In pregnancy, maternal HDL is specifically enriched in, and carries the highest proportion of, DHA in plasma

Nicola Zamai, Colin H. Cortie, Eleanor M. Jarvie, Christopher C. Onyiaodike, Amaal Alrehaili, Monique Francois, Dilys J. Freeman and Barbara J. Meyer

School of Medicine (N.Z., C.C., M.F., B.J.M.), Lipid Research Centre (N.Z., C.C., B.J.M.), Molecular Horizons (B.J.M.), University of Wollongong, Illawarra Health & Medical Research Institute (B.J.M. C.C.), Wollongong, New South Wales 2522, Australia; and School of Medicine (E.M.J., C.C.O.), University of Glasgow, Institute of Cardiovascular and Medical Sciences (A.A., D.J.F.), University of Glasgow, Glasgow G12 8QQ, United Kingdom.

Corresponding Author:

Prof Barbara J. Meyer

School of Medicine,

University of Wollongong,

Northfields Ave,

Wollongong, NSW 2522

Australia

Tel: +61 2 4221 3459

Email: bmeyer@uow.edu.au

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Abbreviations: alpha-linolenic acid (ALA, 18:3n-3), cholesteryl ester transfer protein (CETP), early pregnancy study (EPS), fms-like tyrosine kinase 1 (sFlt1), frozen embryo transfer (FET), In Vitro Fertilisation (IVF), linoleic acid (LA, 18:2n-6), lipoprotein depleted plasma (LPDP), Lipotoxicity in Pregnancy Study (LIPS), long chain polyunsaturated fatty acids (LCPUFA), luteinising hormone (LH), placental growth factor (PlGF), Scottish Index of Multiple Deprivation (SIMD), vascular endothelial growth factor (VEGF-A).

Abstract

Arachidonic acid (AA) and docosahexaenoic acid (DHA) are important for neurological development. The aim was to determine the distribution and relative enrichment of AA and DHA among lipoprotein fractions prior to pregnancy, throughout gestation and in the post-partum period. Our hypothesis was that in pregnancy, in contrast to the non-pregnant state, AA and DHA are carried in highest concentration in the very low density lipoprotein (VLDL) fraction secondary to increased gestational liver triglyceride secretion. Two independent prospective, observational cohort studies carried out in Glasgow were combined; one early in pregnancy and one later in pregnancy with post-partum follow up. Across the pregnancy timeline plasma lipoproteins were isolated using sequential ultracentrifugation and lipoprotein fatty acids were extracted and analysed by gas chromatography. High density lipoprotein (HDL) had the highest concentration of AA and DHA compared to other lipoproteins. HDL became progressively enriched in the proportion of triglycerides at 16 weeks of gestation, which peaked at 35 weeks and returned to baseline at 13 weeks postpartum. HDL DHA per HDL-cholesterol and HDL DHA per apoA-I became progressively enriched at 16 weeks of gestation, peaked at 25 weeks and returned to baseline at 13 weeks postpartum, whereas HDL AA (per HDL-C or HDL-apoA-I) did not differ. DHA is carried primarily in HDL rather than VLDL. HDL has anti-oxidant properties that might afford DHA protection against oxidation.

Keywords: Fatty acid transport, lipoproteins, VLDL, omega-3 fatty acids, arachidonic acid

1. Introduction

The long chain polyunsaturated fatty acids (LCPUFA), particularly docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), have been shown in population and intervention studies to be extremely important for neurodevelopment, learning and cognitive function. DHA constitutes a high proportion of brain fatty acids, mainly found incorporated in membranes, and has particular structural properties that enhance membrane fluidity [1]. DHA promotes neurone survival and is required for neurone connectivity [1]. During the latter stages of pregnancy, DHA is required when the brain accrues its tissue mass [2] and infant premature birth has been linked to neurodevelopmental disorders [3, 4]. AA is also an important structural lipid in the brain that has additional key roles in pregnancy as a source of thromboxane A2, which increases coagulation, and of prostaglandins which are involved in the initiation of labour [5].

In pregnancy, the fetus is dependent on its mother for its supply of DHA and AA via placental transport. Early availability of DHA to the embryo appears to be important at the time of neural tube closure (28 days of gestation) [6]. The fetal-placental circulation is established later at around 9-13 weeks of gestation and allows mass transfer of nutrients from mother to fetus to support growth. The growing fetus preferentially acquires DHA and AA from maternal plasma via placental transport [7-9]. Tracer experiments show that placental transfer of DHA results in fetal plasma enriched in DHA relative to the mother [10]. Maternal plasma and erythrocyte (an indicator of the previous 3 months' whole body DHA status) DHA concentrations increased by the end of the first trimester and continued to significantly increase throughout pregnancy above non-pregnant concentrations by the third trimester [6, 11]. Thus, maternal erythrocyte and plasma concentrations of fatty acids essential for brain development are increased by the end of the first trimester and are available for placental transport to the fetus. In contrast, the essential dietary fatty acids linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), precursors for AA and DHA synthesis respectively, are at best minimally transferred to the fetus [7, 12].

The critical requirement of DHA for fetal brain development, the essential dietary source of its precursor and the poor efficiency of its synthesis in humans [13, 14], means that efficient placental DHA transfer is paramount for optimal fetal nutrition and long-term neurodevelopment of the child. The vehicle that delivers LCPUFA to the placenta has not been specifically determined. In the non-pregnant state, LCPUFA, particularly DHA and AA are primarily transported on high density lipoprotein (HDL) particles in the circulation [15]. However, the three-fold increase in VLDL synthesis by the liver in late pregnancy [16] and the predominance of the liver as a site of LCPUFA synthesis suggests that VLDL may be an important transporter of plasma LCPUFA in pregnancy. Fatty acids such as DHA are mobilised from maternal stores during pregnancy and are potentially directed to the liver for incorporation into TG-rich VLDL-1 [17]. VLDL released into the maternal circulation is thought to be acted upon by placental lipoprotein lipase for direct delivery of fatty acids to the fetus via the placenta. We hypothesised that in pregnancy as compared to the non-pregnant state, DHA and AA are carried in higher concentration in the VLDL lipoprotein fraction than in intermediate density lipoprotein (IDL), low density lipoprotein (LDL), HDL and/or lipoprotein depleted plasma (LPDP) fractions.

The aim of this study was to determine the distribution and relative enrichment of fatty acids, particularly DHA and AA, among five plasma fractions: VLDL, IDL, LDL, HDL and LPDP across the full pregnancy timeline from pre-pregnancy through 4.6, 6.1, 8.4, 16, 25, 35 weeks of gestation and 13 weeks post-partum.

2. Materials and Methods

2.1 Study design, setting and participants

In order to study across the whole timeline of pregnancy, from pre-pregnancy throughout early pregnancy, through later gestation to 13 weeks post-partum, we used a study design where two BMI-matched cohorts of women from Glasgow were followed either from pre-pregnancy to 8.4 weeks of gestation [6] or from 16 weeks of gestation to 13 weeks post-partum. Although these two independent, prospective, observational cohort studies with repeated sampling were individual longitudinal studies we opted to analyse the whole cohort in a cross sectional manner using one way ANOVA rather than repeated measures ANOVA in order not to over-interpret the data.

The early pregnancy study (EPS) was conducted at the Assisted Conception Unit, Glasgow Royal Infirmary between October 2007 and April 2010 [6]. The study was approved by the Glasgow Royal Infirmary Research Ethics Committee (reference 07/S0704/49) and participants provided informed consent. Women were eligible for the study if they were undergoing frozen embryo transfer (FET) and had a regular menstrual cycle not requiring use of exogenous hormones. Participants attended the Assisted Conception Unit to commence daily blood sampling in order to detect the luteinising hormone (LH) surge. At this stage a baseline non-pregnant blood sample was taken (mean 3 days prior to LH surge). FET was performed on day 3 post-LH surge. Patient demographics and fertility history was recorded. Scottish Index of Multiple Deprivation (SIMD) quintile was derived from patient postcode [18]. Patient height and weight data were collected at the initial pre-LH surge visit. Maternal blood samples, after an overnight fast (>10 hours), from 24 women were used from this study (Supplemental Figure 1), each with four time points: pre-pregnant (Pre-P), day 18 post-LH surge, day 29 post-LH surge and day 45 post-LH surge, equating to a total of 93 blood samples (3 women were missing 1 time point each) originating from the EPS. In order to equate gestational weeks between the two independent studies, the time of LH surge was considered to be equivalent to day 14 from last menstrual period in a naturally occurring pregnancy (i.e. 2 weeks of gestation assuming a 28 day menstrual cycle). Pre-P samples were therefore taken 3 days before the LH surge

(i.e. 6 days before FET). The three remaining samples were collected at the equivalence of 4.6, 6.1 and 8.4 weeks of gestation [19].

The Lipotoxicity in Pregnancy Study (LIPS) was conducted in National Health Service Greater Glasgow and Clyde maternity units between March 2010 and November 2011. The study was approved by the West of Scotland Research Ethics Committee (reference 09/S0701/105) and participants provided informed consent. Women were recruited at their first antenatal appointments and represented healthy Caucasian women between the ages of 16-40 years with no significant past medical history. Women were excluded from participating in the study if they had known metabolic disease such as diabetes mellitus, thyroid disease, polycystic ovarian syndrome or cardiovascular disease, had multiple pregnancies, had gestational diabetes, or if they had previous pregnancies through assisted conception. Furthermore, women who developed obstetric antenatal complications throughout the study were retrospectively excluded. Of the 43 women who completed the original study n=20 had sufficient plasma available from each time point for analysis in the current study. The women (n=20) attended after an overnight fast (>10 hours). Blood samples were collected at mean (range) weeks of gestation as follows: 16 (range 14-19); 25 (range 23-28); 35 (range 34-38) weeks of gestation and 13 weeks (range 10-14 weeks) postnatal.

2.2 Blood sampling, plasma analysis and lipoprotein separations

Plasma was collected by low-speed centrifugation and frozen at -80°C within 2 hours of collection. Estradiol and progesterone levels were determined with the commercially available chemiluminescent microparticle immunoassay kits; Architect Estradiol 7K72 and Architect Progesterone 7K77, using the Architect iSR 2000 machine (Abbott Diagnostics). Total cholesterol, triglyceride and HDL cholesterol assays [20] were performed by Vascular Biochemistry, University of Glasgow. Plasma non-esterified fatty acids (NEFA) were quantitated by summing the fatty acids determined by gas chromatography in the lipoprotein depleted plasma fraction as outlined below. Lipoprotein fractions were isolated from 250uL of plasma via sequential ultracentrifugation [21] at the following densities: VLDL <1.006g/mL, IDL 1.006-1.019g/mL, LDL 1.019-1.063g/mL and HDL 1.063-1.21g/mL [22].

The final lipoprotein-depleted plasma (LPDP) fraction was obtained as the HDL infranatant. All volumes were recorded after each separation. Each fraction once recovered was stored at -4ºC and extracted for fatty acid analysis the following day. HDL composition was measured using an autoanalyser (Konelab 20XT) and commercially available kits, reagents and standards from Thermo Electron, USA (total cholesterol, triglyceride, apoA-I); and Wako Pure Chemical Industries, Japan (phospholipids).

2.3 Lipoprotein fatty acid analysis

Recovered lipoprotein fractions underwent direct transesterification [23] after addition of 40µg heneicosanoic acid (21:0) as an internal standard. Fatty acid methyl esters were analysed by flame ionisation gas chromatography (model AOC-20i, Shimadzu) using a 50m x 0.25mm internal diameter capillary column as described previously [11]. The lipoprotein fatty acids esters were compared to the relative retention time of known authentic standards (SIGMA) and quantified by comparison with the internal standard. A total median (interquartile range) recovery of plasma fatty acids was 66 (48, 105) % recovery.

2.4 Statistical Analysis

Fatty acid concentrations in VLDL, IDL, LDL, HDL and LPDP lipoprotein fractions were expressed as median and interquartile range. The two studies were analysed together in an unpaired analysis to give an indication of the changes in fatty acids within each lipoprotein over the whole pregnancy timeline. Proportion of fatty acid within each lipoprotein fraction at each time point was calculated by expressing the concentration in each fraction as a percentage of the total fatty acids in each fraction. HDL fatty acid enrichment was calculated as concentration of fatty acid in HDL divided by HDL-cholesterol or HDL-apoA-I concentration. HDL-total cholesterol measured in mmol/L was converted g/L by multiplying by 0.3867. HDL-triglyceride measured in mmol/L was converted g/L by multiplying by 0.8857. Total HDL composition (g/L) was calculated as the sum of HDL-total cholesterol (g/L) + HDL-triglyceride (g/L) + HDL-phosholipid (g/L) + HDL-apoA-I (g/L). The proportions of total cholesterol, triglyceride, phospholipid and apoA-I in HDL were calculated as

percentages. Data were checked for normal distribution using the Shapiro-Wilk goodness of fit test. Data that were not normally distributed were transformed by log10 or square root. An ANOVA was used to determine if there were any significant differences across the whole pregnancy timeline i.e. pre-pregnant (Pre-P), week 4.6, 6.1, 8.4, 16, 25 and 35 weeks of gestation and 13 weeks postpartum. *Post hoc* analysis used a Tukeys-Kramer test. All analyses were carried out in JMP (Vs11.0.0 2013) and significant significance was set at $P<0.05$.

3. Results

3.1 Demographics and comparability of the two study populations

Table 1 shows the demographic characteristics and plasma lipids of the 24 women from the EPS and the 20 women from the LIPS studies. The EPS women were on average 4 years older than the LIPS women (P=0.0027) but there were no differences in any other characteristics or plasma lipids levels.

Table 2 shows the estradiol, progesterone, plasma lipid and glucose concentrations in the EPS and the LIPS studies. As expected plasma estradiol continually increased during pregnancy and returned back to Pre-P levels in the post-partum period. Plasma progesterone initially increased from Pre-P to 4.6 weeks of gestation, where the concentration remained the same until 8.4 weeks of gestation, after which the concentrations continued to increase to 35 weeks of gestation prior to returning back to Pre-P levels 13 weeks post-partum. As expected plasma triglycerides, total and HDL-cholesterol significantly increased from 16 to 35 weeks of gestation and decreased post-partum. The non-pregnant and post-partum plasma lipids did not differ, suggesting that these two cohorts of women are comparable. NEFA also increased significantly from 16-35 weeks of gestation but remained significantly higher at 13 weeks post-partum compared to the non-pregnant women. There were small changes in plasma glucose across the pregnancy timeline. As expected plasma insulin and HOMA increased from 16 to 35 weeks of gestation and returned back to baseline levels 13 weeks post-partum.

3.2 Plasma AA and DHA concentrations in all plasma fractions across the pregnancy timeline

The median AA and DHA concentration (nmol/mL plasma) across the whole pregnancy timeline indicated that HDL has the highest concentration of both AA and DHA compared to LDL and VLDL (**Figure 1**) and IDL and LPDP (**Supplemental Figure 2**), apart from DHA in HDL at 35 weeks which is not different from LDL. HDL AA does not increase (P=0.27) across the pregnancy timeline

whereas HDL DHA concentration significantly increases across the pregnancy timeline peaking at 25 weeks of gestation ($P<0.0001$).

3.3 Distribution of fatty acids among lipoproteins and LPDP

Figure 2 shows the proportion of AA (Figure 2A), LA (Figure 2B), DHA (Figure 2C) and ALA (Figure 2D) in each of the HDL, LDL and VLDL fractions (with IDL and LPDP fractions shown in **Supplemental Figure 3**). AA is predominant in HDL (between 35-55%) and LDL (22-32%) (Fig 2A), and its precursor LA (Figure 2B) is predominantly in LDL (30-40%) and HDL (28-42%). DHA (Figure 2C) is also predominantly carried in HDL (30-42%) but its precursor (ALA) is evenly distributed among the lipoproteins (Figure 2D and Supplemental Figure 3D). This clearly demonstrates that AA and DHA but not the precursors LA and ALA are preferentially carried in HDL.

3.4 Enrichment of HDL with TG, AA and DHA

The proportion of TG in HDL nearly doubled by 35 weeks gestation compared to pre-pregnancy levels and returned to pre-pregnancy levels at 13 weeks post-partum (Table 3).

In order to assess enrichment of plasma HDL with AA and DHA, the ratio of HDL fatty acid concentration (AA and DHA) to HDL-cholesterol concentration and HDL-apoA-I were calculated. There was more AA in HDL compared to DHA, but HDL AA per HDL-cholesterol (R^2 Adjusted = 1%, $P=0.29$) and HDL AA per HDL-apoA-I (R^2 Adjusted = 4%, $P=0.08$) were not significantly different across the pregnancy timeline. In contrast, HDL DHA per HDL-cholesterol (R^2 Adjusted = 17%, $P<0.0001$) and HDL DHA per HDL-apoA-I (R^2 Adjusted = 16%, $P<0.0001$) became progressively enriched in HDL at 16 weeks of gestation (compared to Pre-P) and peaked at 25 weeks of gestation (triple the Pre-P concentration) before returning to baseline levels at 13 weeks post-partum (**Figure 3 and Table 3**). HDL DHA per HDL-TG did not differ across the pregnancy timeline (Table 3).

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234 *3.5 Relationships between estradiol and progesterone and plasma lipids*

235 The relationships between estradiol and progesterone and plasma lipids is shown in Table 4. As
236 expected both hormones positively correlated with plasma triglycerides, total cholesterol, HDL-C and
237 plasma NEFA. Both hormones positively correlated with HDL AA and HDL DHA, with the
238 correlations being stronger with HDL DHA. However, both hormones only positively correlated with
239 HDL enriched with DHA and not with HDL enriched with AA (**Table 4**).

240

4. Discussion

Contrary to our hypothesis, DHA and AA were primarily carried on maternal HDL rather than VLDL during early and late pregnancy. This study has shown that HDL, and not VLDL, is the preferred lipoprotein in terms of DHA transport across the pregnancy timeline. This is observed as a consistently increasing concentration of DHA carried by HDL peaking at 25 weeks of gestation, a higher proportion of DHA carried in the HDL fraction than any other lipoprotein fraction and the progressive enrichment of HDL in DHA peaking at 25 weeks of gestation. In contrast to DHA, AA concentrations in the lipoprotein fractions do not change throughout pregnancy, a high proportion of AA is carried by both LDL and HDL and there is no enrichment of AA in HDL.

Humans are able to synthesis DHA from its precursor, the essential fatty acid ALA [24], although the fractional conversion rate of ALA to DHA is only 0.04% in men [14] with women being higher at 9% [13]. The ability of women to convert more ALA to DHA has also been attributed to the presence of estradiol [13] with post-menopausal women receiving estradiol therapy increasing the conversion [25]. Conversely, humans have no problem synthesising AA from its precursor, the essential fatty acid LA. The parallel synthetic pathways for AA and DHA share common enzymes and delta-6 desaturase is the enzyme responsible for the initial desaturation of both LA and ALA. Given that human dietary intake of LA is at least 8 times greater than ALA [26], the synthesis of AA predominates. In our study, the AA concentration in HDL remains unchanged across the pregnancy timeline and the proportion is increased in LDL from 16 weeks of gestation to 13 weeks post-partum. Given that AA is carried in both LDL and HDL and there is no AA enrichment in HDL suggests that AA transport in lipoproteins is not rate-limited during pregnancy and lactation.

In contrast DHA is carried primarily in HDL throughout gestation and the HDL particles are enriched in DHA. DHA is also primarily carried in HDL in non-pregnant women. HDL has important anti-oxidant, anti-inflammatory and vasodilatory properties that are potentially accentuated in pregnancy

[27]. Therefore, in pregnancy which is associated with a large degree of oxidative stress [28], HDL is an ideal vehicle in which to carry the additional DHA required for transfer to the fetus [6, 29]. Estradiol and progesterone concentrations were positively correlated with DHA-enriched HDL but not AA-enriched HDL suggesting that HDL enrichment with DHA may be partially controlled by these hormones.

Estradiol increases dramatically during pregnancy and is known to stimulate VLDL and HDL production [16]. In the current study, there was a significant increase in VLDL DHA concentration from the early weeks of gestation to 16 weeks of gestation, coinciding with a significant increase in plasma triglyceride concentrations. Plasma HDL-C increased by 16 weeks of gestation and was significantly higher in later pregnancy (16-35 weeks of gestation) compared to early pregnancy, consistent with previous findings [22, 30]. The triglyceride to cholesterol ratio within HDL continually increased during gestation peaking at trimester 3 before returning to baseline levels post-partum [30]. Therefore, it is possible that newly synthesised maternal DHA packaged into VLDL (as well as DHA released from maternal stores) is incorporated into HDL by transfer from other lipoproteins via cholesteryl ester transfer protein (CETP). CETP activity is highest in the second trimester and then declines in third trimester [31] which correlates with the peak in HDL concentration at 25 weeks of gestation. The increased CETP activity [31] taken together with reduced hepatic lipase activity [30], results in triglyceride enrichment in HDL as seen in this study and others [30]. This triglyceride-rich HDL could be responsible for the transport of DHA to the placenta for the growing fetus. Placenta has both lipoprotein lipase and endothelial lipase activity [9] which may release fatty acids, including DHA from HDL for placental uptake and transfer.

The placenta also has HDL receptors which are regulated by vascular endothelial growth factor (VEGF-A) [32]. VEGF-A is required for the translocation of SR-B1 from the intracellular compartments to the cell surface, which in turn facilitates the binding, uptake and transport of HDL [32] including in placenta [33]. In preeclampsia, placental soluble fms-like tyrosine kinase 1 (sFlt1),

an antagonist of VEGF and placental growth factor (PlGF), is upregulated [34] and binds to plasma VEGF-A. This results in reduced VEGF-A in preeclampsia [34]. Given that VEGF-A is a key regulator in the transendothelial transport of HDL [32] we therefore hypothesise that the decreased fetal DHA in seen preeclampsia [29] could, at least in part, be due to this reduced translocation of HDL. Direct evidence is required to establish this potential pathway.

Fetal growth velocity during pregnancy has been assessed by Grantz et al 2018 [35] and the greatest fetal weight velocity peaked at 35 weeks of gestation (third trimester). The head circumference growth velocity peaked twice during gestation, namely 14 weeks (first trimester) and 19-21 weeks (second trimester). Unfortunately we do not have data at 14 weeks gestation, but our data is consistent with the head circumference growth in the second trimester, as DHA enriched in HDL peaked at 25 weeks of gestation. This supports the importance of HDL delivering the DHA required for fetal neurological development.

4.1 Strengths and limitations

Strengths of this study include the longitudinal study design covering the whole pregnancy timeline and the analysis of all lipoproteins fractions and concurrent hormone measurements. Women participating in the EPS were not given exogenous hormones and their own eggs were harvested, externally fertilised, frozen and in a subsequent cycle were re-implanted in order to mimic natural pregnancy as much as possible [6].

A major limitation is that we did not have one cohort of women throughout the pregnancy timeline. It is extremely difficult in practice to follow free-living women from Pre-P to post-partum. In this study the availability of Pre-P and early pregnancy samples was only possible by the utilisation of an assisted conception cohort. One must be careful when utilising two cohorts not to directly link the timelines and we have indicated this by a broken time axis and we have primarily used unpaired

analysis throughout, which limits our ability to find differences in each subcohort. Any interpretation of data which bridges 8 to 16 weeks of gestation must therefore be viewed with caution. However, both cohorts were recruited from Glasgow city hospitals, and had similar social status, BMI, waist circumference, blood pressures and plasma lipids. The 4 year higher age in the assisted conception population would be expected and is unlikely to have any impact on the metabolic adaptation to pregnancy. In comparison to the average age of 35.5 years (www.hfea.gov.uk May 2019) of women undergoing *in vitro* fertilisation in the UK, the EPS women are of similar age. The LIPS participants were also similar in age to that of the average UK population having their first child, which was on average 30 years of age.

Another limitation is that HDL-C and HDL-apoA-I concentrations are not a reliable measure of HDL particle number. While the concentration of $apoB_{100}$ can be used to accurately determine particle number for VLDL, IDL and LDL, there is no single apolipoprotein suitable to determine HDL particle number. To address this, we used both HDL-C and HDL-apoA-I as surrogates for HDL particle number and the results were similar when expressed as per HDL-C or HDL-apoA-I. We also did not correct for the increase in plasma volume, however, our changes seen in HDL DHA is likely to be an underestimate due to the increase in plasma volume seen during pregnancy.

We did not collect dietary data in the EPS and therefore cannot compare the diets between the two cohorts of women. However, the length of time in the EPS was only 8 weeks whilst the LIPS study was 37 weeks duration and women in the LIPS study did not change their diet throughout pregnancy (data not shown). Furthermore, confirmation of pregnancy outcome for women in the EPS was the detection of a positive fetal heartbeat at 8 weeks of gestation. Prior to this time the women did not know whether or not they were pregnant, let alone whether or not they were carrying twins, yet metabolic responses occurred including a doubling in the mobilisation of DHA concentration in those women with twin pregnancies compared to singleton pregnancies [6] and therefore differences in

346 dietary intake cannot explain the metabolic responses. Moreover, women can experience morning
347 sickness especially in the first trimester of pregnancy, so dietary intake data are not reliable.

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349 Our results have clear implications for the potential benefits of DHA supplementation in DHA
350 deficient mothers. Poor diet in most developed countries, specifically low in oily fish intakes and
351 hence low DHA intake [26, 36] means that many women enter pregnancy with low levels of this
352 essential fatty acid. The fetus can only acquire this important building block for the synthesis and
353 development of neural and brain tissue from the mother and our data demonstrate that the mother
354 mobilises DHA and enriches HDL with DHA at the critical times of neurological development. This
355 ability of the mother to enrich HDL with DHA has increased our understanding of DHA transport
356 during healthy pregnancy. However, women with preeclampsia do not increase their HDL during
357 pregnancy [37] and this, in combination with a potential for reduced transendothelial transport
358 resulting from high sFlt-1 levels, may partially explain why there is less maternal and fetal DHA in
359 women with preeclampsia.

360

361 In conclusion, HDL is the primary transport vehicle of DHA during pregnancy as evidenced by HDL
362 DHA concentration being the highest compared to other lipoproteins; DHA being the highest
363 proportion of all fatty acids in HDL compared to other lipoproteins; and DHA being selectively
364 enriched in HDL peaking at 25 weeks of gestation. DHA is carried primarily in HDL possibly because
365 HDL has important anti-oxidant properties that may protect DHA from oxidation in transit.

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Authors contributions: B.J.M. and D.J.F. conceived and designed the study; E.J. and C.C.O recruited subjects and collected patient data and samples; N.Z. carried out lipoprotein isolations and fatty acid analyses; Statistical analyses were carried out by N.Z., B.J.M., and D.J.F.; manuscript was written by N.Z., B.J.M and D.J.F. with intellectual input and editing from E.J., C.C.O, C.C., M.F, A.A.; B.J.M and D.J.F had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

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Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Appendix A. Supporting information

References

[1] N. Parletta, D. Zarnowiecki, J. Cho, A. Wilson, S. Bogomolova, A. Villani, C. Itsiopoulos, T. Niyonsenga, S. Blunden, B. Meyer, L. Segal, B.T. Baune, K. O'Dea, A Mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: A randomized controlled trial (HELFIMED), Nutr Neurosci, (2017) 1-14.

[2] S.M. Innis, Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids, The Journal of pediatrics, 143 (2003) S1-8.

[3] Johnson S, Evans TA, Draper ES, Field DJ, Manktelow BN, Marlow R, Petrou S, Seaton SE, Smith LK, Boyle EM, Neurodevelopmental outcomes following late and moderate prematurity: a population-based cohort study, Archives of disease in childhood Fetal and neonatal edition, 100 (2015) F301-F308.

[4] Sucksdorff M, Lehtonen L, Chudal R, Suominen A, Joelsson P, Gissler M, Sourander A, Preterm Birth and Poor Fetal Growth as Risk Factors of Attention-Deficit/ Hyperactivity Disorder, Pediatrics, 136 (2015) e599-e608.

[5] C. Besenboeck, S. Cvitic, U. Lang, G. Desoye, C. Wadsack, Going into labor and beyond: phospholipase A2 in pregnancy, Reproduction, 151 (2016) R91-R102.

[6] B.J. Meyer, C.C. Onyiaodike, E.A. Brown, F. Jordan, H. Murray, R.J. Nibbs, N. Sattar, H. Lyall, S.M. Nelson, D.J. Freeman, Maternal Plasma DHA Levels Increase Prior to 29 Days Post-LH Surge in Women Undergoing Frozen Embryo Transfer: A Prospective, Observational Study of Human Pregnancy, The Journal of clinical endocrinology and metabolism, 101 (2016) 1745-1753.

[7] M.D. Al, A.C. van Houwelingen, A.D. Kester, T.H. Hasaart, A.E. de Jong, G. Hornstra, Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status, The British journal of nutrition, 74 (1995) 55-68.

[8] C. Montgomery, B.K. Speake, A. Cameron, N. Sattar, L.T. Weaver, Maternal docosahexaenoic acid supplementation and fetal accretion, The British journal of nutrition, 90 (2003) 135-145.

[9] P. Haggarty, Placental regulation of fatty acid delivery and its effect on fetal growth--a review, Placenta, 23 Suppl A (2002) S28-38.

[10] Gil-Sanchez A, Larque E, Demmelmair H, Acien MI, Faber FL, Parrilla JJ, Koletzko B, Maternal-fetal in vivo transfer of [13C]docosahexaenoic and other fatty acids across the human placenta 12 h after maternal oral intake, American Journal of Clinical Nutrition, 92 (2010) 115-122.

[11] F. Stewart, V.A. Rodie, J.E. Ramsay, I.A. Greer, D.J. Freeman, B.J. Meyer, Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum, Lipids, 42 (2007) 335-344.

[12] R.H. de Groot, G. Hornstra, A.C. van Houwelingen, F. Roumen, Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome, The American journal of clinical nutrition, 79 (2004) 251-260.

[13] G.C. Burdge, S.A. Wootton, Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women, The British journal of nutrition, 88 (2002) 411-420.

[14] G.C. Burdge, A.E. Jones, S.A. Wootton, Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men*, The British journal of nutrition, 88 (2002) 355-363.

[15] A.H. Augustine, L.M. Lowenstein, W.S. Harris, G.C. Shearer, R.C. Block, Treatment with omega-3 fatty acid ethyl-ester alters fatty acid composition of lipoproteins in overweight or obese adults with insulin resistance, Prostaglandins, leukotrienes, and essential fatty acids, 90 (2014) 69-75.

[16] S.S. Huda, N. Sattar, D.J. Freeman, Lipoprotein metabolism and vascular complications in pregnancy, Clinical Lipidology, 4 (2009) 91-102.

[17] Meyer BJ, Stewart F, Brown EA, Cooney J, Nilsson S, Olivecrona G, Ramsay JE, Griffin BA, Caslake MJ, Freeman DJ, Maternal obesity is associated with the formation of small dense LDL and hypoadiponectinemia in the third trimester, Journal of clinical endocrinology and metabolism, 98 (2013) 643-652.

[18] Scottish, Executive, Scottish Index of Multiple Deprivation, in: Summary Technical Support, Edinburgh, 2004.

[19] C.N. Bagot, E. Leishman, C.C. Onyiaodike, F. Jordan, V.B. Gibson, D.J. Freeman, Changes in laboratory markers of thrombotic risk early in the first trimester of pregnancy may be linked to an increase in estradiol and progesterone, Thromb Res, 178 (2019) 47-53.
[20] National, Cholesterol, Education, Program, Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report., Circulation, 106 (2002) 3143-3421.
[21] Havel RJ, Eder HA, Bragdon JH, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, Journal of clinical investigation, 34 (1955) 1345-1353.
[22] N. Sattar, I.A. Greer, J. Louden, G. Lindsay, M. McConnell, J. Shepherd, C.J. Packard, Lipoprotein Subfraction Changes in Normal Pregnancy: Threshold Effect of Plasma Triglyceride on Appearance of Small, Dense Low Density Lipoprotein, Journal of clinical endocrinology and metabolism, 82 (1997) 2483-2491.
[23] G. Lepage, C. Roy, Direct transesterificaton of all classes of lipids in one-step reaction, Journal of Lipid Research, 27 (1986) 114-120.
[24] G.O. Burr, M.M. Burr, On the nature and role of the fatty acids essential in nutrition, Journal of Biological Chemistry, 86 (1930) 587-621.
[25] Ottosson UB, Lagrelius A, Rosing U, von Schoultz B, Relative fatty acid composition of lecithin during postmenopausal replacement therapy–a comparison between ethinyl estradiol and estradiol valerate, Gynecologic and obstetric investigation, 18 (1984) 296-302.
[26] B.J. Meyer, Australians are not Meeting the Recommended Intakes for Omega-3 Long Chain Polyunsaturated Fatty Acids: Results of an Analysis from the 2011-2012 National Nutrition and Physical Activity Survey, Nutrients, 8 (2016) 111.
[27] W.N. Sulaiman, M.J. Caslake, C. Delles, H. Karlsson, M.T. Mulder, D. Graham, D.J. Freeman, Does high-density lipoprotein protect vascular function in healthy pregnancy?, Clin Sci (Lond), 130 (2016) 491-497.
[28] A. Agarwal, A. Aponte-Mellado, B.J. Premkumar, A. Shaman, S. Gupta, The effects of oxidative stress on female reproduction: a review, Reprod Biol Endocrinol, 10 (2012) 49.
[29] V.A. Mackay, S.S. Huda, F.M. Stewart, K. Tham, L.A. McKenna, I. Martin, F. Jordan, E.A. Brown, L. Hodson, I.A. Greer, B.J. Meyer, D.J. Freeman, Preeclampsia is associated with compromised maternal synthesis of long-chain polyunsaturated fatty acids, leading to offspring deficiency, Hypertension, 60 (2012) 1078-1085.
[30] J.J. Alvarez, A. Montelongo, A. Iglesias, A. Lasuncion, E. Herrera, Longitudinal study on lipoportein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women, Journal Of Lipid Research, 37 (1996) 299-308.
[31] A. Iglesias, A. Montelongo, E. Herrera, A. Lasuncion, Changes in cholesteryl ester transfer protein activity during normal gestation and postpartum, Clinical Biochemistry, 27 (1994) 63-68.
[32] S. Velagapudi, M. Yalcinkaya, A. Piemontese, R. Meier, S.F. Nørrelykke, D. Perisa, A. Rzepiela, M. Stebler, S. Stoma, P. Zanoni, L. Rohrer, A. von Eckardstein, VEGF-A Regulates Cellular Localization of SR-BI as Well as Transendothelial Transport of HDL but Not LDL, Arteriosclerosis, Thrombosis, and Vascular Biology, 37 (2017) 794-803.
[33] V. Tsatsaris, F. Goffin, C. Munaut, J.F. Brichant, M.R. Pignon, A. Noel, J.P. Schaaps, D. Cabrol, F. Frankenne, J.M. Foidart, Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences, The Journal of clinical endocrinology and metabolism, 88 (2003) 5555-5563.
[34] S.E. Maynard, J.-Y. Min, J. Merchan, K.-H. Lim, J. Li, S. Mondal, T.A. Libermann, J.P. Morgan, F.W. Sellke, I.E. Stillman, F.H. Epstein, V.P. Sukhatme, S.A. Karumanchi, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia, Journal of Clinical Investigation, 111 (2003) 649-658.
[35] K.L. Grantz, S. Kim, W.A. Grobman, R. Newman, J. Owen, D. Skupski, J. Grewal, E.K. Chien, D.A. Wing, R.J. Wapner, A.C. Ranzini, M.P. Nageotte, S.N. Hinkle, S. Pugh, H. Li, K. Fuchs, M. Hediger, G.M. Buck Louis, P.S. Albert, Fetal growth velocity: the NICHD fetal growth studies, American Journal of Obstetrics and Gynecology, 219 (2018) 285.e281-285.e236.
[36] Y. Papanikolaou, J. Brooks, C. Reider, V.L. Fulgoni, U.S. adults are nto meeting recommneded levesl for fish and omega-3 fatty acid intakes: results of an analysis using observational data from NHANES 2003-2008, Nutrition journal, 13 (2014) 31.

[37] Spracklen CN, Smith CJ, Saftlas AF, Robinson JG, Ryckman KK, Maternal hyperlipidemia and the risk if preeclampsia: a meta-analysis, American Journal Of Epidemiology, 180 (2014) 346-358.

Table 1 Study participants' demographic characteristics shown as mean (±SD) or n (%). Early Pregnancy Study (EPS) Pre-P characteristics (n=24) and Lipotoxicity in Pregnancy Study (LIPS) at booking visit week 16 (n=20).

	EPS (n=24)	LIPS (n=20)	P value
Age (years)	34.8 ± 4.3	30.8 ± 4.1	0.0027
*SIMD quintiles n (%)			0.54
1	2 (8.7)	5 (25)	
2	6 (26)	3 (15)	
3	4 (17)	2 (10)	
4	2 (8.7)	1 (5)	
5	9 (39)	9 (45)	
Weight (kg)	70 ± 9	73 ± 13	0.43
Height (m)	1.64 ± 0.07	1.66 ± 0.07	0.35
BMI (kg/m^2)	26 ± 3	26 ± 5	0.80
Waist (cm)	90 ± 8	94 ± 9	0.25
SBP (mmHg)	116 ± 15	113 ± 11	0.51
DBP (mmHg)	65 ± 7	70 ± 8	0.075
Plasma triglycerides (mmol/L)	1.10 ± 0.42[#]	0.90 ± 0.44[^]	0.06
Plasma cholesterol (mmol/L)	4.77 ± 0.68[#]	5.02 ± 0.80[^]	0.25
HDL-C (mmol/L)	1.47 ± 0.26[#]	1.60 ± 0.38[^]	0.25
NEFA (mmol/L)	0.57 ± 0.47[#]	0.70 ± 1.01[^]	0.57

*SIMD (Scottish Index of Multiple Deprivation), where 1 is affluent and 5 is deprived

[#]Non-pregnant sampled prior to pregnancy; [^]non-pregnant sampled 13 weeks post-partum

Differences between demographic values were tested using a two sample t test. For SIMD a chi-squared test was used.

511 **Table 2** Plasma estradiol, progesterone, lipids, glucose, insulin and HOMA, mean (SD) across the pregnancy timeline (EPS, n=24: pre-pregnant, 4.6, 6.1, 8.4 weeks

512 of gestation and LIPS, n=20: 16, 25, 35 weeks of gestation and 13 weeks post-partum).

Plasma variables	Pre-pregnant (Pre-P)	4.6 weeks$^{\gamma}$	6.1 weeks	8.4 weeks$^{\gamma}$	16 weeks	25 weeks	35 weeks	13 weeks post-partum	R^2 Adj	*P* value
Estradiol (pmol/L)	625 (505)^{a}	821 (361)^{a,b}	1404 (523)^{a,b}	3,597 (2,196)^{b}	26,827 (12,575)^{c}	68,886 (22,021)^{d}	115,601 (45,068)^{e}	164 (78)^{a}	93%	<0.0001
Progesterone (nmol/L)	0.6 (1.3)^{a}	71 (35)^{b}	67 (26)^{b}	65 (18)^{b}	117 (40)^{c}	239 (36)^{d}	555 (107)^{e}	3.6 (0.8)^{a}	94%	<0.0001
Triglycerides (mmol/L)	1.10 (0.42)^{a}	0.89 (0.40)^{a}	0.91 (0.39)^{a}	1.17 (0.42)^{a,b}	1.52 (0.52)^{b,c}	2.06 (0.60)^{c,d}	2.72 (0.66)^{d}	0.90 (0.44)^{a}	56%	<0.0001
Cholesterol (mmol/L)	4.77 (0.68)^{a,b}	4.35 (0.58)^{a}	4.24 (0.65)^{a}	4.32 (0.65)^{a}	5.41 (0.65)^{b}	6.52 (0.95)^{c}	6.78 (1.07)^{c}	5.02 (0.80)^{b}	58%	<0.0001
HDL-C (mmol/L)	1.47 (0.26)^{a}	1.39 (0.25)^{a}	1.40 (0.23)^{a}	1.45 (0.30)^{a}	1.85 (0.37)^{a}	1.94 (0.45)^{a}	1.76 (0.36)^{a}	1.60 (0.38)^{a}	24%	<0.0001

NEFA	0.57	0.38	0.46	0.69	0.89	0.52	0.85	0.50	6%	0.02
(mmol/L)	(0.47)[a]	(0.10)[a]	(0.19)[a]	(0.80)[a]	(0.65)[b]	(0.47)[b,c]	(0.78)[b,c]	(0.44)[c]		
Glucose	4.88	5.02	4.75	5.04	4.56	4.61	4.69	5.03	11%	0.0006
(mmol/L)	(0.41)[a,b,c]	(0.44)[b,c]	(0.46)[a,b,c]	(0.62)[c]	(0.29)[a]	(0.43)[a,b]	(0.43)[a,b,c]	(0.46)[b,c]		
Insulin	9.7	11.2	10.6	12.8	5.6	7.8	13.5	4.90	12%	0.0002
(mU/mL)	(9.0)[a,b,c]	(10.2)[b,c]	(9.3)[a,b,c]	(11.3)[b,c]	(2.8)[a,b]	(4.2)[a,b,c]	(13.4)[c]	(2.50)[a]		
HOMA	2.1	2.5	2.2	3.1	1.1	1.6	3.0	1.11	11%	0.0003
	(2.0)[a,b]	(2.5)[a]	(2.0)[a,b]	(3.0)[a]	(0.6)[b]	(0.9)[a,b]	(3.6)[a]	(0.61)[b]		

*One way analysis of variance across the pregnancy timeline

[γ]Missing data points (n=2 at 4.6 weeks, n=1 at 8.4 weeks)

[a, b, c, d] indicate differences between individual groups using post-hoc Tukeys-Kramer at significance level $p<0.05$

Abbreviations: HDL-C; High Density Lipoprotein Cholesterol, NEFA; Non-Esterified Fatty Acids;

HOMA was calculated as [Insulin (mU/L) x Glucose (mmol/L)]/22.5

519 **Table 3** HDL composition (% of HDL total lipid/protein) and HDL DHA to HDL-TG, HDL-TC, HDL-PL and HDL-apo-A-I ratios across weeks of gestation.

Weeks gestation	1.6 N=23*	4.6 N=20*	6.1 N=23*	8.4 N=22*	16 N=20	25 N=20	35 N=19*	13 weeks post-partum N=19*	P value
HDL-TG	4.1 [a,b]	4.5 [a,b,c]	4.8 [a,b,c]	5.0 [a,b,c]	5.8 [c,d]	7.2 [d,e]	7.9 [e]	3.2 [a]	<0.0001
(% HDL composition)	(1.7)	(1.5)	(1.5)	(1.5)	(2.2)	(2.0)	(2.1)	(1.6)	
HDL-TC	18.9	19.7	18.8	19.1	17.2	17.8	17.6	18.5	0.14
(% HDL composition)	(2.1) [#]	(2.5)	(3.3)	(4.6)	(3.7)	(1.2)	(2.1)	(2.8)	
HDL-PL	34.6	31.7	31.9	32.8	33.4	31.3	31.5	33.0	0.56
(% HDL composition)	(12.6)	(2.6)	(4.7)	(5.1)	(3.4)	(1.8)	(2.4)	(2.7)	
HDL-apoA-I	45.7	44.1	44.4	44.8	43.6	43.6	43.0	45.3	0.11
(% HDL composition)	(3.6)^	(3.4)	(3.3)	(3.3)^	(4.1)	(1.9)	(2.5)	(2.0)	
HDL DHA to HDL-TG	1397 [a,b]	1025 [a,b]	850 [b]	1140 [a,b]	2068 [a,b]	1524 [a,b]	1115 [a,b]	2745 [a]	0.02
ratio (umol/g)	(2591)	(611)	(563)	(553)	(2002)	(1302)	(913)	(3472)	
HDL DHA to HDL-TC	184 [a]	222 [a,b]	203 [a,b]	284 [a,b,c]	565 [c]	556 [c]	483 [b,c]	412 [a,b,c]	<0.0001
ratio (umol/g)	(144) [#]	(142)	(110)	(105)	(426)	(426)	(389)	(443)	

HDL DHA to HDL-PL	108 [a]	133 [a,b]	122 [a]	162 [a,b,c]	286 [b,c]	316 [c]	263 [a,b,c]	225 [a,b,c]	<0.0001
ratio (umol/g)	(62)	(65)	(59)	(66)	(229)	(251)	(205)	(250)	
HDL DHA to HDL	75 [a]	98 [a,b]	85 [a,b]	110 [a,b,c]	220 [c,d]	229 [d]	197 [b,c,d]	161 [a,b,c,d]	<0.0001
apoA-I ratio (umol/g)	(43)^	(53)	(38)	(39)^	(174)	(187)	(162)	(176)	

* missing data points due to low sample volume or no sample; [#]2 samples deleted due to quality control check (HDL-total cholesterol values very low 0.024 & 0.017); ^ 1 sample deleted due to quality control check (HDL-apoA-I values very low).

523 **Table 4** Correlations between plasma estradiol (n=164) and progesterone (n=154) with plasma lipids,

524 HDL AA, HDL DHA, HDL enrichment with AA and HDL enrichment with DHA

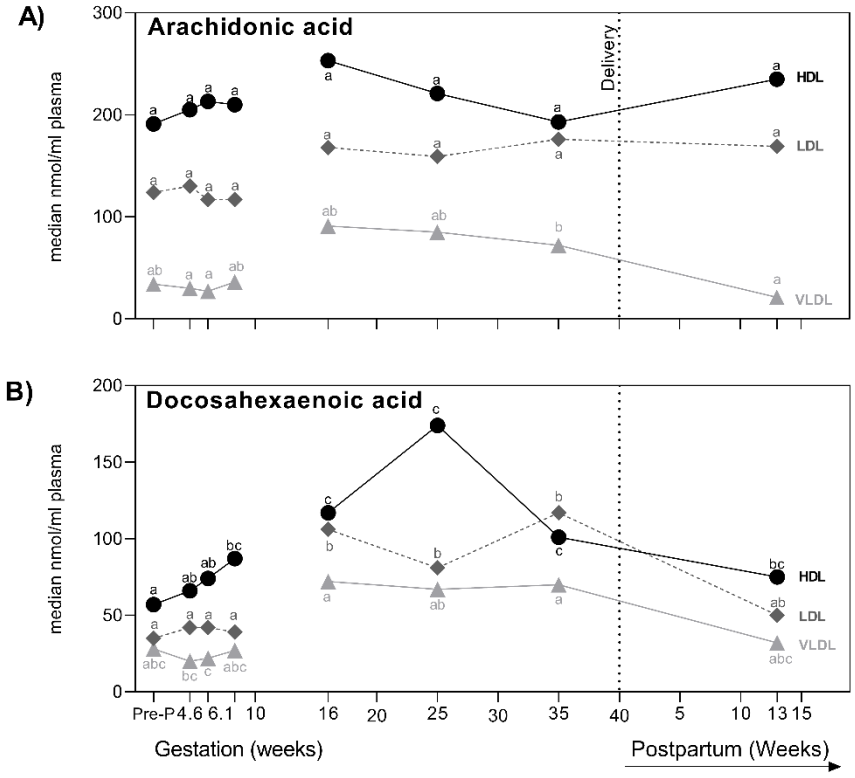
525

	Estradiol * (pmol/L)		Progesterone * (nmol/L)	
	R^2 Adjusted	P value	R^2 Adjusted	*P* value
Plasma triglycerides [#] (mmol/L)	52%	<0.0001	39%	<0.0001
Plasma total cholesterol [#] (mmol/L)	48%	<0.0001	37%	<0.0001
Plasma HDL-C * (mmol/L)	20%	<0.0001	12%	<0.0001
HDL AA * (nmol/mL)	4%	0.0062	3%	0.016
HDL DHA [#] (nmol/mL)	16%	<0.0001	18%	<0.0001
HDL AA per HDL-C * (umol/mmol)	N/A	0.4648	1%	0.26
HDL DHA per HDL-C * (umol/mmol)	10%	<0.0001	13%	<0.0001

526 [#] Log transformed data; * square root data

527

528 **Figure 1**



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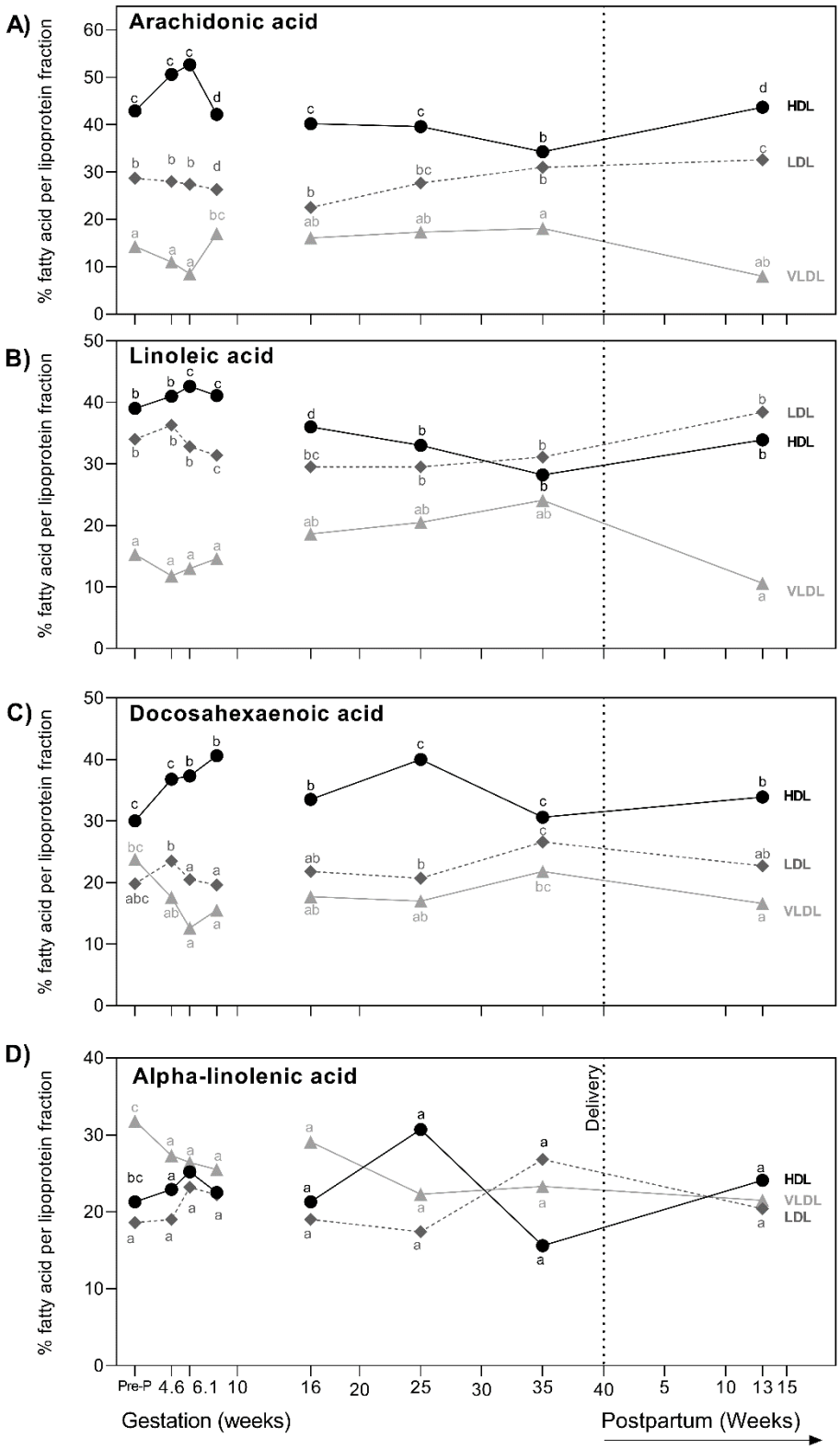
531 **Figure 2**



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534 **Figure 3**

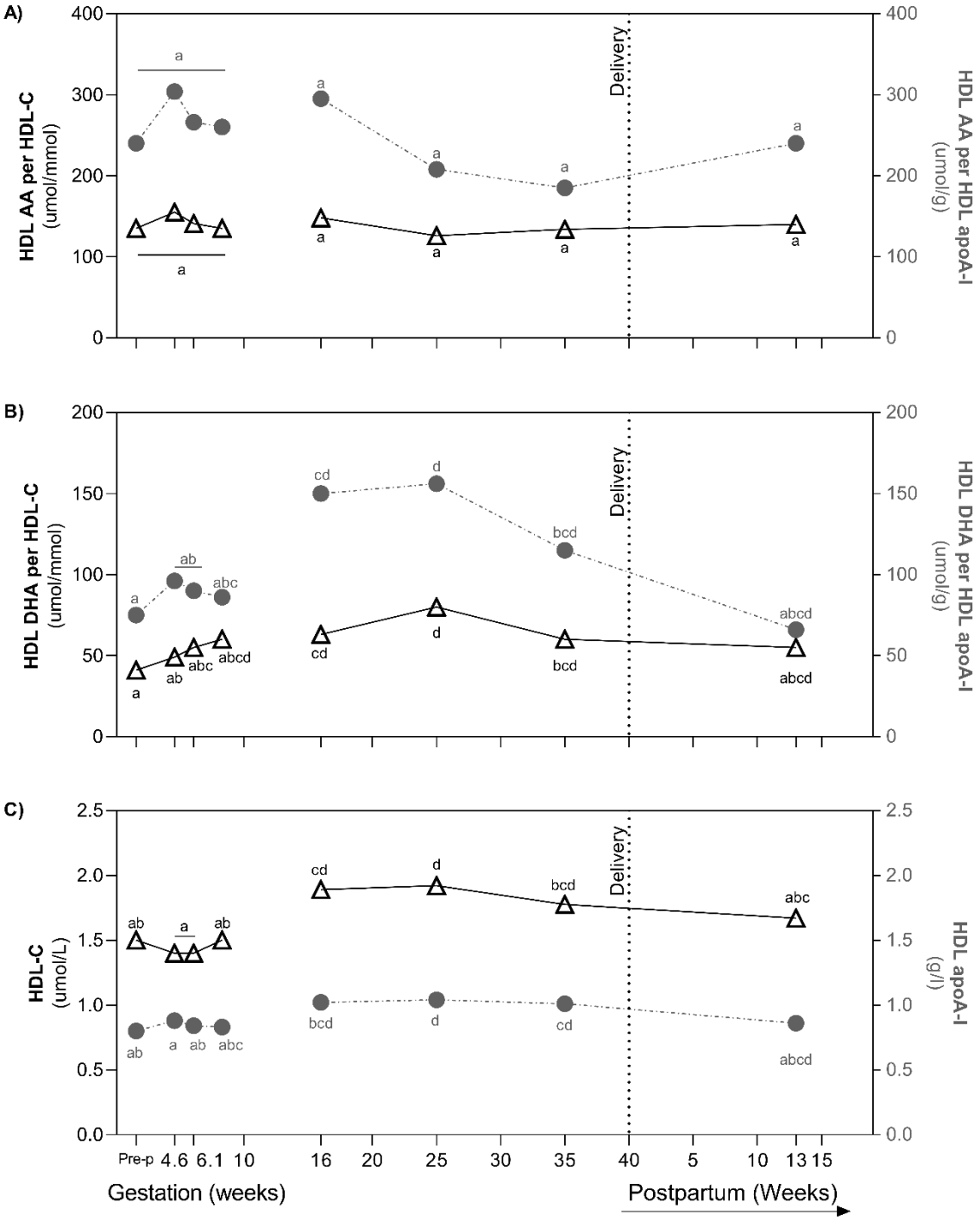

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Figure legends

Figure 1 A: Median AA concentration (nmol/mL plasma) across the pregnancy timeline in HDL, LDL and VLDL. B: Median DHA concentration (nmol/mL plasma across the pregnancy timeline in HDL, LDL and VLDL. EPS, n=24: Pre-P, 4.6, 6.1, 8 weeks of gestation and LIPS, n=20: 16, 25, 35 weeks of gestation and 13 weeks post-partum. [a, b, c] indicate differences between the pregnancy timelines within each lipoprotein using post-hoc Tukeys-Kramer at significance level $P<0.05$.

Abbreviations: DHA; docosahexaenoic acid, AA; arachidonic acid, Pre-P; pre-pregnant, PP; post-partum, VLDL; very low density lipoprotein, LDL; low density lipoprotein, HDL; high density lipoprotein

Figure 2 The relative proportion of fatty acids (% fatty acid in lipoprotein fractions) in HDL, LDL and VLDL across the pregnancy timeline. EPS, n=24: Pre-P, 4.6, 6.1, 8 weeks of gestation and LIPS, n=20: 16, 25, 35 weeks of gestation and 13 weeks post-partum. [a, b, c] indicate differences at each pregnancy timepoint using post-hoc Tukeys-Kramer at significance level $P<0.05$

Abbreviations: DHA; docosahexaenoic acid, AA; arachidonic acid, Pre-P; pre-pregnant, PP; post-partum, VLDL; very low density lipoprotein, LDL; low density lipoprotein, HDL; high density lipoprotein

Figure 3 Enrichment of HDL with AA (Figure 3A) and DHA (Figure 3B) (median umol/mmol HDL-C left Y-axis and median umol/g HDLapoA-I) across the pregnancy timeline. HDL-C (mmol/L) and HDLapoA-I (g/L) concentrations (Figure 3C) across the whole pregnancy timeline. EPS, n=24: Pre-P, 4.6, 6.1, 8 weeks of gestation and LIPS, n=20: 16, 25, 35 weeks of gestation and 13 weeks post-partum. [a, b, c, d] indicate differences between the pregnancy timelines within each lipoprotein using post-hoc Tukeys-Kramer at significance level $P<0.05$

561 Abbreviations: HDL-C; high density lipoprotein-cholesterol, DHA; docosahexaenoic acid, AA;
562 arachidonic acid, Pre-P; pre-pregnant, PP; post-partum, VLDL; very low density lipoprotein, LDL;
563 low density lipoprotein, HDL; high density lipoprotein