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Title: Effect of docosahexaenoic acid enriched fish oil supplementation in pregnant women with type 2 diabetes on membrane fatty acids and foetal body composition – Double-blinded randomised placebo-controlled trial

Authors:

Y. Min[1], O. Djahanbakhch[2,3], J. Hutchinson[1,2], A.S. Bhullar[1], M. Raveendran[2], A. Hallot[1], S. Eram[1], I. Namugere[2], S. Nateghian[1], K. Ghebremeskel[1]

Author Affiliations:

[1] Lipidomics and Nutrition Research Centre, Faculty of Life Sciences and Computing, London Metropolitan University, London, UK

[2] Newham University Hospital, Barts Health NHS Trust, London, UK

[3] Academic Department of Women's Health, Queen Mary's School of Medicine, University of London, London, UK

Corresponding author:

Name: Yoeju Min

Address: Lipidomics and Nutrition Research Centre, Faculty of Life Sciences and Computing, London Metropolitan University, 166-220 Holloway Road, London N7 8DB, United Kingdom

Tel: +44 20 7133 2946

Fax: +44 20 7133 4682

Email: y.min@londonmet.ac.uk or minyoeju@gmail.com

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Conflict of interest disclosures:

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

Novelty Statements

- This study tested the effectiveness of DHA-enriched fish oil supplementation on ameliorating red cell membrane fatty acid composition of pregnant women with type 2 diabetes and their neonates.
- A daily dose of 600 mg DHA supplementation from early pregnancy in women with type 2 diabetes resulted in normalising DHA levels in red cell membrane phospholipids of women and their neonates.
- The DHA-enriched fish oil supplementation helped to minimise maternal DHA depletion in pregnancy with type 2 diabetes.

Abstract

Aims

To test if DHA-enriched fish oil supplementation rectifies red cell membrane lipid anomaly in pregnant women with type 2 diabetes and their neonates, and alters foetal body composition.

Methods

Women with type 2 diabetes (n=88; 41 fish oil, 47 placebo) and healthy women (n=85; 45 fish oil, 40 placebo) were supplemented from the first trimester until delivery. Blood fatty acid composition, foetal biometric and neonatal anthropometric measurements were assessed.

Results

A total of 117 women completed the trial. The women with type 2 diabetes who took fish oil compared with those who received placebo had higher DHA% in red cell phosphatidylethanolamine (PE) in the third trimester (12.0% *vs* 8.9%, p=0.000) and at delivery (10.7% *vs* 7.4%, p=0.001). Similarly, the neonates of the fish oil supplemented women with type 2 diabetes had increased DHA in the red cell PE (9.2% *vs* 7.7%, p=0.027) and plasma PC (6.1% *vs* 4.7%, p=0.020). DHA-rich fish oil had no effect on the body composition of the foetus and neonates of the women with type 2 diabetes.

Conclusions

A daily dose of 600 mg of DHA was effective in ameliorating red cell membrane DHA anomaly in pregnant women with type 2 diabetes and neonates, and in preventing the decline of maternal DHA during pregnancy. We suggest that the provision of DHA supplement should be integrated in the antenatal care of pregnant women with type 2 diabetes.

Introduction

Type 2 diabetes was once regarded as a condition for people over the age of 40s. However, it is now increasingly being diagnosed in young adults in parallel with a continuous rise in childhood obesity [1]. This increase in type 2 diabetes in young adults, particularly women of child bearing age is of major concern because diabetes in pregnancy is associated with a greater risk of childhood obesity, insulin resistance and subsequent diabetes in the offspring [2]. According to the latest UK audit on pregnancy outcome of women with pre-gestational diabetes in 2007/2008, type 2 diabetes accounted for 40.3% of total pregnancies [3], a 1.6-fold increase in four years.

One of the metabolic features of type 2 diabetes is altered membrane fatty acid composition [4,5]. This compositional change is shown to modulate glucose uptake in muscle [6] and insulin secretion/action in adipocytes [7]. Previously, we observed an anomaly in red cell membrane phospholipid in pregnant women with type 2 diabetes and their neonates [8]. This anomaly was characterised by a significant reduction in docosahexaenoic acid (DHA). DHA is a long-chain polyunsaturated omega-3 fatty acid with bioactive properties and it is a vital nutrient for optimal foetal neuro-visual development. Moreover, it is thought to be a potent anti-adiposity agent [9].

Studies have demonstrated that DHA supplementation is effective in raising the level of the nutrient in maternal and neonatal red cell membrane in an uncomplicated pregnancy [10,11]. There are no such studies in pregnancies complicated with type 2 diabetes. Hence, we carried out a double-blind, randomised, placebo-controlled trial to investigate if DHA-enriched fish oil supplementation rectifies red cell membrane anomaly in women with type 2 diabetes and their offspring. The primary outcome measures were red cell DHA level of the women at the beginning of the third trimester and at delivery, and of the neonates at delivery. The secondary outcome measures were foetal body composition and neonatal anthropometry.

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 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 analysis was completed and the data recorded.

Subjects and intervention

The women were recruited during their first visit to the antenatal clinic, Newham University Hospital, London between January 2008 and December 2011. The inclusion criteria were women of 17 – 45 years old with singleton pregnancies with either pre-existing type 2 diabetes or without any known medical condition (uncomplicated pregnancy group). Those who were planning to receive tocolytic or corticosteroid therapy were excluded. A diagnostic oral glucose tolerance test for gestational diabetes was performed during the third trimester in the women with an uncomplicated pregnancy.

Once the subjects consented to participate in the study, they were randomly assigned to receive either "DHA-enriched fish oil (henceforth *fish oil*)" or "placebo (high oleic acid sunflower oil)" and instructed to take two capsules per day until delivery. Two capsules of fish oil provided 600 mg of DHA. The fish oil capsule contained 43.7% of DHA and 7.5% eicosapentaenoic acid (EPA) whilst oleic acid comprised 82.6% of the placebo. Both supplements contained vitamin E (d-alpha tocopherol) as an antioxidant and encapsulated in an identical oblong soft gelatin capsule (750 mg in size). Randomisation was carried out using a random code generated by the supplement provider (Equazen/Vifor Pharma Ltd., Glattbrugg, Switzerland).

Sample size and power calculation

The sample size was calculated based on an observation from our previous study [8]. In this study, we found that the DHA level in red cell phosphatidylcholine (PC) during the third trimester was 3.5% in pregnant women with type 2 diabetes and 5.5% in those without diabetes. We wanted to test if supplementation with 600 mg of DHA would increase the level (3.5%) by 50% to 5.3%. The power calculation indicated that a minimum of 24 subjects per group (fish oil versus placebo) at the third trimester will be required to detect the increase with 85% power. We also included the same number of pregnant women without diabetes in order to assess the effectiveness of supplementation. The sample size and power calculation was performed using G*Power 3 [12] and based on a two independent groups, two-tailed t-test with an α of 0.05.

Blood collection and fatty acid analysis

Non-fasting venous blood (5-10 ml) was obtained from the subjects in the first (≤15weeks) and the third (27-32 weeks) trimesters, and at delivery (maternal and cord) in EDTA treated

vacutainer tubes. The samples collected during the day were sent immediately to the Lipidomics and Nutrition Research Centre (LNRC) laboratory for processing. Blood samples collected during the night or weekend 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 cell and plasma phospholipids was analysed with a standardised method developed in our laboratory [8]. Briefly, total lipids were extracted by homogenising the samples in chloroform and methanol, and phospholipids separated from the resulting total lipid 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).

Foetal body composition and neonatal anthropometric measurements

Detailed foetal ultrasound biometric measurements were carried out by a consultant obstetrician (M.R.) between 34 and 38 gestational weeks based on the protocol described by Salomon et al. [13]. Head circumference, femur and humerus length, biparietal and occipito-frontal diameter, mid-arm and mid-thigh lean mass, mid-arm and mid-thigh fat mass, mid-thigh and abdominal circumference, and abdominal fat mass were measured using a Toshiba Aplio with a 3.5 MHz transducer.

The weight and length of the newborn babies were recorded by a midwife who attended the delivery as per routine practice. The head-, shoulder-, mid-arm-, and abdominal circumferences were measured by research midwives (J.H. and I.N.) using Seca 210 portable measuring mat and Seca 201 ergonomic circumference measuring tape (Seca UK, Birmingham).

Statistical analysis

The data are presented as mean ± SD or SE, median (range), and *n* (%) as appropriate and statistical significance was set at $p<0.05$. The effects of the intervention and diabetes on the primary and secondary outcomes were tested by a two-way ANOVA. A pairwise comparison was performed using Tukey's HSD and Fisher's LSD tests for each dependent variable separately when the F-ratio was significant ($p<0.05$). The changes in DHA level within the subject between the different time points were assessed by a paired-sample t-test. Pearson's

chi-squared test was used to compare the difference in birth outcome. All analyses were carried out with IBM SPSS Statistics version 20 (IBM Corporation, USA).

Results

One-hundred and seventy three women, 88 with type 2 diabetes and 85 with uncomplicated pregnancy (henceforth *healthy*) were randomised to either fish oil or placebo (Figure 1). Ten women were treated for pre-existing medical conditions (4 hypothyroidism, 6 hypertension) and two had sickle cell trait. Blood samples were obtained from 55 women with type 2 diabetes (fish oil, n=25; placebo, n=30) and 49 healthy women (fish oil, n=26; placebo, n=23) in the third trimester. Three women from the healthy group who developed gestational diabetes continued to take the allocated supplement and were included in the final analysis. Fifty-eight women with type 2 diabetes (fish oil, n=28; placebo, n=30) and 59 healthy women (fish oil, n=32; placebo, n=27) completed the trial. Two babies from the healthy group (1 fish oil, 1 placebo) were born with congenital malformation (1 with cleft palate, 1 with extra digit on both hands). Two women with type 2 diabetes from the fish oil group delivered stillborn babies.

The characteristics of the participants and pregnancy outcomes are given in Table 1 and 2, respectively. The majority of the participants were of Asian (45.7%) and African/Afro-Caribbean (28.3%) ancestry. Fifty-seven women had type 2 diabetes for less than 5 years and twenty-four more than 6 years. The women with type 2 diabetes had a shorter gestational duration and a higher rate of caesarean section than those that had an uncomplicated pregnancy. The number of preterm births was lower for women with type 2 diabetes who were supplemented with fish oil than those received placebo ($p<0.01$).

The women with type 2 diabetes who took fish oil had higher DHA level in red cell PC and PE, and plasma PC in the third trimester compared with those who received placebo (Table 3). Fish oil had a similar effect on the red cell PE of the healthy women. When the within-subject change was tested, the proportional increment of DHA by the third trimester was the greatest in the red cell PE as it increased by 53% in the women with type 2 diabetes ($p=0.000$) and 54% in the healthy women ($p=0.000$). In the women with type 2 diabetes and healthy women who received placebo, the DHA level remained unchanged in red cell PC whilst it increased in red cell PE ($p=0.000$).

At delivery, the women with type 2 diabetes who took fish oil had significantly higher DHA level in red cell PE and plasma PC compared with those who received placebo. Interestingly, there was no difference in the red cell PC and PE DHA levels between healthy women who took fish oil and placebo. However, the plasma PC of healthy women who took

fish oil had higher DHA than those who were given placebo (p=0.009). No significant reduction of DHA was found either in the red cell or plasma phospholipids within the individuals with type 2 diabetes who received fish oil. In contrast, the red cell PE DHA level was dropped significantly at delivery (p=0.003) in the women with type 2 diabetes supplemented with placebo. The DHA level of healthy women who received fish oil was reduced in the red cell PC (p=0.001) and PE (p=0.006) but remained unchanged in the plasma. Conversely, it did not change over time in healthy women who took placebo. Likewise, the cord red cell PE (p=0.027) and plasma PC (p=0.020) DHA levels of the women with type 2 diabetes supplemented with fish oil were higher compared with those who were given placebo. However, the cord DHA level of healthy women was similar between the two supplement groups.

Foetal biometric, and neonatal anthropometric measurements adjusted for gestational age are presented in Table 4. Within the fish oil supplemented groups, the foetal mid-arm fat mass of the women with type 2 diabetes was greater than those of the healthy women (p<0.05). The mid-arm, mid-thigh, and abdominal fat mass were significantly higher in the foetus of the women with type 2 diabetes compared with those of healthy women when the data were analysed with the use of Fisher's LSD test (p<0.05). However, these differences did not reach the level of statistical significance when adjustment was made for multiple testing.

Discussion

Our results from previous observation studies [8, 14-16] led us to hypothesise that diabetes might compromise the transfer of DHA to the foetus of pregnant women due to hyperglycaemia induced changes in placental fatty acid transport/transfer mechanism. In normal pregnancy, DHA is preferentially transferred from the expectant mother to her foetus [17]; however, this does not seem to be the case in diabetes. Recently, Pagán et al. [18], with the use of stable isotope tracers, demonstrated that DHA is not preferentially transferred in gestational diabetes. Although this study was restricted to gestational diabetes, their findings would be expected to be applicable to type 2 diabetes since both conditions share similar metabolic features. The enrichment of DHA in cord blood (plasma and red cells) as a result of supplementation in the current study suggests that the impairment of transfer of DHA from the expectant mother to the foetus induced by diabetes can be overcome by supplementation with DHA-rich fish oil. The mechanism by which DHA supplementation enhanced the transfer of the fatty acid in the women with diabetes in this study is not clear. However, there is evidence

that pregnancies affected by type 1 and gestational diabetes [19,20] or maternal obesity [21] are associated with reduced mRNA expression of fatty acid binding protein (FATP 1 and FATP4). These fatty acid binding proteins, which have a high affinity for DHA, have been shown to play a pivotal role in transferring fatty acids across the placenta. Indeed, it has been reported that DHA supplementation enhances the expression of the FATP1 and FATP2 in an uncomplicated pregnancy [22].

The loss of maternal red cell membrane DHA toward the end of pregnancy has been considered a physiological response to pregnancy. However, consistent with the findings in healthy pregnant women supplemented with DHA [11,23] and women from high fish and seafood consuming communities [24], in the current study, the red cell membrane DHA did not decline over the course of pregnancy in the women with type 2 diabetes supplemented with DHA-rich fish oil. Thus, the decline of maternal membrane DHA level during pregnancy appears to be a reflection of an insufficiency of maternal DHA rather than physiological response to pregnancy.

Contrary to our previous finding [8], in mid-gestation, the women with type 2 diabetes who were supplemented with placebo did not have lower DHA compared with their healthy counterparts who received placebo. This may be due to vitamin E which was incorporated in the placebo supplement to prevent peroxidation. Indeed, treatment with vitamin E alone and placebo which was used in the current study resulted in the comparable levels of DHA in phospholipids in the human hepatocellular carcinoma cells (unpublished data). Further supporting evidence comes from a study by Ota et al. [25] who reported an improvement in red cell membrane omega-6 and omega-3 fatty acid levels after 500 mg/d of vitamin E supplementation in patients with hepatitis C virus. However, it is worth noting that the dose which the women in our study would have consumed is minute (about 8 mg/d) compared to what Ota et al. used. Nonetheless, the same effect was not seen in the cord blood as the DHA level in the circulating phospholipids remained low in the neonates of women with type 2 diabetes.

Heavier birth weight and disproportionate body fat distribution are often observed in the offspring of women with diabetes and they are seen as a stepping stone towards childhood obesity and early onset of type 2 diabetes. Consistent with this observation, there was an indication of a disproportionate body fat composition in the foetus of the women with type 2 diabetes. It was not possible to know whether this trend persisted until delivery because the methods we used to assess neonatal anthropometric variables were not sensitive enough to detect differences/changes in body fat composition at birth. The main components of fish oil,

DHA and EPA have been shown to reduce fat mass without affecting lean body mass in patients with type 2 diabetes [26]. However, this effect was not observed in the foetuses of the DHA-rich fish oil supplemented women with type 2 diabetes. It is possible that the dose of the DHA and EPA used in the current study was not high enough to have such an effect.

An unexpected result was the smaller number of cases of preterm and late-preterm births in women with type 2 diabetes who were supplemented with fish oil. Increased gestation after fish oil supplementation has been reported in normal pregnancy [27,28]. Furthermore, the findings from two large cohort studies conducted in Denmark [29] and the USA [30] suggest that moderate fish intake might reduce the risk of preterm birth. Since the sample size in the current study is too small to be conclusive, further research will be required to test if DHA supplementation delays premature labour in pregnancy complicated with type 2 diabetes.

This study has provided some interesting information about the effect of DHA-rich fish oil on birth outcomes in pregnancy complicated with type 2 diabetes but it had limitations which future studies may need to take into account. A significant number of samples were lost during follow-up due to missed appointments, unanticipated delivery, delivery at a different hospital and women moving out of the area without providing contact details. The study was carried out in an area of Greater London with a large and mobile immigrant community. Hence, the problems should have been anticipated and addressed perhaps by recruiting more research midwives. Moreover, there was an imbalance in ethnic distribution between the women with and without type 2 diabetes and this may have had an effect on membrane DHA caused by the difference in fatty acid desaturase gene polymorphisms and/or diet. In addition, the study did not investigate whether or not the placental fatty acid transporter proteins were up-regulated by DHA-rich oil.

In summary, the findings of this study demonstrated that a daily dose of 600 mg DHA supplementation from early pregnancy is effective in ameliorating red cell membrane DHA anomalies in pregnant women with type 2 diabetes and neonates. Moreover, it showed that the decline in maternal DHA which occurs in the final stages of gestation and was thought to be a physiological response to pregnancy can be halted by supplementation. We suggest that the provision of DHA supplement should be integrated in the antenatal care of pregnant women with type 2 diabetes in order to optimise foetal development and avert maternal DHA depletion in pregnancy.

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Figure 1.

* Four women who developed gestational diabetes mellitus (GDM) remained in the originally assigned group and continued to receive the supplement.

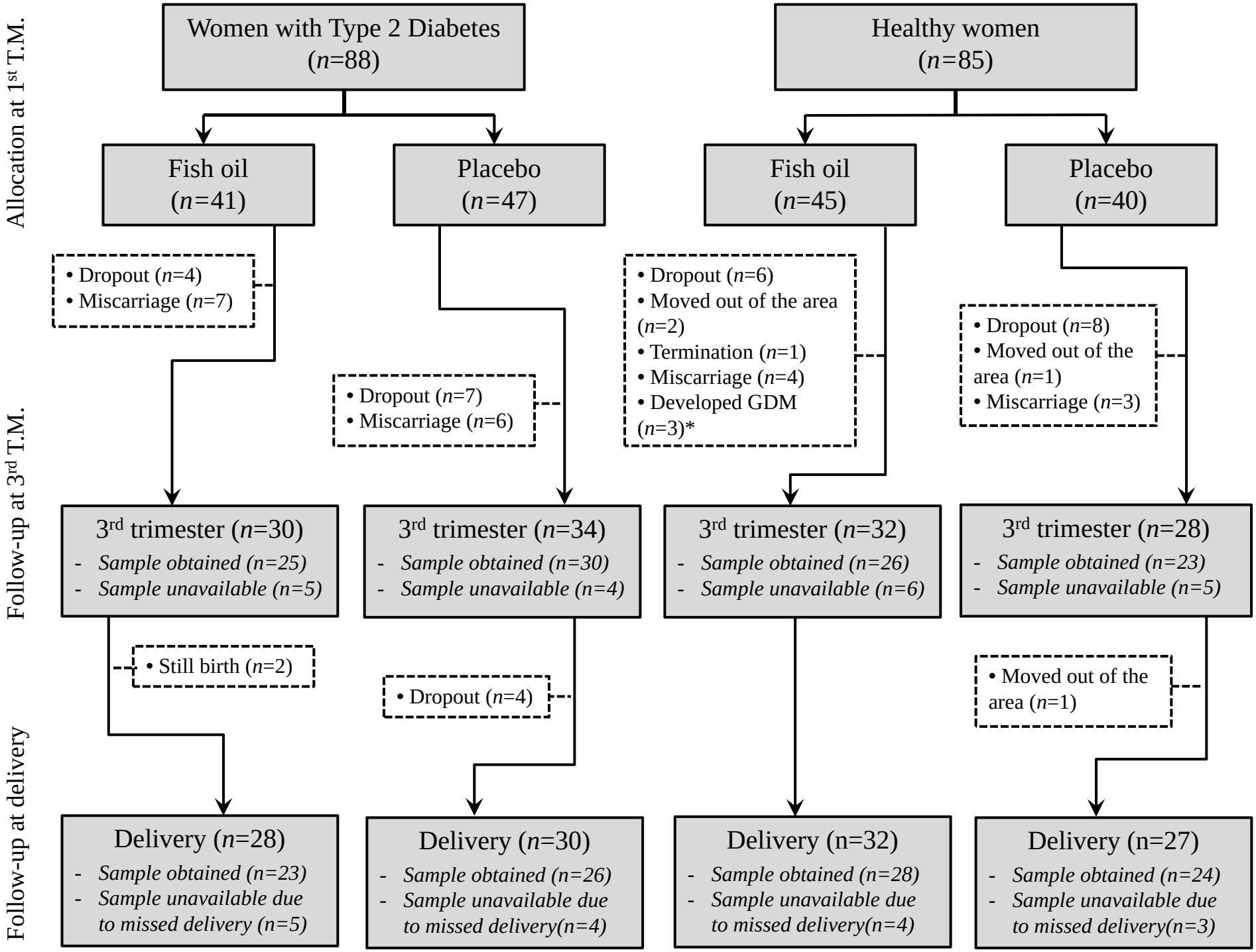
Allocation at 1st T.M.
Follow-up at 3rd T.M.
Follow-up at delivery
Women with Type 2 Diabetes (n=88)
Healthy women (n=85)
Fish oil (n=41)
Placebo (n=47)
Fish oil (n=45)
Placebo (n=40)
• Dropout (n=4)
• Miscarriage (n=7)
• Dropout (n=7)
• Miscarriage (n=6)
• Dropout (n=6)
• Moved out of the area (n=2)
• Termination (n=1)
• Miscarriage (n=4)
• Developed GDM (n=3)*
• Dropout (n=8)
• Moved out of the area (n=1)
• Miscarriage (n=3)
3rd trimester (n=30)
- Sample obtained (n=25)
- Sample unavailable (n=5)
3rd trimester (n=34)
- Sample obtained (n=30)
- Sample unavailable (n=4)
3rd trimester (n=32)
- Sample obtained (n=26)
- Sample unavailable (n=6)
3rd trimester (n=28)
- Sample obtained (n=23)
- Sample unavailable (n=5)
• Still birth (n=2)
• Dropout (n=4)
• Moved out of the area (n=1)
Delivery (n=28)
- Sample obtained (n=23)
- Sample unavailable due to missed delivery (n=5)
Delivery (n=30)
- Sample obtained (n=26)
- Sample unavailable due to missed delivery(n=4)
Delivery (n=32)
- Sample obtained (n=28)
- Sample unavailable due to missed delivery(n=4)
Delivery (n=27)
- Sample obtained (n=24)
- Sample unavailable due to missed delivery(n=3)

Table 1
Demographic and obstetric characteristics of participants[1]

	Women with Type 2 diabetes		Healthy women	
	Fish oil	Placebo	Fish oil	Placebo
Number of participants (*n*)	41	47	45	40
Gestation at recruitment, weeks (range) [a]	9.9 (5.1-15.9)	10.4 (4.3-15.7)	11.0 (6.1-15.4)	12.1 (6.0-15.9)
Age, years (range) [b]	34.0 (20.0-45.0)	37.0 (27.0-45.0)	29.0 (18.0-42.0)	29 (18.0-44.0)
Pre-pregnancy weight (kg)	79.1 ± 17.1	76.0 ± 17.5	68.2 ± 19.4	69.8 ± 13.8
Height (m)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Pre-pregnancy BMI [c]	30.6 ± 5.9	30.4 ± 6.4	25.9 ± 6.6	26.7 ± 4.7
Ethnicity, *n* (%) [d]				
Asian	18 (43.9)	27 (57.5)	16 (35.6)	18 (45.0)
African/Afro-Caribbean	15 (36.6)	10 (21.3)	10 (22.2)	14 (35.0)
Caucasian	5 (12.2)	5 (10.6)	13 (28.9)	6 (15.0)
Others	3 (7.3)	5 (10.6)	6 (13.3) [d]	2 (5.0)
Smoker, *n* (%)	2 (4.9)	0 (0.0)	6 (13.3)	0 (0.0)
Planned pregnancy, *n* (%)	27 (65.9)	19 (40.4)	25 (55.6)	19 (47.5)
Parity, *n* (%) [f]				
0	10 (24.4)	7 (14.9)	18 (40.0)	14 (35.0)
1 - 3	27 (65.9)	32 (68.1)	26 (57.8)	23 (57.5)
> 4	3 (7.3)	6 (12.8)	1 (2.2)	2 (5.0)
Vegetarian, *n* (%)	0 (0.0)	0 (0.0)	1 (2.2)	2 (5.0)
Folic acid supplement, *n* (%) [g]	30 (73.2)	27 (57.4)	27 (60.0)	21 (52.5)
Age when type 2 diabetes was diagnosed (years), *n* (%) [h]				
10 – 20	3 (7.3)	1 (2.1)		
21 – 30	18 (43.9)	16 (34.0)		
31 – 40	18 (43.9)	21 (44.7)		
> 40	1 (2.4)	3 (6.4)		

Diabetes duration, years (range) [i]	3.0 (0-12)	3.0 (0-10)
Years of diabetes duration, *n* (%) [j]		
< 1	2 (4.9)	3 (6.4)
1 - 5	25 (61.0)	27 (57.4)
6 - 9	9 (22.0)	9 (19.1)
≥ 10	4 (9.8)	2 (4.3)
Diabetes treatment before pregnancy, *n* (%) [k]		
Diet only	7 (17.1)	3 (6.4)
Oral hypoglycemic agents	21 (51.2)	25 (53.2)
Oral hypoglycemic agents + insulin	13 (31.7)	17 (36.2)
$HbA1_c$ at recruitment [l]		
%	6.9 ± 0.8	7.0 ± 0.8
mmol/mol	52 ± 9	53 ± 9

[1] No statistical test was performed as per CONSORT guidelines. Data are expressed as mean ± SD, median (range), or number of subjects (%) as appropriate.
[a] Expressed as median (min, max).
[b] Expressed as median (min, max).
[c] BMI, body mass index (kg/m^2)
[d] We used UK Home Office's classification for an individual's ethnicity according to person's self-definition (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).
[e] Included one person whose ethnicity was not declared.
[f] Information was not available from three women with type 2 diabetes (1 fish oil, 2 placebo) and one healthy women (placebo).
[g] Information was not available from eleven women with type 2 diabetes (4 fish oil, 7 placebo) and three healthy women (2 fish oil, 1 placebo)
[h] Information was not available from seven subjects (1 fish oil, 6 placebo).
[i] The year of diagnosis of diabetes was based on subjects' self-report and expressed as median (min, max).
[j] Information was not available from six subjects (1 fish oil, 6 placebo).
[k] Information was not available from two women with type 2 diabetes allocated to placebo.
[k] HbA1c, glycated haemoglobin.

Table 2
Pregnancy outcomes

		Women with Type 2 diabetes		Healthy women	
		Fish oil	Placebo	Fish oil	Placebo
Number of participants retained at delivery (*n*)		28	30	32	27
Gestation at delivery, weeks (range) [a]		37.5 (28.6-40.0)$^{\alpha,\beta}$	37.1 (31.0-40.0)$^{\delta,\gamma}$	39.3 (30.0-42.1)$^{\alpha,\delta}$	39.3 (36.0-42.0) $^{\beta,\gamma}$
Supplementation duration, weeks (range) [a]		26.6 (18.0-34.3)	25.5 (19.4-30.3)	28.4 (17.0-31.6)	26.5 (21.4-31.7)
Male/female, *n*		14 / 14	17 / 13	19 / 13	13 / 14
Preterm birth, *n* (%)[b]		5 (17.9)	12 (40.0)	3 (9.4)	3 (11.1)
Late-preterm birth, *n* (%)[c]		2 (7.1)	8 (26.7)	2 (6.3)	3 (11.1)
Low birth weight, *n* (%)[d]		4 (14.3)	5 (16.7)	4 (12.5)	3 (11.1)
Very low birth weight, *n* (%)[e]		1 (3.6)	1 (3.3)	1 (3.1)	-
Still birth, *n* (%)		2 (7.1)	-	-	-
Macrosomia, *n* (%)		1 (3.6)	-	-	-
Shoulder dystocia, *n* (%)		1 (3.6)	-	-	-
Delivery method, *n* (%)					
Vaginal -	Spontaneous	4 (14.3)	9 (30.0)	20 (62.5)	17 (63.0)
	Assisted	-	1 (3.3)	-	2 (7.4)
	Induced	8 (28.6)	3 (10.0)	3 (9.4)	1 (3.7)
Caesarean section -	Elective	5 (17.9)	10 (33.3)	3 (9.4)	3 (11.1)
	Emergency	11 (39.3)	7 (23.3)	6 (18.8)	4 (14.8)

Tukey's HSD test was used to test the difference in gestational weeks at delivery and the means sharing the same superscript are significantly different at $p<0.01$ ($^{\alpha,\beta}$) and $p<0.0001$ ($^{\delta,\gamma}$).
[a] Expressed as median (min, max).
[b] Preterm birth; born less than 37 weeks of gestation (259 days). Assessed by Kruskal-Wallis test ($p=0.011$).
[c] Late-preterm birth; born between 34 0/7 weeks and 36 6/7 weeks of gestation. Assessed by Kruskal-Wallis test ($p=0.011$).
[d] Low birth weight; birth weight less than 2.5 kg.
[e] Very low birth weight; birth weight less than 1.5 kg.

Table 3
Docosahexaenoic acid level in the red cell phosphatidylcholine and phosphatidylethanolamine, and plasma phosphatidylcholine at baseline, 3rd trimester, and delivery (maternal and cord)

	Women with Type 2 diabetes (T2D)		Healthy women (H)		Significance (*p* value)					
	Fish oil (FO)	Placebo (P)	Fish oil (FO)	Placebo (P)	T2D-FO *vs* T2D-P	T2D-FO *vs* H-FO	T2D-FO *vs* H-P	T2D-P *vs* H-FO	T2D-P *vs* H-P	H-FO *vs* H-P
Red cells										
Phosphatidylcholine										
Baseline	2.82 ± 0.99	2.36 ± 0.64	2.59 ± 0.67	2.87 ± 1.09	ns	ns	ns	ns	0.037	ns
3rd trimester	3.37 ± 0.91	2.53 ± 0.71	3.31 ± 1.40	2.65 ± 1.06	0.006	ns	0.043	0.012	ns	ns[e]
Delivery-Maternal	2.95 ± 1.23	2.16 ± 1.06	2.41 ± 1.11	2.51 ± 1.37	ns[a]	ns	ns	ns	ns	ns
Delivery-Cord	3.68 ± 1.01	3.05 ± 0.97	3.57 ± 0.97	3.62 ± 0.88	ns[a]	ns	ns	ns	ns[d]	ns
Phosphatidylethanolamine										
Baseline	7.87 ± 2.38	7.13 ± 1.84	7.06 ± 1.58	7.41 ± 2.53	ns	ns	ns	ns	ns	ns
3rd trimester	12.0 ± 1.87	8.85 ± 1.82	10.9 ± 2.28	8.98 ± 2.84	0.000	ns	0.000	0.004	ns	0.014
Delivery-Maternal	10.7 ± 3.23	7.41 ± 2.67	8.76 ± 3.10	8.16 ± 2.75	0.001	ns[b]	0.021	ns	ns	ns
Delivery-Cord	9.23 ± 1.83	7.74 ± 1.58	8.99 ± 1.57	8.86 ± 1.99	0.027	ns	ns	ns[c]	ns[d]	ns
Plasma Phosphatidylcholine										
Baseline	4.86 ± 1.93	4.03 ± 1.40	4.57 ± 1.91	5.01 ± 1.96	ns[a]	ns	ns	ns	ns[d]	ns
3rd trimester	5.31 ± 1.35	4.16 ± 1.23	5.00 ± 1.53	3.95 ± 1.44	0.017	ns	0.010	ns[c]	ns	ns[e]
Delivery-Maternal	5.07 ± 0.99	3.72 ± 0.97	4.52 ± 1.53	3.48 ± 1.11	0.001	ns	0.000	0.052	ns	0.009
Delivery-Cord	6.10 ± 1.52	4.68 ± 1.59	6.28 ± 1.87	5.98 ± 1.54	0.020	ns	ns	0.006	0.046	ns

Number of subjects: (a) Baseline: T2D-FO (*n*=41), T2D-P (*n*=47), H-FO (*n*=45), H-P (*n*=40); (2) 3rd trimester: T2D-FO (*n*=25), T2D-P (*n*=30), H-FO (*n*=26), H-P (*n*=23); (3) Delivery (maternal and cord): T2D-FO (*n*=23), T2D-P (*n*=26), H-FO (*n*=28), H-P (*n*=24)

Data are expressed as mean (% wt/wt) ± SD.
A pairwise comparison was performed using Tukey's HSD test for dependent variable (DHA) when the F-ratio was significant ($p<0.05$).
[a,b, c, d, e] The differences between the groups were significant at $p<0.05$ when Fisher's LSD test was used.
ns, not significant.

Table 4

Foetal ultrasound biometric and neonatal anthropometric measurements

	Women with Type 2 diabetes		Healthy women	
	Fish oil	Placebo	Fish oil	Placebo
Biometric measurement[1]				
n	25	23	27	29
Gestation (weeks)	34.7 (34.0-36.3)	34.7 (32.9-36.6)	34.9 (34.0-38.7)	34.9 (34.0-37.9)
Head circumference (mm)	311.7 ± 9.9 (307.6 to 315.8)	312.4 ± 12.4 (306.9 to 317.9)	311.8 ± 15.6 (305.6 to 318.0)	310.9 ± 13.1 (305.9 to 315.9)
Femur length (mm)	67.1 ± 2.9 (65.9 to 66.5)	67.0 ± 4.9 (64.9 to 69.2)	66.4 ± 3.3 (65.2 to 67.7)	66.5 ± 3.2 (65.3 to 67.8)
Humerus length (mm)	58.3 ± 3.9 (56.7 to 59.9)	58.4 ±3.8 (56.7 to 60.0)	57.5 ± 2.8 (56.4 to 58.6)	58.3 ± 2.9 (57.2 to 59.4)
Biparietal diameter (mm)	88.4 ± 3.1 (87.1 to 89.6)	87.5 ± 3.9 (85.7 to 89.2)	87.5 ± 5.1 (85.6 to 89.5)	87.0 ± 3.7 (85.6 to 88.4)
Occipito-frontal diameter (mm)	109.0 ± 4.0 (107.4 to 110.7)	110.6 ± 5.8 (108.0 to 113.2)	110.1 ± 5.7 (107.9 to 112.3)	110.1 ± 5.8 (107.8 to 112.3)
Mid-arm lean mass (cm^2)	6.5 ± 1.6 (5.8 to 7.2)	5.8 ± 1.6 (5.1 to 6.5)	6.1 ± 1.6 (5.4 to 6.7)	6.5 ± 1.8 (5.8 to 7.1)
Mid-arm fat mass (cm^2)	5.3 ± 1.5 (4.6 to 5.9) [a]	5.2 ± 1.2 (4.7 to 5.7)	4.4 ± 0.7 (4.1 to 4.7) [a]	4.9 ± 1.3 (4.4 to 5.4)
Mid-thigh lean mass (cm^2)	8.7 ± 2.2 (7.8 to 9.6)	8.8 ± 2.1 (7.9 to 9.7)	9.0 ± 2.0 (8.2 to 9.8)	8.9 ± 3.1 (7.7 to 10.1)
Mid-thigh fat mass (cm^2)	5.6 ± 2.6 (4.5 to 6.6)	5.6 ± 1.4 (5.0 to 6.2)	4.4 ± 1.1 (4.0 to 4.8)	5.0 ± 1.4 (4.5 to 5.6)
Mid-thigh circumference (mm)	113.8 ± 20.4 (105.3 to 122.2)	123.8 ± 18.9 (115.7 to 132.0)	126.9 ± 22.7 (117.9 to 135.8)	128.5 ± 23.0 (119.7 to 137.2)
Abdominal circumference (mm)	301.7 ± 52.0 (279.7 to 323.6)	313.2 ± 23.6 (302.8 to 323.7)	300.4 ± 24.3 (290.8 to 310.0)	299.8 ± 25.3 (290.2 to 309.5)
Abdominal fat mass (mm)	5.8 ± 1.6 (5.1 to 6.4)	5.8 ± 1.6 (5.0 to 6.5)	4.9 ± 1.2 (4.4 to 5.3)	4.8 ± 1.4 (4.3 to 5.4)
Anthropometric measurements[2]				
n	28	30	32	27
Gestation (weeks)	37.5 (28.6-40.0)[b,c]	37.1 (31.0-40.0)[d,e]	39.3 (30.0-42.1)[b,d]	39.3 (36.0-42.0)[c,e]
Weight (kg)	3.0 ± 0.1 (2.8 to 3.2)	2.9 ± 0.1 (2.7 to 3.2)	3.2 ± 0.1 (3.0 to 3.4)	3.2 ± 0.1 (3.0 to 3.4)
Length (cm)	48.8 ± 0.8 (47.2 to 50.3)	48.6 ± 0.8 (47.0 to 50.3)	49.8 ± 0.7(48.2 to 51.3)	50.9 ± 0.8 (49.2 to 52.5)
Head circumference (cm)	33.7 ± 0.4 (32.9 to 34.5)	33.0 ± 0.4 (32.1 to 33.8)	33.6 ± 0.4 (32.8 to 34.4)	33.5 ± 0.4 (32.6 to 34.3)

Shoulder circumference (cm)	37.3 ± 1.0 (35.3 to 39.4)	34.3 ± 1.0 (32.3 to 36.4)	37.2 ± 1.0 (35.2 to 39.2)	36.0 ± 1.0 (33.9 to 38.1)
Mid-arm circumference (cm)	12.2 ± 0.4 (11.4 to 13.1)	11.0 ± 0.4 (10.2 to 11.9)	10.8 ± 0.4 (10.0 to 11.6)	11.1 ± 0.4 (10.3 to 12.0)
Abdominal circumference (cm)	32.2 ± 0.7 (30.7 to 33.6)	32.1 ± 0.7 (30.7 to 33.6)	32.4 ± 0.7 (31.1 to 33.8)	32.4 ± 0.7 (30.9 to 33.8)

The gestational ages are based on an ultrasound estimate.
[1] Gestation weeks are given as median (min, max) and the biometric measurements are expressed as mean ± SD (95% CI).
[2] Gestation weeks are given as median (min, max) and anthropometric measurements are adjusted for the gestational age and expressed as mean ± SE (95% CI).
A pairwise comparison was performed using Tukey's HSD test for each dependent variable separately when the F-ratio was significant ($p<0.05$).
Means of groups sharing the same superscript letter are significantly different at $p<0.05$ (^{a}), $p<0.01$ ($^{b, c}$) and $p<0.0001$ ($^{d, e}$).