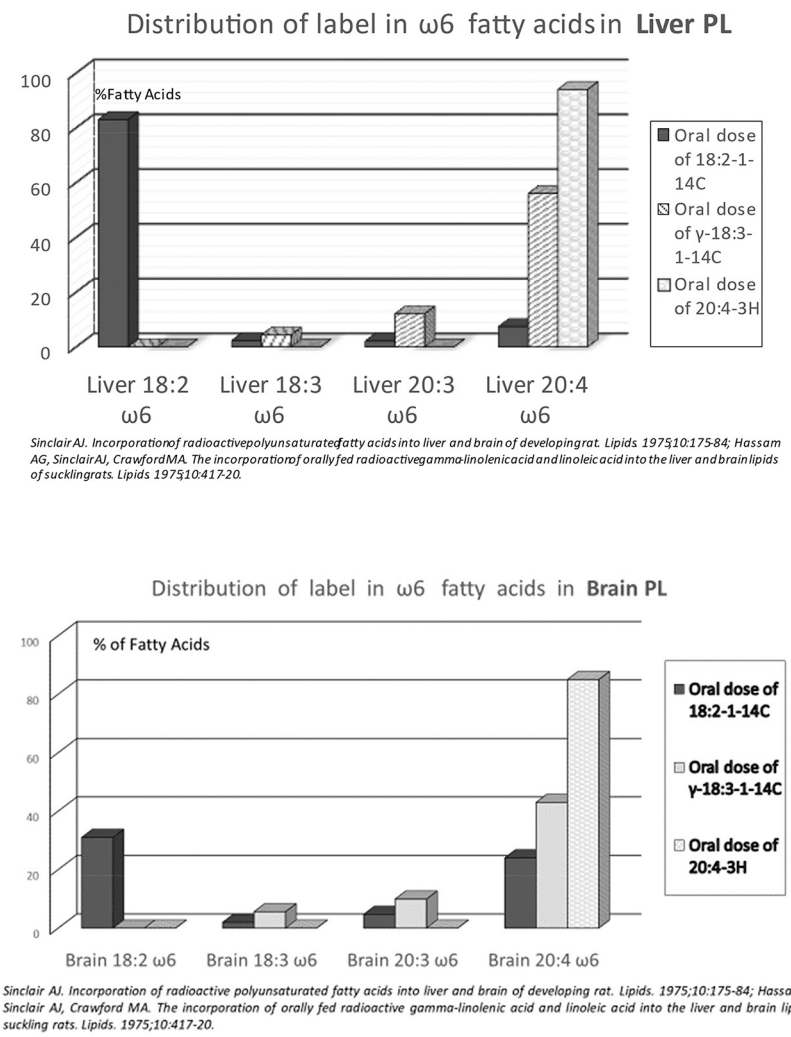

Fig. 1. Percentage of radioactivity in ω6 fatty acids including arachidonic acid relative to total radioactivity in all fatty acids in the phospholipid fractions from liver and brain. The results are shown as mean from at least 4 animals per isotope (data from 10, 11). In these studies, the use of two different isotopes made it possible to determine the extent to which the end product came from one or other or both.

ArA's oxidative derivatives functioning in the regulation of blood pressure, blood flow, inflammation and the resolution of injury [25,26]. In this paper, we review the maternal/foetal, lipid transfers, demonstrating the high selectivity and specificity for ArA but also for nearly all fatty acids transferred to the foetus, with both positive and negative selectivity. We believe the totality of this evidence may shed a new light on ArA and its allies and perhaps also stearic acid which is similarly biomagnified.

Here, we present a review of our findings which describe a dominant contribution of ArA to foetal growth and development that is extended postnatally to the infant by the high concentrations of ArA in human milk. Our work challenges the commonly held negative perception of ArA [12–14].

1.1. Optimum physics hypothesis for cell membranes

The term "OPH" (Optimum Physics Hypothesis) was coined by the late Myer Bloom, professor of Physics and Astronomy at the University of British Columbia, to describe the conditions of the cell membrane pertinent to its optimal function [15,16]. His view was that different cell types and intracellular organelles, would have different compositions depending on their functions [17]. These differences would involve the relationship between lipid composition and proteins specific to the organelle and its specific function. Work by Nicholas Bazan provides an example, with radio-autographic evidence of co-migration of rhodopsin and DHA during the reconstruction of the phagocytized material in the pigment epithelium for the rod. The data indicate non-covalent binding of DHA to rhodopsin [18]. This evidence is further supported by biophysical analysis from Klaus Gawrish's group, illustrating DHA-rich phosphatidylethanolamines accumulating preferentially near the protein in G-coupled membrane receptors [18–20]. As an example of specificity of membrane receptor function, membrane, ArA has been reported to be involved in protein kinase C activation [21].

1.1.1. Early studies

The discovery of the essential fatty acids (EFA) by Burr & Burr (1929,'30) [27] was soon followed by the recognition that deficiency of EFA had dramatic negative effects on fertility, pregnancy outcomes, pup survival, ovulation, prolonged gestation and bleeding and testes atrophy. It was established that both LA and ArA, but not ω 3-alpha linolenic acid, could prevent these effects [28]. It was only in the 1970's that attention turned to the fact that the human brain and indeed the brains of 32 mammalian species had a constant fingerprint of PUFA, namely DHA, ArA and adrenic acid where these fatty acids accounted for approximately 1/3 of total brain FA [29]. Having reported that human milk contained ArA and DHA [30], we were keen to investigate whether these long chain FA were incorporated into the developing brain during the suckling period.

In 1975 we showed the preferential incorporation of ArA into the developing brain of rat pups in comparison to its synthesis from LA. The preferential selectivity for ArA, as opposed to synthesis from LA, was close to an order of magnitude higher (Fig. 1) [31]. This selective incorporation involves each step from LA to gamma-linolenic to di-homo-gamma-linolenic to ArA in which the desaturation steps appear to have a stronger rate limitation than the chain elongation [32]. This selectivity for ArA during prenatal brain development led to an examination of the process in human early prenatal brain development in a study of elective abortions. The data showed ArA was biomagnified across the placenta with nearly twice the proportion in the foetal plasma choline phosphoglycerides (15.6% ± 0.9 SEM) compared to the maternal levels (8.2% ± 0.71). By contrast, the proportion of the parent essential fatty acid, linoleic acid (LA) was halved (23% ± 1.4 to 8.9 ± 0.6). This enhancement of the proportion of ArA was referred to as biomagnification which was continued to neurosynthesis. The proportion of ArA in the foetal brains was 16.6% ± 1.3 in the choline phosphoglycerides (CPG) and 22.6% ± 1.3 $n = 9$ in the ethanolamine phosphoglycerides (EPG) whereas the LA proportion in the CPG was 1.1% and EPG 0.47%. We described the same principle of selective biomagnification for DHA although the maternal-foetal transfer was of a lesser order relying on a more stepwise enhancement from mother to foetal blood, to foetal liver and then to foetal brain [33], as exemplified in Fig. 2. These data were from elective abortions with the caveat that the data refer to an early period in pregnancy and in the figure the data for ArA and DHA are from different tissues..

In 2015 Shlormann et al. [34], reported that the levels of trans isomers were lower in cord blood compared to the mothers, again suggesting the placenta is screening for foetal requirements. The data we present in this review are not new. Previous publications focus on the polyenoic fatty acids only as in a recent 2021 paper [35]. In this review we draw attention to the wide scale of selectivity expressed which includes the saturated, monounsaturated, DHA precursors and the allies of ArA, demonstrating selectivity consistent with Bloom's OPH. This wide range of data would have been present in previous publications of ours and other authors but thus far, not mentioned. Hence we include the full set of fatty acids in certain tables because we believe that their significance has been overlooked.

2. Data validating the optimum physics hypothesis (OPH)

The fatty acid compositional data in this review are presented as a % of total fatty acids. We acknowledge that expression of data in this way necessarily means that increases in the % of one FA will mean decreases in % of other FA. However, our data clearly show specific and major alterations in the proportions of LA and ArA in the plasma lipoproteins and cellular compartments, with little if any change in the proportions of other FA.

In Tables 1 and 2, summarised in Fig. 3, we suggest that the biomagnification data are relevant to foetal nourishment and cell structural utilisation for growth. The ArA precursor, linoleic acid (LA), is processed in the reverse manner with its proportion in the foetal plasma being less than half that of the maternal side. The difference is more striking in the plasma choline phosphoglycerides (Table 2). The same is true of all DHA precursors, with the small proportions of α-linolenic and EPA, also reduced in the foetal circulation. At the same time our RBC data show that the saturated fatty acids are bio-magnified and the monounsaturated are bioreduced. These movements represent a formidable, selective action that has evolved in mammalian evolution for prioritising nutrients for foetal nourishment: the OPH operating on a grand scale.

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

The fatty acid analysis of the RBC 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, metabolism including oxidation to CO2, fatty acid desaturase (FADS), compartmentalisation into polar/non-polar lipids with their various partitions and selectivity for membrane synthesis and maintenance with genetic variations in all compartments [36].

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". 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. Although diet and genetics play basic roles both regarding the

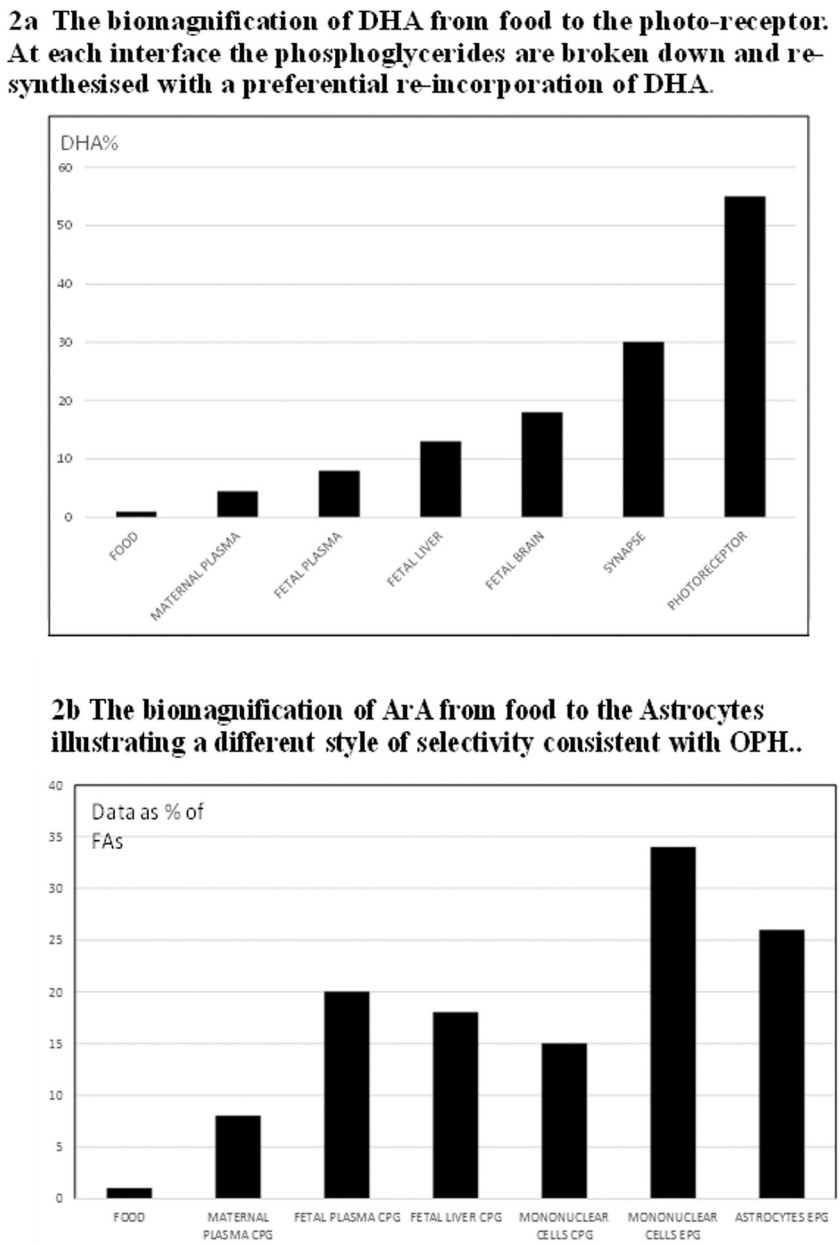

Fig. 2. Biomagnification of ArA and DHA at every step from food to its super-saturation in the photoreceptor. Figures constructed based on widely accepted data.

amount of the EFAs, their derivatives and the competing/enhancing dietary factors, it is the end-product in the membrane that ultimately matters: "*the tissue is the issue*".[1]

2.1.1. Analysis of maternal and cord blood fatty acids

A study of arterial-venous differences across the placenta described a "*considerable venous-arterial difference for the RBC membrane fatty acids*". Hence it is plausible that both RBC and plasma contribute to foetal nutrition [37]. Table 1 presents compositional data of principal RBC fatty acids determined in healthy women from a study of 296 pregnancies at Chelsea and Westminster Hospital, London. UK FOSS1.[2]

In Table 1 it can be seen that the change from recruitment to delivery identified a notable increase in oleic acid and all monounsaturated fatty acids (MUFAs). There was a reduction in ArA, DGLA and adrenic acids from recruitment to delivery. Previously, we found that RBC membrane oleic acid was a predictor of preterm birth [38]. Membrane oleic acid is known to increase in response to a fall in long chain PUFA. Hence the drop in the proportion of ArA (12.8% ± 1.59 to 10.6% ± 2.34 $P < 0.005$) and increase in oleic (13.8% ± 1.58 to 15.2% ± 1.74 $P < 0.005$) and other monounsaturated fatty acids from recruitment to delivery are consistent. However, there was no significant difference seen in DHA proportion from recruitment to delivery, raising questions regarding the reduction in the proportion of ArA. Is this reduction the consequence of an inadequacy of preformed ArA in face of the biomagnification of ArA which is significantly greater than that of DHA? Alternatively, the increase in blood volume could explain the reduction in plasma ArA from recruitment to delivery. However, there was no significant reduction in DHA. It is plausible that the transplacental movement of ArA was at least in part, responsible for the decline.

Table 2 presents data from maternal and foetal, plasma choline

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

[2] 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

Table 1
London (UK) recruitment, delivery and cord blood RBC fatty acids with bidirectional selectivity. Note the proportion of ArA falls from recruitment to delivery whereas that of DHA does not. All precursors of DHA. ALA, EPA and ω3DPA proportions fall across the placenta whilst DHA rises. Similarly, with the ω6 LA falls whereas the DGLA, ArA and adrenic acid are all higher in the foetal circulation compared to maternal.

	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	0.05 ± 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 ± 0.53* ↑	2.49 ± 0.65^^^###
22:5ω6	0.29 ± 0.13	0.36 ± 0.14↑	0.73 ± 0.27^^^###
$\sum\omega6$	29.9 ± 2.56	27.4 ± 3.35	25.4 ± 4.54^^^#
$\sum\omega6LC$	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
$\sum\omega3$	7.96 ± 1.55	7.07 ± 2.08	7.01 ± 1.78
$\sum\omega3LC$	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^^^###
Ω3 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^^

M = maternal; $\sum$ = 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: ### $P < 0.005$; ## $P < 0.01$; # $P < 0.05$.
[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.

phosphoglycerides to illustrate the more significant 2-fold biomagnification of ArA compared to the 1.33 amplification in the RBC. The bioreduction of LA is also more prominent than in the RBC with the reduction in the plasma being almost 3-fold. It also confirms the biomagnification of stearic acid which is in similar proportion to that which is seen in the RBC data in Table 1.

The data in Table 2 are from a study of 58 Vietnamese uncomplicated pregnancies and healthy births at full term as confirmed by ultrasound assessment for gestational age. The study was a part of Dr. Ivan Golfetto's PhD thesis [39] and was conducted during a neurological survey. The location was the Maela camp in Vietnam [40]. The data are presented to illustrate the difference in plasma and RBC and are similar to our published data from London, UK [33,41]. It also confirms the cross-cultural identity of the biomagnifications and reductions discussed later.

In Table 3 we summarise key data comparing selected plasma and RBC fatty acids from Hackney (a multi-ethnic region of London), Vietnam and Thailand [42] to confirm as in Tables 1 and 2, the cross-cultural identity in the principle of biomagnification of ArA and the modest amplification of DHA across the placenta.

These data also illustrate the greater amplification and bioreductions that occur in the plasma CPG compared to the fatty acids of the RBC. The proportionate biomagnification of plasma ArA CPG (8.76% ± 1.49 to 17.5% ± 3.22 $p < 0.0001$) is greater than the RBC (10.6 ± 2.34 to 14.1 ± 3.30) $P < 0.0005$) and interestingly greater than the corresponding data for RBC DHA (4.82 ± 1.54 to 5.74 ± 1.59 ns). Opposite to ArA there was a striking bioreduction of linoleic acid (RBC 12.6 ± 1.78 to 4.18 ± 2.01 $p < 0.0001$) and in the plasma CPG (18.6% ± 3.05 to 6.31 ± 1.27 $p < 0.0001$).

All the long chain ω6 fatty acids, C20:3ω6, C20:4ω6, C22:4ω6 and C22:5ω6 were biomagnified. In contrast, all DHA precursors (18:3ω3, 20:5ω3 and 22:5ω3) were bioreduced. Some authors refer to the biosynthetic conversion of precursor to DHA, including suggestions of that this process is occurring in the brain. Whilst in vitro studies may illustrate the potential, in reality there is so little precursor as to make biosynthesis in the foetus unlikely or at the least to be less significant than the use of the biomagnified PUFA (ArA and DHA) (see Fig. 2).

Table 2
Plasma choline phosphoglycerides fatty acid composition of mothers and cord from Vietnam (% Total Fatty Acids, Mean ± SD).[1]

Fatty acids	Mothers (n = 44)	Fetus (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
24.0	0.32 ± 0.11	0.51 ± 0.12	<0.05
ΣSFA	45.29 ± 3.60	45.92 ± 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 ± 0.18	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
20:3ω9	0.17 ± 0.06	0.70 ± 0.35	<0.0001

[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 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.

2.1.2. Monounsaturated (MUFA) and saturated fatty acids (SAFA).

In transit from maternal to foetal blood, the RBC MUFAs were bioreduced, except for 18:1ω7 and nervonic acid. On the other hand, the SAFA were bioamplified except for the small amount of myristic acid (C14:0) which seldom features in cell membranes. These SAFA biomagnifications and MUFA bioreductions have not been commented on before other than our specific reference to stearic acid. The significant difference for stearic from maternal to cord blood was similar in both RBC and plasma (RBC 11.7% ± 1.18 to 15.2% ± 1.98 $p < 0.005$, plasma CPG 9.80% ± 1.84 to 14.6% ±1.94 $p < 0.001$). The biomagnification of the SAFA which included lignoceric likely provide for the sn-1 positions of the phosphoglycerides, and ceramide and myelin synthesis. Interestingly, although the MUFAs were bioreduced, nervonic acid was amplified, again consistent with the OPH and likely to promote myelination.

2.1.3. Cultural similarity

Table 3 summarises comparative cross-cultural data obtained from women in London, Thailand and Vietnam which confirms the general principle of bio-reduction/magnification with selected data for plasma CPGs and RBC. Transplacental selectivity is the same across different cultures and socio-economic status as the Hackney data are from a disadvantaged, multi-ethnic region in London, where poor nutrition was a risk factor for low birthweight independent of ethnicity [43],compared to more affluent population from the Chelsea and Westminster Hospital in London (Table 1). The data for ArA from all three centres is remarkably close to identical.

In Table 4 we present data for matched fatty acid totals of maternal RBCs compared to plasma sampled from the mothers at delivery [44] again as an illustration of OPH at work. It is interesting that the difference between plasma and red cells is somewhat similar to the difference between maternal and foetal cord bloods in that LA is present in the RBC at a significantly lower proportion than in the plasma whilst ArA and DHA are at higher proportions. In the ω 3 family ALA and EPA are both almost at trace levels in the RBC. On the other hand, DGLA, adrenic, and the ω6-docosapentaenoic acids are present in significantly higher proportions than in the plasma (for adrenic RBC 2.45% (1.38–4.58) compared with plasma 0.10% (0.02–2.01). The plasma is the source or exchange pathway for the RBCs. Hence, a relative deficiency of ArA would be seen as a greater difference between plasma and RBCs.

2.1.4. Gestational age and birthweight

Table 5 summarises the correlations for placental fatty acid composition with subsequent gestational age, and Aberdeen birthweight centiles, 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 [45], was associated with gestational age. There was no independent relation with birthweight and ArA 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, and this ratio is often more sensitive than the individual fatty acids [46]. It is plausible that the negative correlations with gestational age, and the decline in plasma ArA from recruitment to delivery (Table 10a 20:4ω6[b]-20:4ω6 [a]) and several regional brain volumetrics of the girls, is due to the extraction of ArA by the placenta, in face of an inadequate dietary provision. Fig. 1 highlights the difference in utilisation of preformed ArA compared to biosynthesis.

2.1.5. Plasma compared to RBC ethanolamine and choline phosphoglycerides (Table 6)

In this table we present the comparison of the principal 20 and 22 carbon chain length polyenoic fatty acids in plasma CPG and RBC CPG and RBC EPG in mothers and their neonates from the studies in Hackney, London (UK). Whilst it describes much as has been seen above, we considered it worth presenting, as the 20 and 22 chain length ω6 fatty acids are represented in the brain CPG and EPG. In the ω3 family, however, EPA is present in only trace amounts in the brain which might arise from contamination by blood. In each fraction, ArA, 22:4ω6 and 22:5ω6 were present in significantly higher proportions in the neonatal than the maternal samples.

2.1.6. Early and term placentas compared (Table 7)

The placental tissue data in Table 7 summerise 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 largely represent the placental tissue membranes. The placenta develops ahead of the foetal brain thrust of the last trimester. Our starting point is the placenta itself. These data are from mothers with no adverse health issues, 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[3] 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 DGLA, ArA and adrenic acid and their metabolites in the cell regulation [47] 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.

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

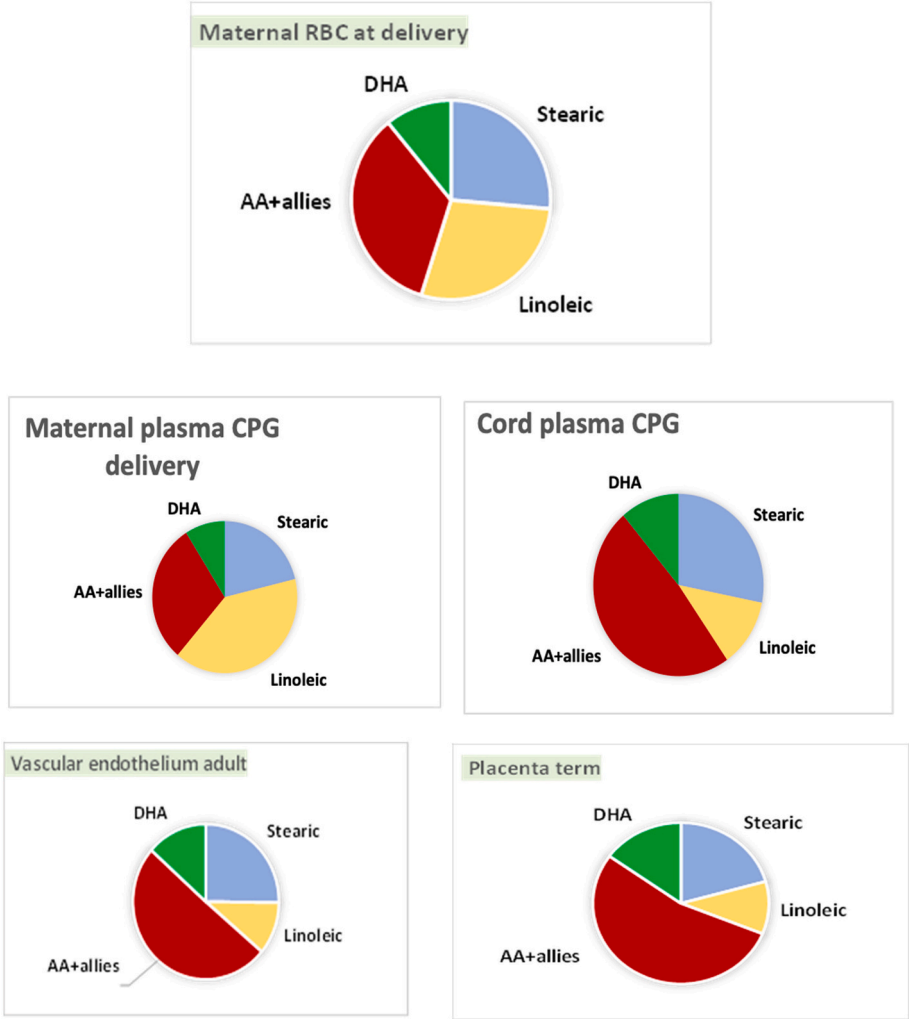

Fig. 3. The plasma choline phosphoglycerides illustrate the biomagnification of ArA and bioreduction of linoleic acid are seen more starkly than the same contrast in the red cells. The plasma choline phosphoglycerides are more in contact with metabolic events rather than the red cell lipids which are sequestered in the membrane. Jean-Marie Bourre used to refer to the membrane lipid as the "long term parking".

Experimental studies have demonstrated that ArA and its eicosanoid metabolites are involved in follicular development and ovulation and corpus luteum function [48].

2.1.7. Placental choline phosphoglycerides

The data from the placental choline phosphoglycerides for the early and term placentas makes the same point as seen in RBC total fatty acids versus plasma and maternal versus cord bloods (Table 7). We saw a dominance of ArA and its allies in the placental cell membranes. There was remarkably little DHA, 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. There has been limited research on PGE1, derived from DGLA [49], however it is known to have antiplatelet activity [50].

2.1.8. Placental ethanolamine phosphoglyceride (EPG)

The differences between the early and term placental, plasma membrane bi-layer, EPG were minimal (Table 7). The EPG is the principal location for the long-chain PUFA and is in the inner leaflet of the membrane 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 $p < 0.0001$) DHA was present but at less than a third of the proportion of ArA and was not different in the early compared to the term placental EPG. It was better represented than in plasma and RBCs (8.24 ± 1.8 vs 8.87 ± 1.99 ns) although in the early placentas all C20 and C22 ω6 were present at nearly 4 times the total C 20 and C22 ω3 fatty acids.

2.1.9. 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 (Table 7). However, contrary to expectations from general membrane data, ArA and its acolytes remained dominant. In the early placentas SPG the ArA proportion was 11.2% compared to 3.88% for DHA which is usually found to dominate the PUFA content in SPG (especially in neural tissue) [51]. The major difference was in DGLA (3.96% $\pm$ 0.91 compared to 9.15% $\pm$ 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.

2.1.10. Placental inositol phosphoglycerides (IPG)

The inositol phosphoglycerides are typically rich in ArA (Table 7). Indeed, ArA and all long chain ω6 LCPUFA accounted for 42% of the

Table 3
Summary data comparing selected fatty acids from Hackney (London), Vietnam and Thailand plasma choline phosphoglycerides.

SAMPLE	FATTY ACIDS	PLASMA CPG MATERNAL	PLASMA CPG CORD	P
HACKNEY $n = 54$	18:0	10.2 ± 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 ± 1.01	<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	17.54 ± 3.22	<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
	FATTY ACIDS	RBC EPG MATERNAL	CORD	P
VIETNAM	C20:4ω6	16.0 ± 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
	FATTY ACIDS	RBC CPG 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

The data are means followed by standard deviation. Selected fatty acid data comparing maternal and cord plasma CPG from three countries, and maternal and cord RBC EPG and CPG from two countries.

fatty acids whereas the LCPUFA ω3 amounted to 3.7%, so the ω6 LCPUFA were 12 times the proportion of the ω3LCPUFA in the IPG. Note again the higher proportion of DGLA at term with the lower amounts of ArA and adrenic acids. These differences seem common to all the phosphoglycerides and are unrelated to prematurity as the mothers were all term deliveries and 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 proportion of ArA was slightly less at term in the EPG (28.5 ± 2.12 to 24.7 ± 1.83 $p < 0.001$) but more so in the inositol phosphoglycerides (35.9% ± 2.58 to 31.9% ± 2.48 $p < 0.005$).

The results did not support the notion that the levels of ArA increase prior to delivery to allow prolabour eicosanoid synthesis. The early placenta ArA was, if anything, marginally higher than term. Indeed, 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 that the process of labour would drive further prostaglandin synthesis in a positive feed-forward cycle ensuring that once started, labour would continue until delivery is achieved [52].

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 the development of the placenta and fetus, adding to the plethora of biological control functions attributable to ArA and its various, cell regulatory products [53].

The data in Table 7 imply that the changes in placental composition from the 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 was 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 was also a significant difference in the CPG for

Table 4
The OPH of selectivity for matched maternal RBC fatty acids compared to plasma at delivery, Note the paucity of ALA and EPA.

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)	0.13 (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
$\sum\omega 6$↓	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
$\sum\omega 3$↑	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
Ω 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

*P < 0.05, **P < 0.01, ***$P < 0.001$.

[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] ω-3 index represents the sum of DHA and eicosapentaenoic acid (EPA) proportions.

Table 5
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).

	Gestation age at delivery ($n = 73$) ($N = 71$)		Aberdeen birth centiles ($n = 49$)[2] (N = 49)	
Placental fatty acids (% 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	0.43
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	0.0004	−0.21	0.15
ω6/ω3	−0.35	0.003	−0.24	0.10
Ω 3 Index[4]	0.34	0.003	0.21	0.16

[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] Ω-3 index represents the sum of DHA and eicosapentaenoic acid (EPA) proportions.

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 accumulation of DGLA reported from the early to the term placentas 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 explanation is that the maternal capacity to supply ArA is exhausted by the demands of placental and foetal growth. The placenta processes great quantities of blood through its extensive endothelial system supporting the placental terminal villi, which are the functional unit of maternal-foetal 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 CPG and SPG are trivial yet the reduction in the IPG and EPG 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 may be tending to outstrip the metabolic conversion of DGLA to ArA.

Powell et al. [54] analysed the fatty acids in the total phospholipid of the polar brush border microvillous membrane and basal membrane 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 [54].

2.1.11. Immunity: cord blood mononuclear cells (CBMC)

Survival of the foetus 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 8 and 9, consistent with the observation that in ex vivo experiments, ArA has been found to support mononuclear cell function [55].

ArA is important for early immune cell and organ development and its striking accretion into thymus phospholipids [56]. The consequences of the ArA deficit in pre-term cord blood CBMCs phospholipids (Table 8) could therefore be on both the early, and post-natal growth and development of immune cells and organs e.g., thymus development as well as cellular immunological deficits/dysregulation. During early pregnancy, the Th1 Th2 balance is tilted in favour of the Th2 anti-inflammatory response, balancing the need to maintain the immune response to infection while protecting the fetoplacental unit from maternal Th1 responses, resulting in a poor pregnancy outcome [57]. One of the mechanisms by which a shift towards a Th2 like response could be physiologically mediated is via ArA series 2 prostaglandins such as PGE2 and PGJ2, which are known to inhibit Th1 responses e.g., suppress interferon-gamma [58,59]. Thus, deviation of immunity towards a beneficial Th2 anti-inflammatory response during pregnancy may be dependent on cellular phospholipid ArA.

2.1.12. Immune cells CPG and EPG

The mononuclear cells isolated from cord blood at birth, can be expected to represent the prenatal state. As with the placenta, ArA and its

Table 6
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, London UK 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

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".
[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.

Table 7
Long chain, fatty acid composition of phosphoglycerides of early (8–14 wk) and term placentas illustrating the dominance of ArA and its C20 and C22 partners in the membrane bi-layer (Newham General Hospital in East End of London).[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	0.55 ± 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	3.88 ± 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. 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.

allies dominated. Even in the EPG fraction, which has the highest proportion of DHA at 6.61%, in term cord blood mononuclear cells (CBMCs), the proportion of ArA was 6-times greater than DHA which was similar to the ratio in CBMC CPG at 9-times greater than DHA (Table 8).

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 [60] consistent with their greater susceptibility to infection [45,61,62].

2.1.13. Astrocytes and ArA

The fatty acid data on astrocyte phospholipids, presented by Christine Bénistant et al. [63], 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 Fig. 4). 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 [64]. Whilst the neurons and synapses may have more DHA than ArA, the quantitative dominance of the astrocytes and their pivotal role in brain health, means that the supply of ArA may be critically important to astrocyte and so brain function.

2.1.14. Fatty acids as markers of prematurity

In Fig. 4 we summarise the polyenoic acid content of human aortic endothelium to illustrate the dominance of ArA. A capillary gas-liquid chromatographic trace of the endothelial cells scraped from the cord of very low birthweight, preterm infant is presented in Fig. 5. 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 [65]. It also shows a similarly large peak of the Mead Acid elongation product C22:3ω9 [45]. which has rarely been reported. This surely signifies a significant deficit of EFA and of 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 extraordinarily high proportions raise the question as to the role of EFAs in foetal nutrition and the stability of the endothelium referred to above. There were also significant levels of both these ω9 fatty acids in normal term births, in the order of 2% and 1%, respectively.

The ratio of Mead acid/ArA (triene-tetraene ratio 20:3ω9/20:4ω6) has been used as a quantitative assessment of the degree of EFA deficiency. This study, although small (14 births), there were none-the-less strong correlations between endothelial EPG and birth weight (20:3ω9/20:4ω6, Pearson coefficient at −0.87 t value 6.46 $n = 14$ $p < 0.001$) and head circumference (Pearson −0.83 $t = 5.14$ $n = 12 < 0.001$) [45] That is, the smaller the weight at birth the greater the classical measure of EFA deficiency.

The umbilical endothelium is a piece of foetal tissue and although it is a throw-away tissue, the data represent a significant message regarding prematurity. If the data reflect the state of the endothelium in the developing brain, then that would be a matter of concern as it is known that preterm birth carries a high prevalence of neurodevelopmental disorders. Further research is needed to assess the significance of the Mead acid in the umbilical endothelium of preterm infants and whether there is a relationship to the high prevalence of neurodevelopmental disorders and cerebral palsy.

Interestingly, oleic acid is the precursor of the Mead acid and is a marker of preterm birth, and it also is a biomarker for low levels of long chain PUFA [37,62]. In the comparisons in all the Tables, oleic acid and

Table 8
Comparison of fatty acids in cord blood mononuclear cells[1] choline phosphoglycerides (CPG) from term, preterm and definitely not infected (DNI) (Newham General Hospital).

CPG 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	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

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 [55].

most MUFAs were raised when ArA was lowered and vice versa. In dealing with percentages, when something goes up something else goes down. However, a specific rise and a specific decrease is likely to be meaningful.

The early neogenesis of the embryonic cardio-vascular system serves organogenesis and emergence of the placenta which is a vascular network. It will also be required to serve the prodigious energy needs of the third trimester foetal brain growth thrust. Inadequacy of vascular development is involved in the complications of prematurity. These include 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/function. ArA is almost certainly involved in the earliest cardio-vascular development and angiogenesis delivering the nutrients for organogenesis, promoting and maintaining the rapidly expanding vascular networks. These will require the needed regulation of function through prostacyclin, possibly assisted by DGLA and PGE1. In an MRI study of intrauterine growth restriction (IUGR), evidence was presented implicating substandard blood flow [66].

2.1.15. A plausible explanation for a misunderstanding of ArA and inflammation

As ArA is the precursor to PGE2, which is suggested to be pro-inflammatory, and to thromboxane, which is prothrombotic, ArA has been unfairly linked to these adverse effects., within the immune system PGE2 is immune regulatory and has a range of anti-inflammatory effects from decreasing Th1 lymphocyte cytokines to inducing tolerogenic effects on dendritic cells and reducing inflammatory cytokines [67,68]. The thrombotic role of thromboxane derived from ArA is a response to injury, which is vital for survival. Other ArA derivatives can be beneficial [69] 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 [70]. Mediators, e.g., eicosanoids and cytokines, act differently in maintenance, the response to injury (whether physiological or traumatic) and its resolution. Inflammation is a response to injury or a disease process which causes roughening of the arterial wall, damage to the face of bone joints, haemorrhage or tissue breakdown. Both would be expected to elicit an arachidonic acid-dependent eicosanoid response driven by the disease process, not ArA. Indeed, ArA and its eicosanoids maintain the normal function of a range of essential processes, including vascular tone and preventing platelet adhesion ensuing proper blood flow [71,72].

2.1.16. 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 making it a cell type mass weighing an aggregate of at least 1 Kg [73]. Whilst these are data for adults, the velocity of prenatal endothelial development must be high and as mentioned earlier, will be achieved before, and in preparation for the acceleration in foetal brain growth during 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 and are compositionally and precisely determined by the genome. By contrast, the composition of the lipid component is susceptible to nutritional and other external influences.

Table 9
Comparison of the fatty acid composition of cord blood mononuclear cells[1] ethanolamine phosphoglycerides (EPG) from term, preterm and definitely not infected (DNI) (Vietnamese samples).

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: 0.92–0.21	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: 063–1.46	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. 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.

Moreover, the composition of the lipid affects membrane protein function and signalling [74]. A major variable in membrane composition is diet especially as regards the EFAs and their longer chain derivatives. This places membrane lipids 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 due to an EFA deficiency [75]. 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 characterised by reduced contraction/relaxation and vasodilation ability [76] 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.

To test the idea that ArA had a role in arterial relaxation, small mesenteric arteries ex vivo were used in the myograph technique [77]. The isolated mesenteric arteries were perfused with individual fatty acids bound to the albumin [39]. 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 were washed with 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 Fig. 6 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. Fig. 7 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.

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

We studied the impact of a supplement (DHA 300 mg, 8.4 mg ArA /day) on the Magnetic Resonance Imaging (MRI) of the brains of newborn infants. The supplement was started shortly after booking for pregnancy care (around 12 weeks after conception), in a randomised trial with a placebo of olive oil (ISRCTN 240687 [37]. We found that DHA supplementation increased male but not female brain growth. The data here, are presented for the placebo groups only (Table 10).

2.1.18. *Correlation of fatty acids in maternal recruitment, delivery and cord RBC and regional brain development in girls (Table 10)*

Significant Pearson correlations between maternal LA/ArA were observed in the girls 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 $p < 0.0019$ $n = 12$; Table 10A). There was only one significant correlation in the data on the boys. This was the proportional difference ($20{:}4\omega6^{b}$-$20{:}4\omega6^{a}/20{:}4\omega6^{a}$) between ArA at term and recruitment (0.500 $P < 0.035$, $n = 18$) (Table 10B).

The cord/maternal DHA ratio in the boys is like the ArA/LA ratio of the girls signify the separate provision for DHA and ArA for foetal 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 unlikely to be chance but evidence for the

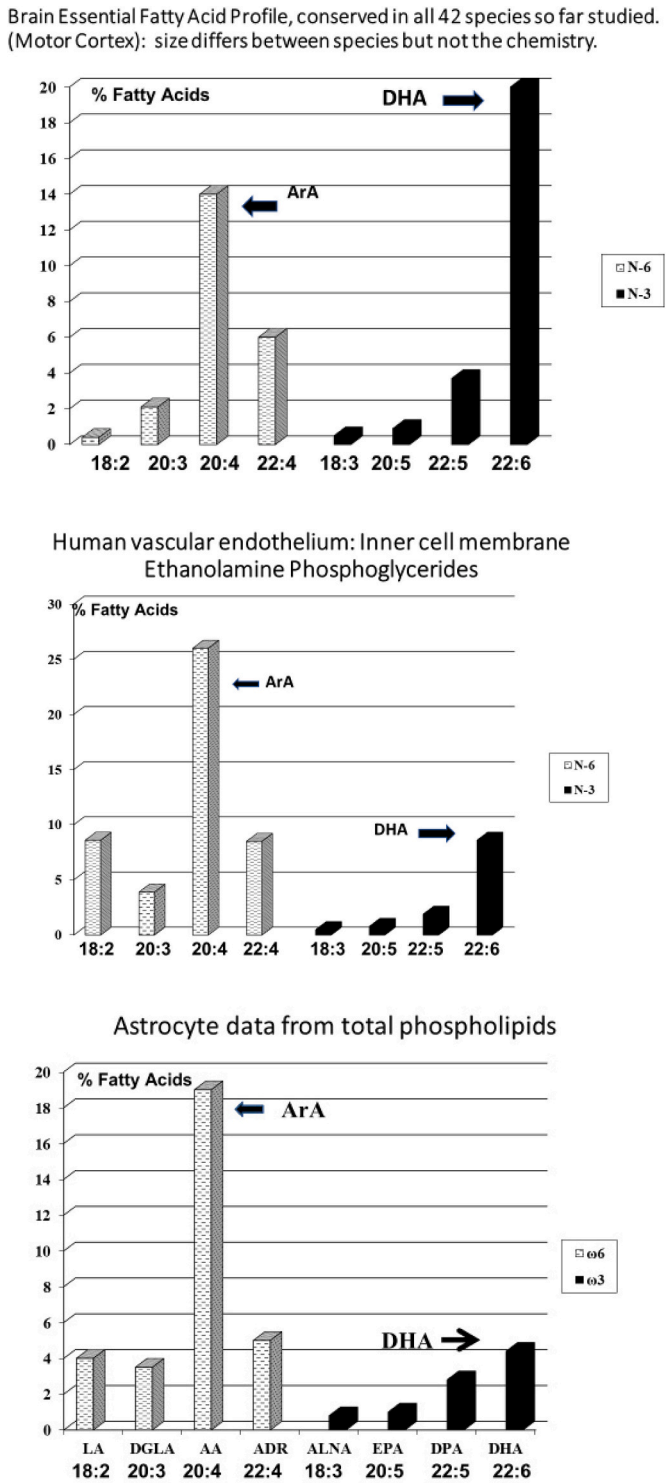

Fig. 4. ArA is the major acyl component of inner cell membrane lipids of the endothelium which lines the arteries. As the heart beats, the pressure waves cause the arteries to expand and contract. This action likely brings the inner membrane into intermittent contact with the lysosomes and contact with 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 DHA by contrast is concentrated in the signalling systems. ArA in the brain is more concentrated in the astrocytes [63] which are responsible for maintenance and myelination.

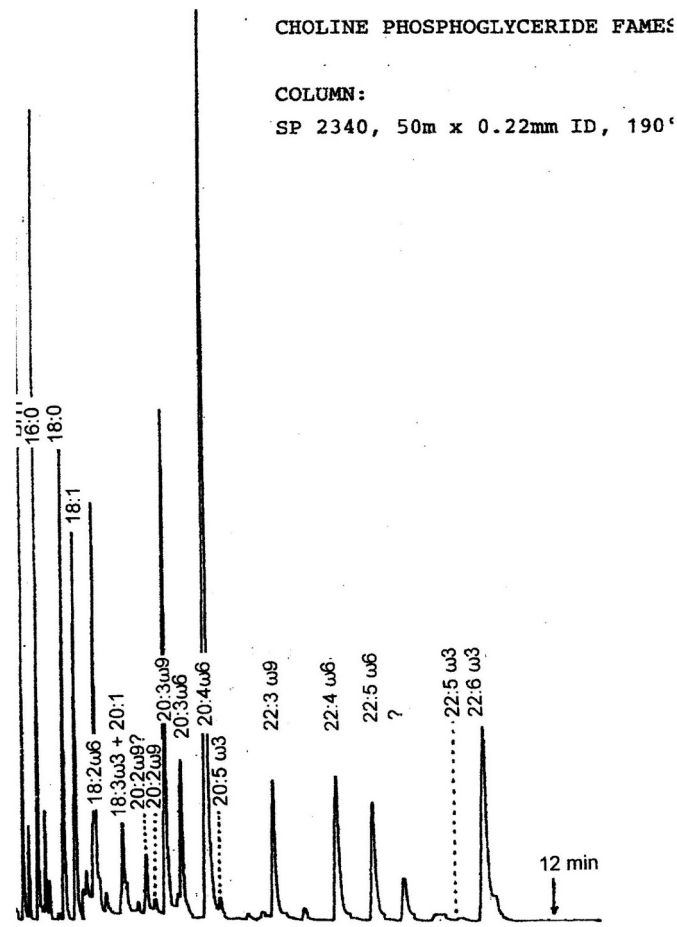

Fig. 5. The striking presence in the cord artery endothelium of the Mead acid (20:3ω9) and its 22-carbon elongation product is a classical sign of essential fatty acid deficiency. It is from a piece of foetal tissue and this demonstration really requires further investigation with regard to the vascular incited pathophysiologys 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 and that of DHA with the boys, indicates EFA and long chain PUFA needs to be investigated as a likely aetiological disorders of prematrity which can adversely affect child for life.

robustness of the data contrasting male and female prenatal biology consistent with the significantly greater prenatal sensitivity of the boys to EFA status [78].

2.1.19. *Fatty acid composition of human milk*

In Table 11 we present data from 9 previous studies on the composition of human milk. These include some 2000 samples in a study for WHO [79] comparing the effect of steroidal contraceptives on the composition of human milk carried out in Hungary and Thailand in which milk samples were collected over a period of 6 months [80]. We also include details of the Karen (Vietnamese) mothers' milk for comparison in Table 11b. In all cases, the sum of DGLA, ArA and adrenic acids were close to twice the proportion of EPA and DHA. The composition of human milk globally with the same results has recently been reviewed by Thomas Brenna [81]. He also commented on the misinformed recommendation by the EFSA to omit ArA from infant formula [82] a position also questioned by Koletzko and others [14].

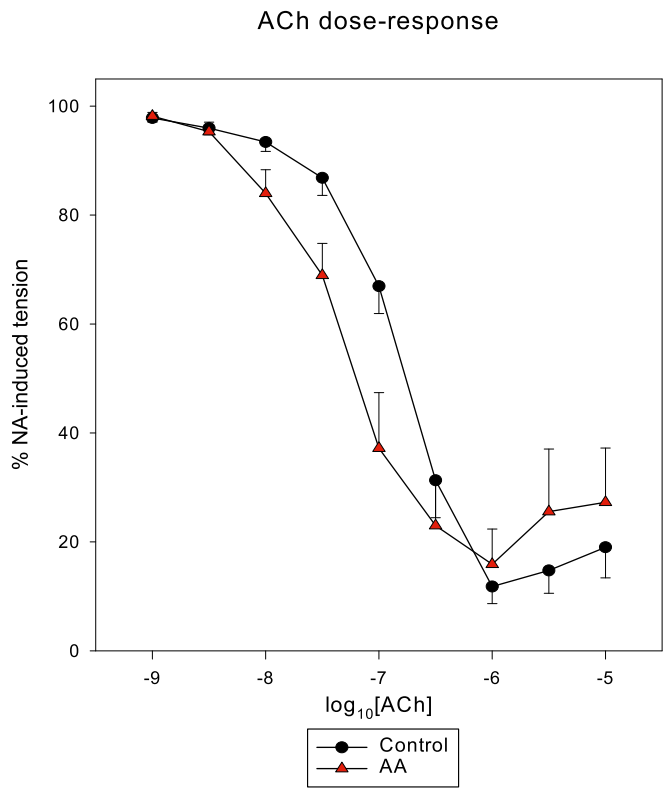

Fig. 6. This study of the relaxation of the mesenteric arteries by ARA is in stark contrast to the absence of effect of DHA (Fig. 7), implying this is a property of ArA in normal vascular function.

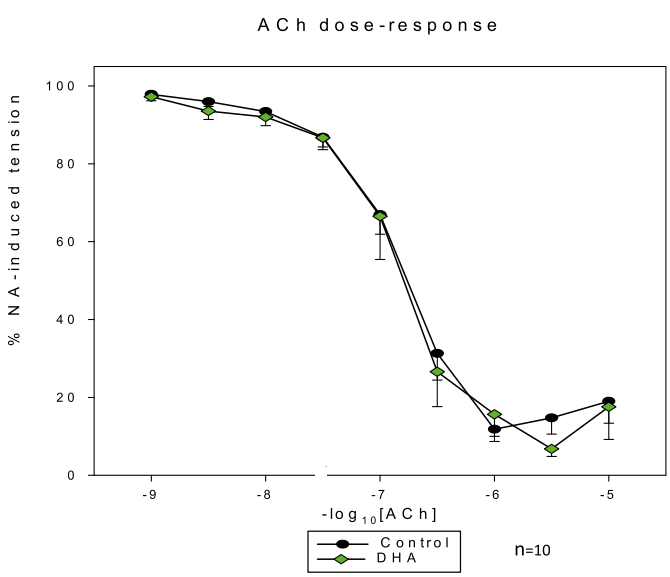

Fig. 7. DHA was tested in this study of the relaxation of the mesenteric arteries and is in stark contrast to ArA (Fig. 6) with and absence of effect.

3. Discussion

The data presented here have highlighted that ArA is the predominant PUFA in foetal red cells, prenatal mononuclear cells, placental membranes, astrocytes, and the umbilical cord endothelium, in contrast to significantly lower proportions of LA, DHA and EPA. Despite this, the current recommendations for maternal and infant nutrition are almost entirely focussed on DHA and EPA. Indeed, ArA is not even recommended to be included in infant formulas by EFSA despite its presence in human milk in all countries so far studied. This is despite the WHO and FAO view that human milk should be the gold standard.

Although the biomagnification for ArA was first reported in 1976, the biomagnification and bioreduction process of the SAFA, MUFA and the C20 and C22 carbon chain length allies of ArA and the ω3 family have all been overlooked. The fact that the placenta selectively transfers all biologically relevant fatty acids of even chain length from C16 upwards has not been commented on previously although supportive data have been published [83]. The interesting movement of RBC SAFA, except for myristic acid, raises a question about their nutritional role, especially for stearic acid. During foetal growth they will be needed for the sn-1 of the phosphoglycerides and as mentioned above, for myelin synthesis. The selective treatment of fatty acids by the placenta extends our understanding of foetal nourishment involved in cell membrane growth and integrity.

That the biomagnification process is through selection rather than via metabolic conversion processes is supported by the lack in the placenta of the desaturation and elongation enzymes involved compared to the liver [84]. Moreover, the double isotope studies shown in Fig. 1 illustrate the point that the conversion is restricted by the rate limitations, especially of the two desaturations steps but also of the elongation in the synthesis of ArA from LA. The data show that the efficiency of incorporation of preformed ArA into brain lipids is close to an order of magnitude greater than ArA synthesised from LA. The proof that ArA can be synthesised from LA was carried out by the late James Mead using isotope recovery methodology. And his paper shows clearly how little ArA is synthesised from the ingested isotopically labelled LA [85].

It is evident that ArA and its allies would have played significant roles in cardio and vascular development as a pre-requisite for organogenesis, placental growth and function, together with foetal cellular membrane growth, including the most membrane-rich organ, the brain. There again, they would need to be accompanied by SAFA, with stearic acid especially as an accompaniment to ArA and DHA in the phosphoglycerides, to satisfy the OPH.

The data derived from the RBC are from a plasma membrane which would be similar to the endothelium of the blood vessels [86]. To what extent the compositional changes seen in pregnancy (Table 1) also happen in other endothelial cells is a question which requires an answer. That the changes from recruitment to delivery are mirrored in other similar membrane types is supported by Fig. 5, which shows the extraordinary level of the 20:3ω9, Mead acid and its 22-carbon elongation product in the foetal cord endothelium of a very preterm male infant. Hornstra et al. [86] also reported Mead acid in umbilical cord arteries and veins from Dutch infants born at term. Mead acid is a classical indicator of EFA deficiency [65].

The data also raise the question: does this mean that present maternal dietary intake is physiologically insufficient and could 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 7). Is the depletion of these fatty acids involved in the reports of shrinking of the brain in pregnancy? [60,87].

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 [88]. The implication is that ArA and adrenic acids have a prominent role in early development which will include immune cell, astrocyte development and neural connectivity (Fig. 4).

During early development, differentiation and cell division requires the nourishment, including lipids, for building plasma membranes, mitochondria, nuclear envelopes, reticulo-endothelial systems, lysosomes, Golgi apparatus and other structural organisations of the cells.

Table 10
Correlation of fatty acids in maternal cord RBC and regional brain development (brain volumes based on MRI) in girls and boys from refs, 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/ArA[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 callosum	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	0.188	0.150	0.209
LA/ArA[b], $N = 19$	−0.145	−0.025	−0.0139	−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	0.39	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	−0.036	−0.173	0.209	−0.0374	−0.0293	−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

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. * <0.05 ** <0.01. *** <0.005.

Importantly they will be proceeding at a rapid pace from a zygote weighing less than a milligram, turning into a 3.5 Kg new-born in a matter of 9 months. That is a velocity of 4.7 Kg in 12 months.

In response to the rapid growth of the foetus, there will be a high demand for membrane liquidity and elasticity during cell division as shown elegantly by Atomic Force microscopy [89]. People often conclude that adding cis double bonds lowers melting point and liquidity. However, adding additional double bonds after the first two makes little difference to melting properties similar (melting point has usually been taken as an index of liquidity). Both ArA and DHA will contribute similarly to cell membrane elasticity. In fact, ArA has a lower melting point than DHA (PUBCHEM gives the melting point of ArA as −49.3 °C and -44 °C for DHA). Inadequate flexibility during the extremes of curvature involved in cell division would be expected to be a candidate for membrane rupture and a consequent response to injury. It is noteworthy that the complications of prematurity involve blood vessels as in intraventricular haemorrhage, periventricular leukomalacia, the leaking of fibrin in broncho-pulmonary dysplasia and the consequences of inflammation in necrotising enterocolitis and retinopathy of prematurity.

3.1. ArA and its metabolites in the brain

The effect of ω3 PUFA deficiency on neural function has been extensively characterised in rodent and primates [90]. 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 tests.

Table 11
Composition of human milk.

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 2000+ samples each. Data are means followed by standard error of the mean (SEM). LCPUFA = long-chain polyunsaturated fatty acids.

SATURATES			Ω 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			Ω 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 11b
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.

	Camp 1 $N = 36$	Camp 2 $N = 53$
Total lipids (g/100 mL)	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	1.29 ± 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

The best performing groups were those fed DHA alone and DHA plus ArA [91]. 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) [92] and short- and long-term-memory and spatial learning (in rats) [93,94]. 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 [95].

There has been a sustained interest in lipoxin modulation of the proinflammatory responses in neural tissue [96]. Additional and critical neural derivatives of ArA are the endocannabinoids (2-arachidonoyl-glycerol and anandamide), lipid messengers involved in fine tuning of synaptic function [97] and the release of important signal transduction molecules, arachidonoyl-diglycerides, from phosphoinositides [98].

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 [99], though there are indications of exaggerated metabolism of neural ArA by COX-2 to PGE2 in mood disorders [100–102] and in ω3 deficiency [103]. This, and much of the anti-inflammatory role of the long chain ω3 PUFA is different from the role of ArA in cell regulation via prostacyclin, lipoxins and other eicosanoids [104], foetal tissue assembly 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 [21] which would be relevant to foetal growth and likely to its resolvin activity [105]. ArA as an inflammatory agent is too simplistic a view.

3.2. 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 [8] 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 [106]. In the brain, adrenic acid proportions decline with age [107]. Retroconversion of adrenic acid to ArA has been shown in astrocytes and bovine aortic endothelial cells in vitro [108,109]. While adrenic acid is metabolised by cyclooxygenase, lipoxygenase and cytochrome P450's, little is known of the role of these metabolites in vivo [110].

3.3. Cretaceous–Paleogene (K–Pg) boundary: the evolution of mammals and ArA

The conservation of ArA and its allies in the brain and human milk from different cultures (Table 11), where the proportions of DHA are more variable, and the dependence of mammals on ω6 for reproduction [28], raises the question as to the evolutionary implications. Prior to mammals the primary food for the giant reptiles were the giant trees, ginkgoes, the ferns and similar. These reproduced by spreading spores and by their root systems. The vegetarian dinosaurs would eat leaves and bark and possibly dig for roots. 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 [111]. All studies thus far on the composition of the brain in different species demonstrate the highly conserved presence of ArA and adrenic acids in equal measure to DHA. Prior to the K-Pg boundary a brachiosaurus, weighing up to 80 metric tons had a tiny brain said to be not much bigger than a walnut. With the coming of the mammals, brain size increased dramatically.

So, what was the trigger? The mammals require ω6 for reproduction. Perhaps it was the new abundance of the flowering plants with their protected seeds containing oils rich in ω6 linoleic acid? Was this switch in food and anatomical physiology just a coincidence?

It is plausible that ArA's adhesive derivatives resulted in the egg sticking to the uterus instead of being excreted in a hard shell as dominated before the K-Pg extinction event. This would explain the

dependence of mammals on ω6. The emergence of an abundant source of linoleic as the precursor to ArA, together with the ArA-rich membranes encouraging endothelial/vascular proliferation, with the emergence of the placenta nourishing the product of conception would have filled the missing gap to result in a range of mammals with larger brains than ever seen before.

It is plausible that the egg laying system and paucity of ω 6 would have restricted brain growth, as evidently happened (eg brachiosaurus), whereas the abundance of ω 6 would have filled the missing gap to achieve the highly conserved balance between ArA and its allies to DHA (Fig. 4). That is a new abundance of ω 6 filled the gap for both mammalian reproduction and brain size .

The dependence of mammals on ω 6 might be questioned by the fact that about 10 million years after the extinction event and the appearance of an abundance of mammals, several species migrated to live in the sea. The marine habitat is considered to be ω 3 rich, not ω 6. Surprisingly, the muscle and liver levels of the Grey Whale (*Eschrichtius robustus*) [112] and Dolphin (*Tursiops truncates*) [113] are ArA-rich. The oil from the Grey 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, surplus storage site of excess lipid, and serves a purpose insulating against cold and providing buoyancy, energy and some fat-soluble nutrients.

The dependence on ω6 is the likely explanation for the visits of the Grey Whale to the warm waters of its Baja breeding lagoons. The food web in warm waters contains ArA and other ω6 fatty acids [114]. 50 million years of evolution in the ω3-rich sea do not seem to have changed the requirement for ArA in mammalian reproduction. Similar findings on ArA in vital organs of the Harbour Porpoise and Grey Seal have been reported [115] and are likely to be common amongst marine mammals.

3.4. Lipids and the OPH

Lipids are often considered just 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 [114,116].

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 (Fig. 6). Its functional requirement for the brain is incontrovertible. The message of the P-Kg boundary is that both DHA, ArA and its allies were required for the optimal chemistry of the brain and mammalian encephalisation.

Moreover, in considering the needs for reproduction, the health and nutrition of the mother prior and around the time of conception is important and is a principle known for some time [117,118]. The implantation of the zygote and its development, takes place 6 days after conception. When a woman reports for pregnancy care at 10 weeks post conception, the cells that will form the brain are already migrating to the correct site. The evidence here is that these early events are ArA dominated.

The oleic acid levels reflect the ArA and DHA status of the mother can predict prematurity [37] and is of potential clinical utility for preconception care and the prevention of low birthweight and prematurity [119,120]. Women with high RBC oleic acid levels could be recruited for targeted interventions to reduce their risk of prematurity.

In addition to the discussion on the LCPUFA, the message from reproductive biology also draws attention to the saturated fatty acids which are biomagnified alongside the LCPUFA, doubtless to fill the sn-1 positions to accompany the sn-2 LCPUFA. Radio-isotope studies showed that stearic acid was conserved whilst oleic, LA and ALA were largely oxidised to CO_2 in 24 h [121]. This coincided with stearate uptake by brain membrane lipids [122].

Our knowledge is scant as regards the specific lipid-protein interactions with the creation of optimal, local lipid domains for optimal membrane/protein function. 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 foetal growth restriction and mortality [123]. 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 and could be central to many of the neuro-developmental and health issues of concern today.

3.5. Conclusions and perspective

The topic of this review is the role of ArA in foetal growth and development. We have summarised the data identifying ArA and its allies (20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6) as major players in the growth of cell systems in the reproductive process in a set of pie charts (Fig. 3) comparing the biomagnification of ArA and allies with DHA, linoleic and stearic acids (Figs. 3 and 8). 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 being returned to the mother.

The pie chart of maternal CPG at delivery compared to cord plasma CPG is given independently as it is the contents of the plasma lipid which is most readily taken up by the foetus and shows the greatest biomagnifications and bioreductions. Fig. 8 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 [117,118]. With the oleic acid status inversely reflecting the ArA and DHA status of the mother and predicting preterm birth [37], 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. This optimal ratio has now been exceeded and reaches up to 20:1 in some cases. It is this lack of understanding of the difference between the risk posed by excessive dietary exposure (to ω6 fatty acids, particularly linoleic acid in this case), the balance between the different fatty acids and the exposure or profile best suited to the biological necessity for membrane synthesis or the natural physiological response of ArA to injury, for example, that has led to the "inflammatory" label attached to ArA. 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 demonstrate the selectivity and biomagnification of stearic acid which has received little attention. The dominant presence of ArA in the products of conception and the universal positive or negative selection by the placenta of all fatty acids measured, suggest it is time to reconsider its implications 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, 3, and 5.

The population for the FOSS study consisted of 300 women, recruited

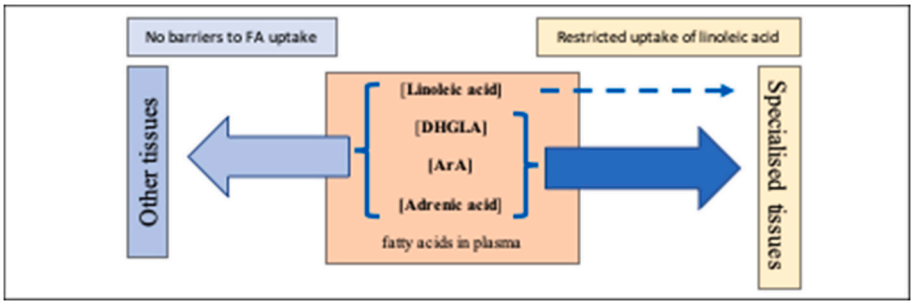

Fig. 8. Schematic showing the restricted uptake of linoleic acid compared with ArA and its allies in specialised tissues (foetus, placenta, vascular endothelium, brain) compared with ubiquitous presence of linoleic acid in most other tissues.

for a randomised trial of ω3 and 6 fatty acids, has been described previously. The study group was drawn from 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 are only from healthy controls with birthweights between 3200 and 4500 g.

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 [37].

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 (20 mL) was collected at cannulation by the obstetric anaesthetist and cord blood was collected within 10 min of delivery in a 10 mL 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 placentas

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. Placentas 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. Placentas 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 placentas by vacuum aspiration is a preferred process as it captures a substantial mass of (4-10 g) 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 [124].

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 and 40 years with a parity of 0–3. Prior to delivery women in preterm labour were treated with dexamethasone sodium phosphate (dexamethasone, 12 mg); a majority of women ($n = 14$) received a second dose approximately 12 h 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, infection acquired in utero, or inflammatory reactions. Further details of this study were published in 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, 8 mL, was obtained at delivery time and at 12 weeks post-delivery. Cord blood was also collected at birth from healthy term babies. Vietnam samples: 8 mL 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. Data are presented in 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 [79].

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 (Fig. 6).

Female Sprague-Dawley rats (200-250 g) 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 [125]. 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 were pre-incubated separately with 40 μM AA, EPA or DHA. They were then solubilized in dimethyl sulfoxide (DMSO) (with a final concentration of DMSO 0.4%) and 0.3% albumin in 10 mL 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 37 °C, 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–3 × 10^{-6} mol/L). Arteries pre-constricted with NA (5 × 10^{-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 [77].

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 [126,127].

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 [128] and after its closure transferred to the Homerton Hospital, Hackney in East London [129], 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 [130]. 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 [131]. The methodology for the magnetic imaging of the brain in relation to maternal lipid nutrition and sex differences were carried out by Professor David Edwards and Nora Tusor at Centre for the Developing Brain, King's College London, London SE1 7EH, UK with the study designed and executed at the Chelsea and Westminster Hospital Campus of Imperial College, London SW10 9NH. UK by Dr Enitan Ogundipe and Professor Mark Johnson, [132,133].

Ethical approval

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.

Author contrbution

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, acquisition of data, or analysis and interpretation of data, (2) drafting the article and all contributed to revising.

Michael A. Crawford. Writing original draft. Conceptualization, funding acquisition, design of the study.

Andrew J. Sinclair, Methodology, Vizualization, Writing - Review & Editing - Historical contribution back the 1970s, including double labelled studies for precursor and end product comparisons for linoleic vs arachidonic and linolenic compared to DHA preferential incorporation in the brain during growth. Figs. 1, 2, 3, 4 and 8.

Barbara Hall validation and Writing, Review & Editing. Historical contribution back the 1970s discussion on human milk and Table 11.

Enitan Ogundipe Data curation. Sex difference in neurodevelopment Table 10.

Yiqun Wang Formal analysis throughout Tables 1, 4, 11 and editing.

Dimitrios Bitsanis Formal analysis for placental data and discussion Tables 6 and 7, Writing - Review & Editing.

Ovrang.B. Djahanbakhch Project administration: obstetrician responsible for the overseeing the work in the East end Tables 3, 7, 8 and 9.

Laurence Harbige Resources, - Review & Editing.

Kebreab Ghebremeskel Investigation responsible for the biochemistry for the work in the East end data, Tables 3, 7, 8 and 9.

Ivan Golfetto Investigation - work in Thailand and Vietnam as well as the vascular response for arachidonic and docosahexaenoic acids not published other than in his thesis. Tables 2 and 3 and Figs. 6 and 7.

Therishnee Moodley Investigation - specifically on foetal mononuclear cells Tables 8 and 9.

Ahmed Hassam Investigation - data identification of the rate limitation of the first desaturation (FADs 2) with double labelled studies comparing linoleic and gamma-linolenic acids f or synthesis of brain arachidonic acid. Figs. 1 and 2.

AnnieBelle Sassine Investigation. OPH at work in plasma and RBC comparison, Aberdeen centiles/birthweights Tables 4 and 5.

Mark R Johnson design of the study. Project administration obstetrician responsible for the work at Imperial College and MRI for neurodevelopment Tables 1, 4, 5 and 10. Writing - Review & Editing..

Declaration of Competing Interest

The authors have no conflicts of interest.

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