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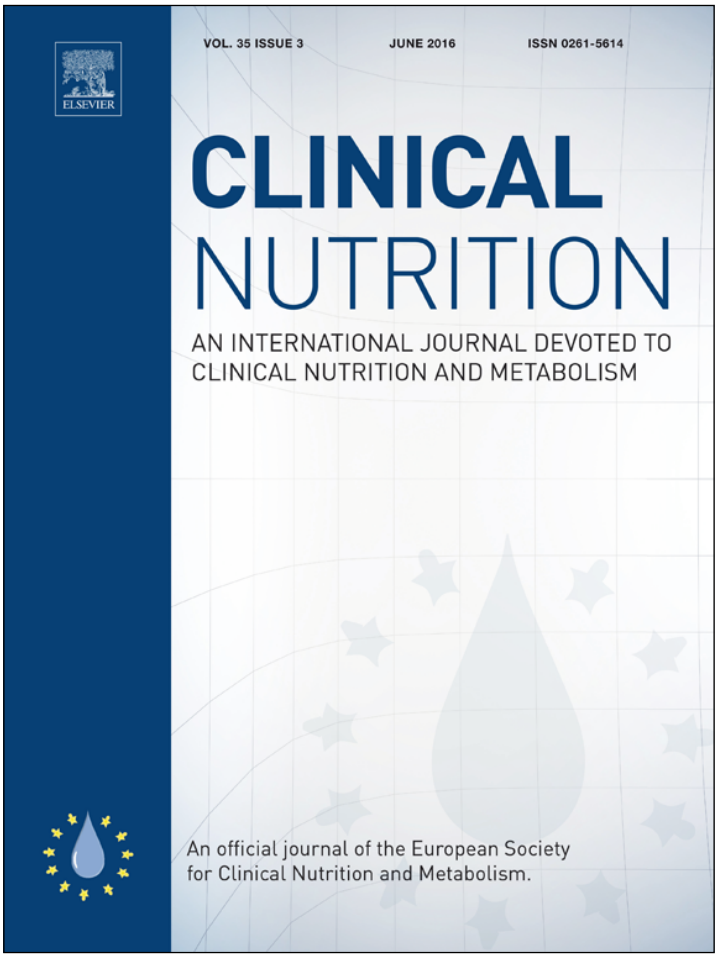

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Randomized control trials

Efficacy of docosahexaenoic acid-enriched formula to enhance maternal and fetal blood docosahexaenoic acid levels: Randomized double-blinded placebo-controlled trial of pregnant women with gestational diabetes mellitus

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SUMMARY

Background & aims: Gestational diabetes mellitus (GDM) compromises the level of docosahexaenoic acid (DHA) in phospholipids of maternal and fetal red blood cells and fetal plasma. This is of some concern because of the importance of DHA for fetal neuro-visual development. We have investigated whether this abnormality could be rectified by supplementation with DHA-enriched formula.

Methods: Women with GDM (n = 138) recruited from Newham University Hospital, London received two capsules of DHA-enriched formula (active-group) or high oleic acid sunflower seed oil (placebo-group) from diagnosis until delivery. Maternal (baseline and delivery) and fetal (cord blood) red blood cell and plasma phospholipid fatty acid composition, and neonatal anthropometry were assessed.

Results: One hundred and fourteen women (58 active, 56 placebo) completed the trial. The active-group compared with the placebo-group had significantly enhanced level of DHA in plasma phosphatidylcholine (4.5% vs 3.8%, $P = 0.011$), red blood cell phosphatidylcholine (2.7% vs 2.2%, $P = 0.022$) and phosphatidylethoanolamine (9.5% vs 7.6%, $P = 0.002$). There was no difference in cord plasma and red blood cell phospholipid DHA between the two groups. The neonates of the two groups of women had comparable anthropometric measurements at birth.

Conclusion: Daily supplementation of 600 mg DHA enhances maternal but not fetal DHA status in pregnancy complicated by GDM. The inefficacy of the supplement to improve fetal status suggests that the transfer of DHA across the placenta maybe impaired in women with the condition. Regardless of the mechanisms responsible for the impairment of the transfer, the finding has implications for the management of neonates of women with GDM because they are born with a reduced level of DHA and the condition is thought to be associated with a risk of neuro-developmental deficits. We suggest that babies of women with GDM, particularly those not suckling, similar to the babies born prematurely require formula milk fortified with a higher level of DHA.

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1. Introduction

The impacts of gestational diabetes mellitus (GDM) on short- and long-term health of women and their offspring are increasingly recognized [1]. In Europe, the estimated prevalence of GDM ranges from 2 to 6% and it is expected to rise with increasing obesity, a strong risk factor for developing the condition [2]. Other determinant factors are a family history of type 2 diabetes, advanced maternal age and ethnicity. Certain ethnic groups are more predisposed to GDM [3].

We have previously reported that GDM reduces the levels of docosahexaenoic acid (DHA) and arachidonic acid (AA) in maternal red blood cell [4,5] and fetal red blood cell and plasma [5–7]

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phospholipids. DHA and AA, which are highly unsaturated fatty acids of the omega-3 and omega-6 family, respectively, are vital structural and functional components of cellular and sub-cellular membranes as well as precursors of diverse bioactive compounds. In addition, based on animal models of obesity and cell culture studies, it is postulated that DHA might be a potent anti-adiposity agent [8] and enhance glucose utilization by modulating insulin secretion and action [9,10].

The importance of DHA and AA in fetal life has been extensively documented [11,12]. Of these two fatty acids, DHA is considered to be the most limiting nutrient in pregnancy and lactation because it is scarce or absent in land animal and plant food sources. Moreover, the synthesis of DHA from the parent compound α-linolenic acid is inefficient. In normal pregnancy, DHA is preferentially transferred by placental selection from maternal to placental circulation. However, recent studies have reported that the placental uptake and transfer of DHA is impaired in pregnancy complicated by GDM [13,14]. There are no published communications whether or not the impact of this impairment could be ameliorated by DHA supplementation. In this report, we present the findings of a randomized, double-blind, placebo-controlled trial in which women with GDM were supplemented with DHA-enriched formula or high oleic acid sunflower seed oil. The primary outcome measure was the red blood cell membrane phospholipid DHA levels in the women and their neonates at delivery.

2. Materials and methods

The study was approved by East London & The City HA Local Research Ethics Committee 3 (REC reference no. 06/Q0605/89) and registered with ISRCTN Register (registration no. ISRCTN68997518). Written informed consent was obtained from all participants and the investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2007. Participants, midwives, and all investigators were blinded to allocation until all the data collated and analyzed.

2.1. Subjects and intervention

The study was carried out in Newham Borough, an inner city area of Greater London with a high proportion of immigrant community, predominantly South Asian and African/Caribbean ethnic group. Subjects were recruited during their visit to the antenatal or diabetic clinics at Newham University Hospital between October 2007 and February 2012. The inclusion criteria were 17–45 years old women with singleton pregnancies diagnosed with GDM. Those who were planning to receive tocolytic or corticosteroid therapy or had been taking omega-3 supplement during the period of time leading to their current pregnancy were excluded. Because of the high incidence of GDM and type 2 diabetes in the area, the hospital tests for GDM for women with high risk factors for the condition from early weeks of pregnancy. The test involves an overnight fast followed by drinking a solution of 75 g of glucose and drawing blood at 60 and 120 min for glucose determination. GDM is diagnosed if the glucose level at 120 min is greater than 7.8 mmol/l.

The eligible women who consented to participate in the study were randomized to receive daily 2 capsules of either “DHA-enriched formula” or “placebo (high oleic acid sunflower seed oil)”. Each active supplement capsule contained 300 mg of DHA, 42 mg of eicosapentaenoic acid (EPA) and 8.4 mg of AA, and placebo 721 mg of oleic acid. Randomization was carried out using a random code generated by the supplement provider (Equazen/Vifor Pharma Ltd., Glattbrugg, Switzerland). Both supplements contained vitamin E (d-alpha tocopherol) as an antioxidant and were encapsulated in an identical oblong soft gelatin capsule (750 mg in size).

2.2. Sample size

Sample size was calculated based on our previous case–control study [7] which found a 35% difference in red blood cell phosphatidylcholine DHA between neonates of pregnant women with GDM (4.0%) and those without the condition (5.4%). We assumed that supplementation of women with GDM with DHA-rich fish oil would increase the level of the nutrient in their neonates to the level of those born to women who had uncomplicated pregnancy. The power calculation indicated that a minimum of 40 subjects (neonates) per group would be required to detect the changes in red blood cell phosphatidylcholine DHA level with 80% power. The sample size and power calculation was performed using G*Power 3 [15] and based on a two independent groups, two-tailed t-test with an alpha of 0.05.

2.3. Blood collection and fatty acid analysis

Non-fasting venous blood samples (5–10 ml) were obtained from the subjects at recruitment (17^{th}–33rd gestational weeks) and delivery (maternal and cord) in EDTA vacutainer tubes. Samples collected during the day were transported promptly to the Lipidomics and Nutrition Research Centre (LNRC) laboratory for processing. Blood samples collected at night or on weekends were processed at the Pathology Laboratory, Newham University Hospital and subsequently transported to the LNRC laboratory. All samples were stored at −70 °C until analysis.

Fatty acid composition of red blood cell and plasma phospholipids was determined using the standardized method developed by our laboratory [7]. Briefly, total lipids were extracted by homogenizing the samples in chloroform and methanol, and the phospholipid fractions from the resulting total lipid were separated by thin-layer chromatography. Fatty acid methyl esters prepared from the phospholipids were separated using a gas–liquid chromatograph (HRGC MEGA 2 Series; Fisons Instruments, Milan, Italy) and quantified using a chromatography data system (Agilent EZChrom Elite Chromatography Data System v3.2, Scientific Software, Inc., Pleasanton, CA, USA).

2.4. Neonatal anthropometric measurement

Weight and length of the newborn babies were recorded by a midwife who attended the delivery as per routine practice and head-, shoulder-, mid-arm-, and abdominal circumferences by research midwives (JH and IN). Seca 210 portable measuring mat and Seca 201 ergonomic circumference measuring tape (Seca UK, Birmingham, UK) were used.

2.5. Statistical analysis

Data are presented as mean ± standard deviation (SD), median (range), and number of occurrence as appropriate and statistical significance was set at $P < 0.05$. All analyses were performed based on intention-to-treat principle. Independent t-test was used to compare the difference in fatty acids and anthropometric measurement between the active and placebo groups. The differences in pregnancy outcome were tested with Chi-square test. The change in DHA level within the group (baseline versus delivery) was assessed both by independent (including all subjects) and paired-sample (including only matched samples) t-test. All analyses were carried out with IBM SPSS Statistics version 20 (IBM Corporation, Armonk, NY, USA).

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3. Results

One hundred thirty eight women with GDM were randomized to receive either active or placebo, and 114 (58 active-group, 56 placebo-group) completed the trial (Fig. 1). The last delivery took place in May 2012.

The ethnic composition of the groups was similar and reflected the population diversity of the Borough (Table 1). 22.4% of the active-group and 25.4% of the placebo-group had gestational diabetes in their previous pregnancy. Over 60% of women were treated with oral hypoglycemic agents (active-group 49.3%; placebo-group 50.7%) or insulin (active-group 11.9%; placebo-group 14.1%). There was a wide variation in the duration of supplementation. This was a result of number of women being diagnosed with GDM either early or late stage of pregnancy. Five women (4 active and 1 placebo) claimed to have taken occasionally omega-3 supplement purchased from health shops during the course of the study. However, their DHA and EPA levels were not different from the mean values of their respective groups. Compliance was monitored by regular home visits by the research midwives and telephone contact (including out-of-office hour), and by counting unused capsules which the women are asked to bring with them when they come to collect the next batch of supplements.

Pregnancy outcomes and neonatal anthropometry are shown in Table 2. From the placebo-group, one woman had miscarriage and another delivered stillborn baby, and there was one case of cleft lip and palate in the active-group. There was no difference in the anthropometric measurement between the two groups. However, the numbers of babies born at preterm and low birth weight were higher in the active-group but the difference did not reach statistical significance level.

Percentages of the major fatty acids of plasma and red blood cell phosphatidylcholine and red blood cell phosphatidylethanolamine are presented in Table 3. There was no difference in fatty acid composition of plasma or red blood cell phospholipids between those randomized to receive active supplement or placebo at baseline. At delivery, the women supplemented with active supplement compared with those who were given placebo had higher level of DHA in plasma phosphatidylcholine ($P = 0.011$), red blood cell phosphatidylcholine ($P = 0.022$) and phosphatidylethanolamine ($P = 0.002$), and a lower level of AA in plasma phosphatidylcholine ($P = 0.045$). Both groups had comparable levels of saturated and monounsaturated fatty acids in plasma and red blood cell phospholipids. Moreover, they had a similar red blood cell unsaturation index (sum of the % unsaturated fatty acids multiplied by their number of double bonds).

Within-group baseline and delivery comparison in plasma phosphatidylcholine revealed an increase in palmitic ($P < 0.01$) and oleic ($P < 0.001$) acids and decrease in stearic acid ($P < 0.001$) in both groups. There was a decrease in the level of DHA in the placebo-group (−17.4%, $P = 0.009$) and AA in the active-group (−12.3%, $P = 0.000$). The level of DHA in the active-group and AA in the placebo-group did not change between baseline and delivery.

In red blood cell phosphatidylcholine, oleic and stearic acids in the active-group ($P < 0.05$) and α-linolenic acid, EPA, DHA in the placebo-group ($P < 0.01$) decreased between baseline and delivery. The changes in red blood cell phosphatidylethanolamine fatty acids with the progress of pregnancy mirrored broadly that of plasma phosphatidylcholine. There was an increase in saturated ($P < 0.05$) and monounsaturated ($P < 0.01$) fatty acids, and a decrease in AA ($P < 0.01$) in both groups of women. The level of EPA and DHA did not change between baseline and delivery in the women who were given active supplement. In contrast, the level of both fatty acids decreased significantly at delivery in those who received placebo ($P = 0.001$). These changes led to a reduced unsaturation index ($P < 0.01$) in both groups.

Plasma and red blood cell phospholipid fatty acid profiles of the neonates from both groups were similar (Table 4). Within the active

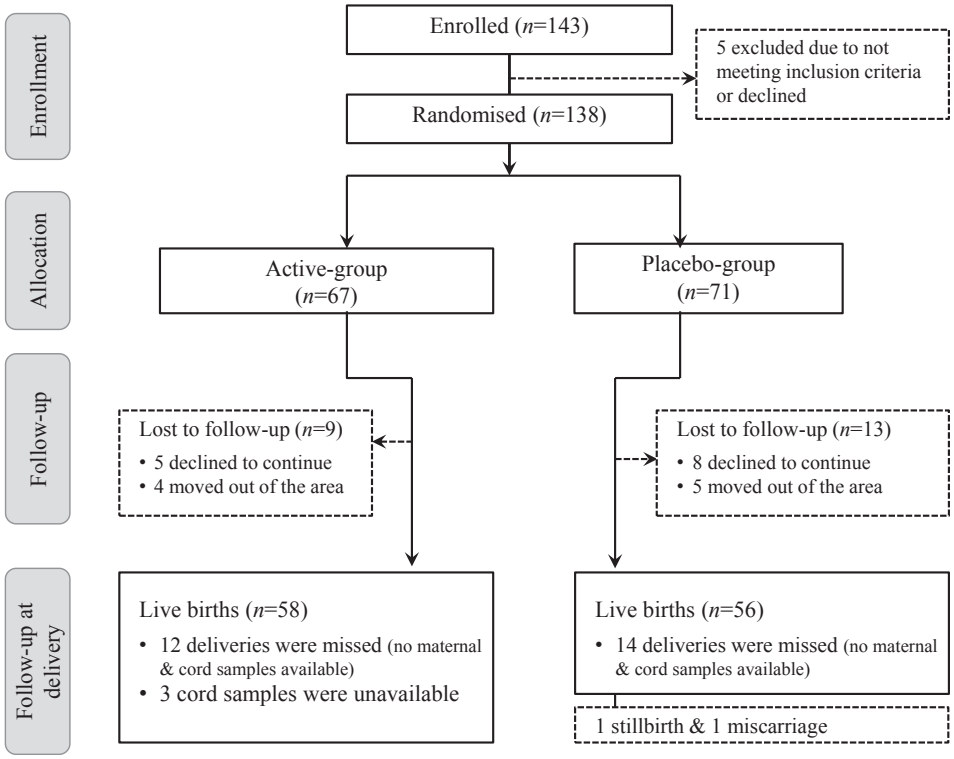

Fig. 1. Flowchart of participants during the course of the randomized trial.

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Table 1
Demographic and obstetric characteristics of the participants.[a]

	Active-group	Placebo-group
Number of participants (*n*)	67	71
Gestation at recruitment (weeks)[b]	28.3 (17.4–33.4)	28.3 (17.0–32.9)
Age (years)[b]	31.0 (21.0–41.0)	32.0 (21.0–44.0)
Pre-pregnancy weight (kg)[c]	73.9 ± 15.8	72.6 ± 15.3
Height (m)[c]	1.6 ± 0.1	1.6 ± 0.1
Pre-pregnancy BMI[c,d]	29.0 ± 5.6	29.2 ± 5.7
≤25.0	17	19
25.1–30.0	23	24
≥30.1	27	28
Ethnicity (*n*)[e]		
Asian	40	44
African/Afro-Caribbean	18	18
Caucasian	5	5
Others	4	4
Parity (*n*)[f]		
0	33	24
1 - 3	27	40
≥4	6	5
Smoker (*n*)	2	0
Planned pregnancy (*n*)	42	37
GDM in previous pregnancy (*n*)	15	18
Folic acid use (*n*)	18	18
Glucose tolerance test		
Gestation (weeks)[b]	18.7 (11.4–30.1)	20.6 (13.3–31.9)
Glucose at 0 min (mmol)[c]	5.6 ± 1.6	5.5 ± 1.3
Glucose at 120 min (mmol)[c]	9.6 ± 2.2	9.3 ± 1.8
Diabetes treatment (*n*)		
Diet only	26	24
Oral hypoglycemic agents	33	36
Insulin	8	10
Oral hypoglycemic agents + insulin	–	1
Family history of diabetes (*n*)		
Mother	29	21
Father	20	26
Both parent	6	12

[a] No statistical test was performed as per CONSORT guidelines.
[b] Gestation week and age are presented as median (min – max).
[c] Values are presented as mean ± SD.
[d] BMI, body mass index (kg/m^2).
[e] The UK Home Office's classification for an individual's ethnicity according to person's self-definition was used; Asian (Bangladesh, Bengali, Sri Lankan, Indian, Pakistani), African/Afro-Caribbean (Black African, Black British, Caribbean, Afro-Caribbean), Caucasian (English, Polish, Irish, European), Others (Filipino, Arab, North African, Latin American, Mixed-race).
[f] Information was not available from 3 women (1 active-group, 2 placebo-group).

Table 2
Pregnancy outcomes and anthropometric measurement.[a]

		Active-group	Placebo-group[b]
Live births (*n*)[c]		58	56
Gestation at delivery (weeks)[d]		38.1 (33.3–41.1)	38.1 (35.9–41.1)
Supplementation duration (weeks)[d]		10.0 (4.0–22.0)	10.0 (4.0–20.0)
Gender (*n*)			
Male		29	32
Female		29	24
Delivery method (*n*)			
Vaginal -	Spontaneous	19	11
	Assisted	5	3
	Induced	6	13
Caesarean section -	Elective	7	9
	Emergency	21	20
Preterm birth (*n*)[e]		12	5
Late-preterm birth (*n*)[f]		10	5
Low birth weight (*n*)[g]		8	4
Macrosomia (*n*)[h]		3	3
Neonatal hypoglycemia (*n*)		2	3
Congenital defect (*n*)[i]		1	0
Anthropometric measurement[j]			
Weight (kg)		3.2 ± 0.6	3.1 ± 0.4
Length (cm)		49.0 ± 7.8	50.3 ± 5.7
Head circumference (cm)		33.9 ± 1.6	33.8 ± 2.5
Shoulder circumference (cm)		36.3 ± 5.5	36.0 ± 5.6
Mid-arm circumference (cm)		11.9 ± 2.1	11.5 ± 2.6
Abdominal circumference (cm)		33.1 ± 4.1	33.0 ± 4.3

[a] The pregnancy outcome and neonatal anthropometric measurement did not differ between active and placebo groups.
[b] There was one stillbirth and one miscarriage in the placebo-group.
[c] This number included all the subjects who completed the supplementation regardless of the availability of blood samples at delivery.
[d] Gestation week at delivery and supplementation duration are given median (min – max).
[e] Preterm birth; born less than 37 weeks of gestation.
[f] Late-preterm birth; born between 34 0/7 weeks and 36 6/7 weeks of gestation.
[g] Low birth weight; birth weight less than 2.5 kg.
[h] Macrosomia; body weight greater than 4 kg.
[i] One baby from the active-group was born with cleft lip and palate.
[j] Anthropometric measurement are presented as mean ± SD. The measurement for one baby from the active-group was not obtained.

supplemented group, the level of DHA in the neonates whose mothers had less than 10 weeks of supplement did not differ from those supplemented for a longer period.

4. Discussion

Mature red blood cells have a limited ability of synthesizing phospholipids de novo from glycerol or sphingosine backbone. Therefore, the renewal/remodeling of red blood cell membrane phospholipids, which occurs continuously, is reliant on plasma lipid pool. The renewal mechanisms include transfer of fatty acids from plasma neutral lipids to phosphatidylethanolamine through phosphatidylcholine [16], deacylation–reacylation cycles of endogenous fatty acids and passive exchange of intact phospholipids, primarily phosphatidylcholine, with plasma phospholipids [17]. Hence, it is evident that the fatty acid moieties of red blood cell phospholipids have their origin in plasma lipids [18] in individuals without underlining clinical conditions.

We have previously observed that women with GDM, regardless of their ethnic origin and dietary background, had reduced level of DHA in red blood cell but not plasma phospholipids [4,5,7]. These findings led us to postulate "the incorporation of DHA from plasma into red blood cells is compromised in women with the condition due to hyperglycemia". In addition, we postulated "this impairment is ameliorated by a rigorous glycemic control". Consistent with our postulation, DHA supplementation enriched the level of the fatty acid in plasma and red blood cell phospholipids in the GDM women whose blood glucose was well-controlled by using diet, oral hypoglycemic agents or/and insulin.

Maternal DHA level declines in the later part of pregnancy in healthy "well-nourished" women without obstetric complications. This is often described as a physiological response to pregnancy. There was no such decrease in the women with gestational diabetes supplemented with DHA-enriched formula. Moreover, the phenomenon does not occur in pregnant women from communities who consume high amounts of fish and seafood [19], or in those supplemented with fish oil or fish [20,21]. Hence, the decline of DHA in pregnancy signifies an imbalance between maternal status and mother-fetus requirement.

Working groups have recommended that expectant and nursing mothers have to consume 300 mg of DHA daily [22]. The recommended value which is based on breast milk, and maternal, neonatal and infant blood DHA data from supplemented and unsupplmented women primarily from the developed countries are essentially guesstimates. In our study of a cohort of unsupplmented pregnant British and Sudanese women, the levels of DHA in maternal plasma phosphatidylcholine were 4.3% [7] and 2.3% (Nyuar KB and Ghebremeskel K, unpublished data) respectively.

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Table 3
Maternal plasma and red blood cell phospholipid fatty acid composition at baseline and delivery.[a]

	Baseline			Delivery			Baseline *vs* delivery[c] (*P* value)	
	Active-group (*n* 67)	Placebo-group (*n* 71)	*P* value[b]	Active-group (*n* 46)	Placebo-group (*n* 42)	*P* value[b]	Active-group	Placebo-group
Plasma phosphatidylcholine								
Palmitic	29.8 ± 3.6	30.4 ± 3.1	NS	32.0 ± 2.1	32.7 ± 2.6	NS	0.000	0.000
Stearic	10.2 ± 1.4	10.0 ± 1.2	NS	9.1 ± 1.0	9.0 ± 1.2	NS	0.000	0.000
Oleic	10.9 ± 3.8	10.7 ± 2.0	NS	13.0 ± 2.4	13.0 ± 2.6	NS	0.001	0.000
Linoleic	24.7 ± 3.5	24.8 ± 3.0	NS	23.7 ± 3.0	22.7 ± 3.6	NS	NS	0.002
α-linolenic	0.3 ± 0.1	0.3 ± 0.1	NS	0.4 ± 0.1	0.3 ± 0.1	NS	NS	NS
Arachidonic	10.6 ± 2.1	10.6 ± 1.9	NS	9.3 ± 1.8	10.1 ± 2.3	0.045	0.000	NS
Eicosapentaenoic	0.7 ± 0.7	0.8 ± 0.7	NS	0.7 ± 0.4	0.6 ± 0.5	NS	NS	NS
Docosahexaenoic	4.5 ± 1.0	4.6 ± 1.5	NS	4.5 ± 1.0	3.8 ± 1.2	0.011	NS	0.009
Σ Saturates	41.0 ± 3.4	41.3 ± 2.5	NS	42.0 ± 1.9	42.6 ± 2.4	NS	NS	0.006
Σ Monoenes	11.5 ± 4.0	11.4 ± 2.1	NS	13.9 ± 2.5	13.9 ± 2.7	NS	0.001	0.000
Σ n-6	40.4 ± 3.4	40.0 ± 3.5	NS	37.2 ± 2.8	37.2 ± 4.0	NS	0.000	0.000
Σ n-3	6.1 ± 1.5	6.3 ± 2.3	NS	6.0 ± 1.4	5.3 ± 1.7	0.031	NS	0.015
Σ n-6/Σ n-3 ratio	7.1 ± 1.9	7.3 ± 3.4	NS	6.5 ± 1.9	7.9 ± 3.4	0.028	NS	NS
Red blood cell phosphatidylcholine								
Palmitic	36.4 ± 2.3	36.3 ± 3.4	NS	35.8 ± 4.0	36.4 ± 4.3	NS	NS	NS
Stearic	8.1 ± 1.2	8.1 ± 1.6	NS	8.3 ± 1.5	8.5 ± 1.7	NS	0.018	NS
Oleic	15.5 ± 1.6	15.5 ± 2.3	NS	16.9 ± 2.3	17.0 ± 2.8	NS	0.000	0.000
Linoleic	22.5 ± 2.8	21.8 ± 3.7	NS	22.4 ± 3.3	21.2 ± 4.2	NS	NS	NS
α-linolenic	0.2 ± 0.1	0.2 ± 0.1	NS	0.2 ± 0.1	0.2 ± 0.1	NS	NS	0.007
Arachidonic	7.2 ± 1.5	7.5 ± 2.0	NS	6.8 ± 1.8	7.3 ± 2.7	NS	NS	NS
Eicosapentaenoic	0.4 ± 0.4	0.6 ± 0.5	NS	0.5 ± 0.3	0.4 ± 0.3	NS	NS	0.003
Docosahexaenoic	2.5 ± 0.7	2.7 ± 1.0	NS	2.7 ± 1.0	2.2 ± 1.0	0.022	NS	0.002
Σ Saturates	45.5 ± 2.2	45.3 ± 3.6	NS	44.9 ± 4.1	45.7 ± 4.5	NS	NS	NS
Σ Monoenes	16.1 ± 1.7	16.2 ± 2.3	NS	17.6 ± 2.3	17.7 ± 3.0	NS	0.000	0.000
Σ n-6	33.4 ± 2.7	33.0 ± 4.1	NS	32.7 ± 4.5	32.0 ± 5.9	NS	NS	0.036
Σ n-3	3.6 ± 1.0	4.0 ± 1.5	NS	3.8 ± 1.3	3.2 ± 1.4	0.044	NS	0.000
Σ n-6/Σ n-3 ratio	10.3 ± 5.0	9.7 ± 4.5	NS	9.6 ± 3.4	11.6 ± 4.7	0.03	NS	0.023
Unsaturation index[d]	121.5 ± 8.2	123.3 ± 14.7	NS	121.5 ± 15.7	118.1 ± 19.1	NS	NS	0.036
Red blood cell phosphatidylethanolamine								
Palmitic	14.9 ± 1.6	14.9 ± 1.8	NS	15.9 ± 2.8	16.5 ± 3.7	NS	0.02	0.004
Stearic	5.5 ± 0.9	5.6 ± 1.1	NS	6.3 ± 1.3	6.5 ± 1.7	NS	0.000	0.003
Oleic	16.4 ± 1.2	16.4 ± 1.4	NS	17.9 ± 2.9	18.6 ± 4.5	NS	0.004	0.009
Linoleic	5.5 ± 1.2	5.6 ± 1.4	NS	6.0 ± 1.4	6.1 ± 1.6	NS	0.02	NS
α-linolenic	0.2 ± 0.1	0.2 ± 0.1	NS	0.2 ± 0.1	0.2 ± 0.1	NS	NS	NS
Arachidonic	19.7 ± 2.4	19.4 ± 2.6	NS	17.7 ± 3.3	17.7 ± 4.1	NS	0.000	0.002
Eicosapentaenoic	0.9 ± 0.5	1.0 ± 0.7	NS	0.9 ± 0.6	0.8 ± 0.7	NS	NS	0.001
Docosahexaenoic	8.8 ± 1.9	9.2 ± 2.2	NS	9.5 ± 2.3	7.6 ± 3.0	0.002	NS	0.001
Σ Saturates	21.3 ± 2.7	21.3 ± 1.6	NS	23.2 ± 4.0	24.0 ± 5.3	NS	0.001	0.001
Σ Monoenes	16.9 ± 1.3	17.0 ± 1.5	NS	18.5 ± 2.9	19.2 ± 4.5	NS	0.004	0.011
Σ n-6	33.5 ± 3.5	33.4 ± 4.2	NS	31.1 ± 4.6	31.6 ± 6.6	NS	0.000	0.003
Σ n-3	13.6 ± 2.7	14.0 ± 3.4	NS	13.7 ± 3.2	11.7 ± 4.5	0.028	NS	0.001
Σ n-6/Σ n-3 ratio	2.6 ± 0.7	2.6 ± 1.1	NS	2.4 ± 0.7	3.5 ± 3.9	NS	0.047	NS
Unsaturation index[d]	215.2 ± 12.5	217.6 ± 13.0	NS	207.1 ± 24.5	198.7 ± 33.6	NS	0.009	0.000

NS, not significant ($P > 0.05$).
[a] Data are expressed as relative percentage of total fatty acids identified (% wt/wt).
[b] Independent t-test was used to compare the differences between the active and placebo groups at baseline and delivery.
[c] Paired t-test was used to compare the changes between the baseline and delivery within the group.
[d] Unsaturation index is the sum of the % unsaturated fatty acids multiplied by their number of double bonds.

The corresponding values in cord were 7.1% and 3.3%. The question is whether supplementation of Sudanese pregnant women with 300 mg of DHA daily would be sufficient to forestall the decline of the fatty acid in late pregnancy. We suggest that the DHA requirement for healthy pregnant women should be based on the amount needed to prevent this decline.

Improved neonatal DHA status after maternal supplementation with high DHA during uncomplicated pregnancies has been consistently reported. Similarly, we have recently reported maternal supplementation-induced enrichment of cord blood DHA in pregnancy complicated by type 2 diabetes [23]. In this study, the neonates of the DHA supplemented women did not have increased level of the nutrient. The reason for this intriguing finding is not clear since type 2 and GDM share similar metabolic features and both groups of women were given 600 mg of DHA daily. However, impaired placental uptake [13,14] and maternal–fetal transfer [14] of DHA, and considerable placental abnormalities than in type 1 and 2 diabetes [24] have been reported in pregnancy complicated by GDM. It is worth noting that the shorter supplementation duration (median 10 weeks) in the current study compared with our previous investigation of pregnant women with type 2 diabetes (median 26 weeks) [23] could be germane to the outcome.

As in our previous studies [4,5], there was a significant decline in the level of AA in red blood cell phosphatidylcholine in both groups of women between diagnosis and delivery. Although the DHA-enriched supplement contained about 2% of AA (% total fatty acids), there was a similar decrease in plasma phosphatidylcholine AA in the group who received DHA-enriched supplement. The DHA + EPA/AA ratio of the capsule was 26:1 and there is evidence that supplementation-induced increase in tissue level of DHA, EPA or DHA plus EPA is associated with a concomitant decrease in AA [25]. Hence, it appears that AA in both plasma and red blood cell

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Table 4
Neonatal plasma and red blood cell phospholipid fatty acid composition.[a,b]

	Plasma phosphatidylcholine		Red blood cell phosphatidylcholine		Red blood cell phosphatidylethanolamine	
	Active-group	Placebo-group	Active-group	Placebo-group	Active-group	Placebo-group
Palmitic	30.3 ± 2.3	30.3 ± 2.1	36.8 ± 2.5	36.5 ± 2.9	17.8 ± 2.9	17.4 ± 2.6
Stearic	13.4 ± 1.8	13.3 ± 1.5	9.2 ± 1.4	9.6 ± 1.5	6.1 ± 1.7	5.8 ± 1.2
Oleic	12.0 ± 1.9	11.8 ± 1.9	18.2 ± 2.2	17.6 ± 1.9	13.8 ± 3.0	13.4 ± 2.5
Linoleic	10.3 ± 2.3	9.9 ± 2.6	9.3 ± 2.2	8.9 ± 1.4	2.5 ± 0.6	2.4 ± 0.5
α-linolenic	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.04
Arachidonic	16.9 ± 3.0	18.0 ± 3.4	13.4 ± 2.6	14.3 ± 2.6	23.8 ± 3.9	24.4 ± 3.0
Eicosapentaenoic	0.5 ± 0.3	0.5 ± 0.4	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	0.3 ± 0.3
Docosahexaenoic	5.8 ± 1.7	5.5 ± 1.7	3.5 ± 1.2	3.4 ± 1.1	8.4 ± 1.9	8.2 ± 1.6
Σ Saturates	44.5 ± 2.3	44.4 ± 2.3	46.9 ± 2.8	47.0 ± 2.4	25.5 ± 3.7	24.7 ± 2.4
Σ Monoenes	13.0 ± 2.2	12.7 ± 2.0	19.2 ± 2.4	18.5 ± 2.0	14.4 ± 3.1	13.9 ± 2.6
Σ n-6	34.2 ± 3.3	34.8 ± 3.1	28.0 ± 3.9	28.6 ± 3.4	35.9 ± 4.3	37.3 ± 4.0
Σ n-3	6.9 ± 1.9	6.7 ± 2.0	4.2 ± 1.4	4.2 ± 1.3	9.8 ± 2.3	9.7 ± 2.2
Σ n-6/Σ n-3 ratio	5.4 ± 1.6	5.7 ± 2.2	7.5 ± 2.9	7.5 ± 2.7	3.9 ± 1.3	4.1 ± 1.2
Unsaturation index[c]			134.3 ± 16.5	136.5 ± 13.6	213.2 ± 21.6	217.4 ± 16.7

[a] Data are based on 43 neonates from active-group (3 cord samples were lost) and 42 placebo-group and expressed as relative percentage of total fatty acids identified (% wt/wt).
[b] No statistically significant differences were found between the two groups when tested with an independent t-test.
[c] Unsaturation index is the sum of the % unsaturated fatty acids multiplied by their number of double bonds.

phospholipids was displaced, to a certain extent, by DHA and EPA. The significance of reduction in blood AA level in women compromised by GDM is yet to be fully investigated. However, because AA is a ubiquitous constituent of most tissues and plays an important role in biological functions under normal physiological conditions, there may be a need to provide AA in order to help prevent the decline.

Nutritional intervention and epidemiological studies have reported that increased intake of long-chain omega-3 fatty acids during pregnancy reduces preterm births by prolonging gestation period [26–29]. We have observed a similar trend in a small number of pregnant women with type 2 diabetes [23]. Paradoxically, in the current study, the number of preterm births was higher (statistically insignificant) in the active-group. To find possible explanations for the observation, past obstetrics histories of the women who delivered prematurely were examined. In the active-group, 8 of the 12 women who delivered early had obstetric complications (pregnancy-induced hypertension, GDM, miscarriages) in their previous pregnancies. There was only one woman who had a history of GDM in the placebo-group. Hence, it is possible that the higher number of preterm births in the active-group may have been a reflection of the predisposition of these women to a recurrent preterm delivery due to genetic-environmental factors or unidentified underlying medical conditions.

There is indication that the provision of DHA during pre and postnatal period influences adiposity in infants [30,31] and preschool children [32,33]. We have assessed whether a similar effect was apparent in this study. Shoulder, mid-arm and abdomen circumference measurements were used as an indirect indictor of adiposity. There was no difference in the values of the aforementioned parameters between the neonates born to the two groups of mothers. This finding was not surprising because maternal supplementation did not enhance fetal DHA status.

There was a high number of a delivery sample loss resulting from unanticipated births before due date, women moving out of the area without providing a contact address and too-busy labor or delivery midwives to collect samples. This limitation would have been minimized by recruiting additional midwives but it could not be done because of financial constraints. Another limitation was the inability to determine placental fatty acids and the activity and expression of placental fatty acid binding and transport proteins. Lastly, despite our efforts to obtain information about a habitual diet of the women using a 4-day food diary, we were unable to extract valuable data about their nutritional intake. This was because a large number of diaries were returned incomplete.

This randomized, double-blind, placebo-controlled study demonstrates that supplementation of women with GDM is effective in enhancing maternal but not fetal DHA status. The failure of the supplementation to improve the level of fetal DHA is of some concern because the offspring of women with GDM are born with a lower level of the nutrient and the condition is thought to be associated with a risk of neuro-developmental deficits. It is plausible that maternal supplementation with more than 600 mg of DHA may trickle down to the fetus and mitigate the insufficiency. Regardless, we suggest that babies of women with GDM, particularly those not suckling, similar to the babies born prematurely require formula milk fortified with a higher level of DHA.

Statement of authorship

YM and KG conceived the idea and designed the study. OD contributed clinical aspect of the study design. YM prepared the protocols, performed data analysis and its interpretation and drafted the manuscript. KG contributed to the interpretation of findings. JH and IN recruited and followed up the patients, collected biological samples and clinical and demographic information relevant to the study. SE and ASB carried out sample processing and fatty acid analysis. All authors approved the final manuscript.

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Conflict of interest statement

All authors declare that there is no competing financial interest in relation to this work.

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