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Multiple micronutrients and docosahexaenoic acid (DHA) supplementation during pregnancy: a randomized controlled study

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1. INTRODUCTION

During pregnancy, an adequate maternal dietary intake is essential to meet the increased nutritional demands required to maintain metabolism and support fetal development. Micronutrients, such as folic acid and B vitamins, vitamin C and vitamin D, calcium, copper, magnesium, iodine, selenium, zinc, iron all have vital roles throughout all stages of pregnancy (Cetin et al., 2019)

Nutritional requirement increases during pregnancy in order to maintain maternal metabolism and to support fetal growth and development (Mousa et al., 2019). Poor dietary intakes or deficiencies in key micronutrients and macronutrients can have an adverse effect on pregnancy outcomes and neonatal health (Berti et al., 2016): including an increased risk of neural tube defects, preeclampsia, miscarriage, and low birth weight (Ramakrishnan et al., 2012). Even though a healthy and diverse diet remains the preferred way of meeting nutritional needs, some requirements of pregnancy are challenging to meet with diet alone. Many women are at risk of insufficient nutrient intake, in industrialized as well as developing countries (Blumfield et al., 2013; Parisi et al., 2014).

As such, micronutrient supplementation is frequently recommended during pregnancy to help improve pregnancy outcomes in the mother and child (Gernand et al., 2016; Schaefer ,2016).

International guidelines recommend supplementation of the micronutrient folic acid in the periconceptional period, as this is critical for prevention of fetal neural tube defects (NTDs) (Bale, 2003; de Benoist, 2008; Berry et al., 1999). Furthermore, iron supplementation is indicated in presence of maternal deficiency and anemia (World Health Organization, 2016; Peña-Rosas et al., 2015). Recently, there have been extensive scientific and medical discussions around the need to include vitamin D as a standard nutrient to be supplemented during pregnancy, due to low intake. Vitamin D regulates calcium and phosphate body stores and is therefore critical for bone health (US Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). Furthermore, low concentrations of blood vitamin D in pregnant women have been associated with pregnancy complications (Mulligan et al., 2010; Dovnik et al., 2018).

In addition to micronutrients, a proper balance in intake of macronutrients, such as energy, protein, carbohydrates and fatty acids, is recommended. Benefits of supplementation with long-chain n-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), during pregnancy have attracted substantial interest in recent years (Mousa et al., 2019). Indeed, over the course of gestation, maternal concentrations of essential fatty acids decreases (Al et al., 1995) and therefore, their dietary intake is important to meet maternal and fetal requirements. Dietary sources of PUFAs include oil-rich fish as well as fish oil supplements (mainly omega-3) (Mousa et al., 2019). Epidemiological and interventional studies have suggested that consumption of a diet high in fish oil-derived n-3 PUFAs may be beneficial in reducing the incidence of common pregnancy complications such as preterm birth, preeclampsia and intrauterine growth restriction (Larqué et al., 2012; Rogers et al., 2013). Maternal n-3 PUFAs supplementation has also been shown in some studies to be beneficial to the fetus/neonate by improving neurodevelopment and behavioral outcomes (Campoy et al., 2011; Middleton et al., 2019; Rogers et al., 2013) and reducing the risk of developing allergic diseases in childhood (Dunstan and Prescott, 2005; Klemens et al., 2011; Palmer et al., 2012). However, overall evidence about the beneficial effects of PUFAs remains contrasting and no specific consensus recommendation concerning their use in pregnancy is available yet (Makrides et al., 2006; Middleton et al., 2019; Saccone and Berghella, 2015; Saccone et al., 2015). Additional studies evaluating the use of PUFAs during pregnancy are currently ongoing (Mousa et al., 2019).

The use of multiple micronutrients/macronutrients supplementation may be more effective than the use of single micronutrient supplements in preventing non-predictable inadequacies or deficiencies and in reducing oxidative stress (Parisi et al., 2014). Indeed, retrospective studies indicate that women following a diet rich in antioxidants and fish have a reduced risk for intrauterine growth restrictions, preeclampsia and preterm deliveries (Englund-Ögge et al., 2014). Moreover, a Cochrane review suggests that women taking multiple-micronutrient supplements have better pregnancy outcomes than women taking folate or iron alone (Keats et al., 2019). Nevertheless, a consensus is yet to be reached concerning the replacement of iron and folic acid supplementation with multiple-micronutrients supplementation (Keats et al., 2019), such as multiple micronutrients and DHA.

Therefore, given the great interest in the potential beneficial effects of food supplementation with micronutrients and DHA during pregnancy and since the accumulation of DHA in human brain takes place mainly during the fetal period (Lauritzen et al., 2016), we wanted to assess the effects of supplementation with a micronutrient plus DHA preparation during the second and third trimesters of gestation on biomarkers of mothers during pregnancy. In literature indeed there are few data about the relationship between nutritional intake and biochemical nutrient status which are essential for determining potential nutrient deficiencies or excesses. The primary variable of the study investigated the DHA concentration of erythrocytes, as the marker is considered indicative for long-term dietary intake and therefore long chain polyunsaturated fatty acid (LCPUFA) status. Moreover, we evaluated maternal nutritional intake and status of selected micronutrients and aimed to correlate all these variables to maternal and infant's health outcomes of interest in the supplemented and non-supplemented groups. In a selected subgroup of women, we planned to assess additional parameters in cord blood and placental tissue in relation to micronutrient supplementation, in order to gain more knowledge about whether multi-micronutrient supplements and DHA taken during second and third trimesters of pregnancy, have a beneficial effect on metabolomic patterns of cord blood and placental tissue.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this study was to examine the effects of micronutrient (multi-vitamin/mineral) & DHA supplements during the 2nd and 3rd trimester of pregnancy on maternal DHA status in blood.

2.2 SECONDARY OBJECTIVES

The secondary objectives of this study were:

- To examine the effects of micronutrient (multi-vitamin/mineral) & DHA supplements during the 2nd and 3rd trimester of pregnancy on maternal FA parameters in blood.

- To examine the effects of micronutrient (multi-vitamin/mineral) supplements & DHA on the anthropometric parameters of infants at delivery.

- To assess the safety and tolerability of micronutrient (multi-vitamin/mineral) & DHA supplements in the form of Adverse Events (AE) and clinical parameters.

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives of this study were:

- To examine the effects of micronutrient (multi-vitamin/mineral) & DHA supplements during the 2nd and 3rd trimester of pregnancy on maternal micronutrient status via maternal blood, cord blood and placental tissue biomarkers in a subset of 10 women per study group undergoing elective caesarean section.

- To evaluate the maternal intake habits of energy, macro- and micro-nutrients during the 2nd and 3rd trimester of pregnancy.

3 INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN DESCRIPTION

This was a multicenter, randomized, controlled study to investigate the effects of once daily micronutrient plus DHA supplementation (MMS soft gel capsules) on maternal biomarkers and on the anthropometric parameters of infants in a study group of pregnant women starting supplementation with MMS soft gel capsules (Elevit supplementation) from gestational week 13-15 until delivery versus a control group of pregnant women not supplemented with MMS soft gel capsules.
A subset of subjects (approximately 10 subjects per study group) undergoing elective Caesarean section (for reasons independent from the study) were also studied at Caesarean section.
Five visits plus a follow-up visit were conducted during the study.

At the first visit (Visit 1; Screening; GA week 11-14), pregnant women were screened for eligibility to the study and baseline blood collection for efficacy and safety analyses were performed. Eligible pregnant women were selected prior to the start of the supplementation period. For this purpose, pregnant women returned to the site for Visit 2 (Baseline; GA week 13-15).

At Visit 2 (Baseline), eligible pregnant women were equally randomized in one of the two study groups (supplemented study group or non-supplemented study group). In order to have 70 evaluable subjects per arm, it was planned to have approximately 82 pregnant women entering into each of the two study groups. At Baseline, inclusion and exclusion criteria were confirmed. The Elevit soft gel capsules were assigned to the pregnant women belonging to the supplementation study group and they were instructed to take the supplement once daily until delivery. Blood was collected for safety parameters and baseline nutritional status was assessed through the submission of a questionnaire. All pregnant women were instructed on when they should return for the next visit.

At Visit 3 (GA week 24-26) and Visit 4 (GA week 34-36) pregnant women belonging to both study groups returned to the site for blood sampling to analyze efficacy and safety parameters. The DHA level measured at Visit 4 was compared to the value measured at baseline (Visit 1) to assess the primary endpoint.

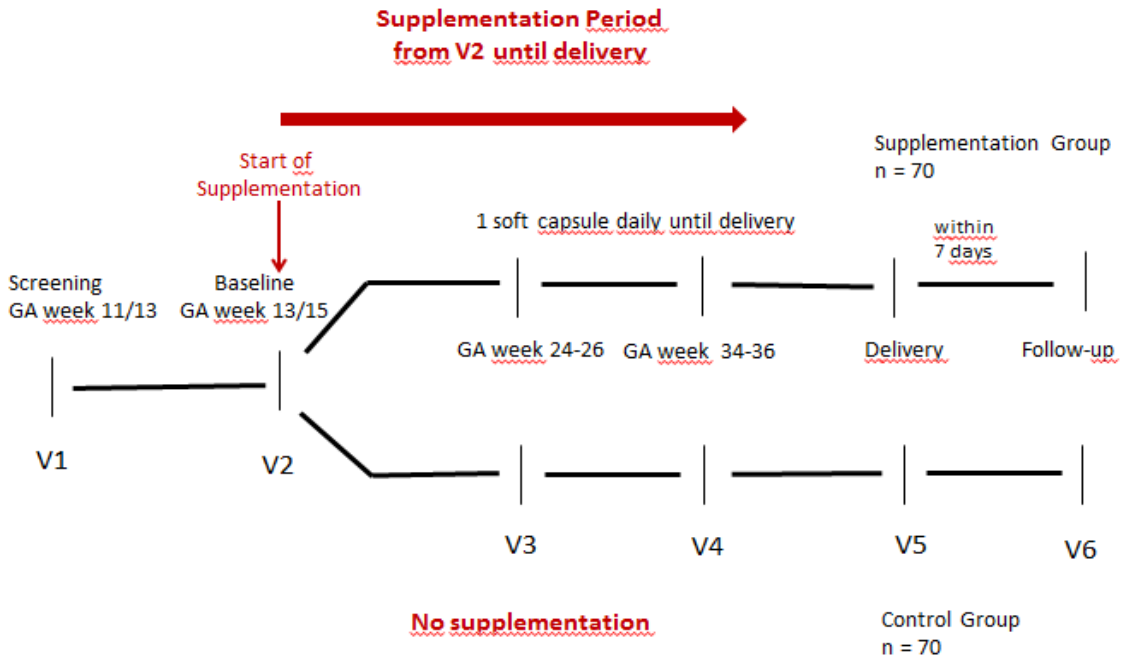
At Visit 5 (Delivery) obstetric evaluations (e.g. gestational length, type of delivery) were performed in all women. Infants' anthropometric parameters evaluation was measured. In the subset of women (approximately ten (10) planned women per study group) undergoing Caesarean section, blood sampling for efficacy parameters and evaluations of cord blood and placenta tissue biomarkers were planned to be performed.

At each visit concomitant medications and adverse events were assessed.

Food consumption, diet and physical activity habits were reviewed by using a semi-quantitative Food Frequency Questionnaire (FFQ) at Visits 2, 3 and 4.
At Visit 6 (Follow-up; within 7 days after delivery) a follow-up evaluation was conducted. Adverse events were recorded.

An overview of the study design is presented in the Figure 3-1.

Figure 3-1: Study design



3.2 SELECTION OF STUDY POPULATION

Healthy pregnant Caucasian women aged 18 to 42 years (inclusive) in their 1st -2nd trimester (GA week 11-14 at enrollment) were enrolled.

3.2.1 Inclusion criteria

1. Healthy pregnant Caucasian women aged 18 to 42 years (inclusive) in their 1st - 2nd trimester (gestational age (GA) week 11-14 at screening);
2. Hemoglobin > 105g/L;
3. Inconspicuous fetal anomaly screening;
4. Normal ultrasound examination (Ultra Sonography (USG));
5. Singleton pregnancy;
6. Taking at least 400 mcg folate per day;
7. Seronegative for Human Immunodeficiency Virus (HIV), Hepatitis B and Hepatitis C at screening;
8. Pregnant women who, in the opinion of the Investigator, are willing and able to participate in all scheduled visits, to adhere to the supplementation plan, to laboratory tests and to all other study related procedures according to the clinical protocol;

9. Pregnant women providing a personally signed and dated given informed consent to participate in the study and to adhere to all study procedures indicating that they have been informed of all pertinent aspects of the trial and that they understood and accepted these, prior to admission to the study.

3.2.2 Exclusion criteria

1. Physical (including vital signs e.g. blood pressure and pulse rate), hematological and clinical-chemical parameters deviating from normal and with clinical relevance;
2. Any infection (acute or chronic) at screening and baseline;
3. Any current metabolic diseases (e.g. diabetes, hypothyroidism);
4. Less than 12 months from previous delivery;
5. Any history or current diseases, which are associated with malabsorption, or other severe diseases of the gastrointestinal tract (e.g. chronic inflammatory bowel disease, iron accumulation, iron utilization disorders);
6. Any history or current neurological, cardiac, endocrine or bleeding disorders;
7. Specific diets (e.g. vegan vegetarian, celiac, lactose free);
8. Body mass index (BMI) < 18 or >30 kg/m2;
9. Pregnant women already taking DHA/multivitamin supplements (except folate or iron);
10. Diagnosed or suspected malignant or premalignant disease;
11. Current clinically significant depression;
12. Current intake of pharmaceuticals or dietary supplements which may interact with any of the ingredients of the trial treatment (i.e. fluoroquinolones, bisphosphonates, levodopa, levothyroxine, penicillamine, antibiotics containing tetracycline or trietine);
13. History of or current diseases where vitamin, mineral, trace element or DHA supplementation might be not recommended /contraindicated [such as sickle cell anemia, copper metabolism disorders (Wilson's disease), renal disease, nephrolithiasis, urolithiasis, hypercalcemia, hypercalciuria, hepatobiliary diseases, existing hypervitaminosis, iron metabolism disorders, hypermagnesemia];
14. Severe Hyperemesis gravidarum;
15. Previous adverse birth outcomes (e.g. small for gestational age, low birth weight, premature birth, still- birth, more than two consecutive spontaneous abortions);
16. Previous adverse pregnancy outcomes (e.g. gestational diabetes);
17. Diagnosed congenital abnormalities in current or previous pregnancy;
18. Known carrier or affected with a genetic disease or condition (e.g. mutation carrier for autosomal recessive diseases);
19. History of or current abuse of drugs, alcohol or other substances;
20. Current smokers and women who smoked during current pregnancy;
21. Any history of hypersensitivity or known allergy to any of the ingredients of the study supplement.

3.2.3 Removal of subjects from therapy or assessment

Withdrawal criteria

Subjects had to be withdrawn from the study if any of the following occurred:

- At their own request or at the request of their legally acceptable representative, at any time during the study and without giving reasons, without suffering any disadvantage as a result.
- If the subject developed preeclampsia, eclampsia, gestational diabetes, oligohydramnios, polyhydramnios, premature rupture of membranes, placental insufficiency.
- If, in the investigator's opinion, continuation of the study would be harmful to the subject.
- At the specific request of the sponsor and in liaison with the investigator.

Depending on the time of withdrawal, a withdrawn subject was referred to as either a “screening failure” or a “drop-out” as specified below.

Screening failure

Screening failures were defined as pregnant subjects who consent to participate in the clinical trial but were not subsequently randomized, for any reason (e.g. failure to satisfy the selection criteria).

Participation of an initial “screening failure” subject at a later time, even if she met all selection criteria upon re-screening, was not acceptable.

Drop-out

A subject who discontinued study participation prematurely for any reason was defined as a “drop-out” if the subject was already randomized.

General procedures

In all cases, the reason for withdrawal was recorded in the subject's medical records. The subject’s data that have been collected until the time of withdrawal were retained and statistically analyzed in accordance with the statistical analysis plan.

Withdrawn subjects were not replaced.

3.2.4 Subject identification

The subject identifier (Subject ID) was a 9-digit number consisting of:

- Digits 1 to 2 Country code
- Digits 3 to 5 Center number within the country
- (Digits 1 to 5 Trial unit Identifier)
- Digits 6 to 9 Current subject number within the center

3.3 TREATMENT

3.3.1 Supplement administered

The study food supplement (Elevit supplementation), MMS soft gel capsules, consisted of 12 vitamins, 6 minerals and LCPUFA DHA. The product was formulated to meet the requirements of women during pregnancy (especially during the second and third trimester).
The MMS product composition together with upper tolerable level and recommended dietary allowance is shown in Table 3.1.
The study treatment was administered only to the supplementation group of pregnant women; the investigational product (IP) was taken orally once daily with liquids from randomization (at Visit 2) until delivery (Visit 5).
The control group of pregnant women did not receive any treatment, neither MMS soft gel capsules nor a matching placebo.

3.3.2 Identity of study supplement

The identity of study supplement is outlined below (Table 3-2).
The study product was labeled according to the requirements of local law and legislation. Label text was approved according to the sponsor's procedures, and a copy of the labels was made available to the study sites upon request.

Table 3-1 Elevit product composition and upper tolerable levels and recommended dietary allowances

Nutrient	Units	Elevit soft gel capsule	EU RDA	EFSA UL	IOM RDA*	IOM UL*
Vitamins						
Vitamin A	IU	2566	2666	10000	2567 (2500)#	10000 (9333)#
Vitamin C	mg	85	80	ND	85 (80)#	2000 (1800)#
Vitamin D	IU	200	200	2000	600	4000
Vitamin E	IU	15	12	201	22.35	1490 (1192)#
Vitamin B1	mg	1.4	1.1	ND	1.4	ND
Vitamin B2	mg	1.4	1.1	ND	1.4	ND
Vitamin B3 Niacin	mg	18	16	900	18	35 (30)#
Vitamin B5	mg	6	6	ND	6	ND
Vitamin B6	mg	1.9	1.4	25	1.9	100 (80)#
Folic Acid	µg	200	200	1000	600	1000 (800)#
Folic Acid (as L-5-MTHF)	µg	226	200	1000	600	1000 (800)#
Vitamin B12	µg	2.6	2.5	ND	2.6	ND
Biotin	µg	30	50	ND	30	ND
Minerals and trace elements						
Iodine	µg	150	150	600	220	1100 (900)#
Magnesium	mg	57	375	250	350-360	350
Zinc	mg	10	10	25	11	40 (34)#
Selenium	µg	60	55	300	60	400
Copper	mg	1.0	1.0	5	1.0	10 (8)#
Iron	mg	14	14	50	27	45
Others						
DHA	mg	200	≥ 200*	1000*	ND	ND
EPA	mg	80	DHA+EPA: 350-450*	EPA: 1800 DHA+EPA: 5000	ND	ND

RDA: Recommended dietary allowance; UL: Upper tolerable level; EU RDA: European RDA as set by EU commission Directive 2008/100/EC; EFSA UL: EFSA (European Food Safety Authority), Tolerable upper intake levels for vitamins and minerals; IOM RDA/UL: Institute of Medicine, Dietary reference intakes; ND: Not determined (insufficient scientific data); *: recommendation for pregnant women; ()#: Recommendation for pregnant women below the age of 19y, if different from general recommendation during pregnancy.

Table 3-2 Trial treatment

Treatment	Elevit soft gel capsules
BAY number	BAY987765
Dose	One capsule daily
Pharmaceutical Form	Soft gel capsules
Strength	13 vitamins, 6 minerals, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)
Formulation	Multi-micronutrient & DHA
Route of Administration	Oral
Batch Number	Available in the study file
Manufacturer	Berlimed

3.3.3 Treatment assignment

At Visit 2, pregnant women fulfilling eligibility criteria, were randomized into either the supplementation group (Elevit supplementation once daily) or the control group (no supplementation) in a 1:1 ratio. Randomization was stratified by the status of planned elective Caesarean section (yes/no) in order to balance treatment assignment between the two strata. Subsequent changing to the elective Caesarean section group by keeping the originally assigned treatment was allowed at the investigator's discretion to maximize the number of subjects belonging to the subgroup of elective Caesarean section. A randomization number was assigned to each woman.
A subject pack containing three subject kits of the IP, was provided to the women assigned to the supplementation group according to their randomization number.

Supplement compliance

Subjects returned any unused study product to the study site at delivery. Any discrepancies between actual and expected amount of returned study product was discussed with the subject at the time of the visit, and any explanation documented in the source records.

Permitted / prohibited prior and concomitant therapy

Any additional supplementation other than MMS soft gel capsules (except for folate and/or iron) in the supplementation group was to be avoided during the study. Subjects in the control group were to abstain from any DHA/multi-vitamin/nutritional supplementation (except for folate and/or iron) during the study.
Any intake of dietary supplements and/or pharmaceuticals and/or herbal products except for folate and/or iron which could have interfered or interacted with any of the ingredients of the trial treatment e.g. fluoroquinolones, bisphosphonates, levodopa, levothyroxine, penicillamine, antibiotics containing tetracycline or trientine were to be avoided during the study.

3.4 PROCEDURES AND VARIABLES

3.4.1 Schedule of procedures

A detailed tabular schedule of procedures is provided in Table 3-3, which lists all the procedures and assessments and when they were performed.

Screening Phase (from GA week 11/14 to GA week 13/15)

Visit 1 (Screening)

At the Screening Visit (Visit 1), the Principal Investigator or designee discussed with each potentially eligible pregnant woman the nature of the trial, its requirements and its restrictions.

Subjects being invited for Screening (Visit 1) were asked to fast for at least 10 hours (overnight) prior to the scheduled Visit and assessments done at Visit 1.

A list of assessments performed during Visit 1 can be found in Table 3-3.

Supplementation Phase (from GA week 13/15 until delivery)

Visit 2 (Baseline)

After the screening period, pregnant women visited the site for Visit 2 (Baseline) (after an overnight fast).

Upon verifying inclusion/exclusion criteria for subject eligibility, based on the information collected at Visit 1 and the clinical and safety laboratory results, randomization in one of the two groups according to the randomization list occurred.

The food supplementation was dispensed to women in the supplementation group and they were instructed on how to take the supplement.

The assessments listed in Table 3-3 were performed.

Visit 3 (GA week 24-26)

Pregnant women were asked to come back to the site for Visit 3 (after an overnight fast) and the assessments listed in Table 3-3 were performed.

Visit 4 (GA week 34-36)

Pregnant women were asked to come back to the site for Visit 4 (after an overnight fast) and the assessments listed in Table 3-3 were performed.

Visit 5 (Delivery)

The list of assessments performed on women and infants at Visit 5 is available in Table 3-3.

Further examinations were performed in the subset of pregnant women undergoing elective Caesarean section.

Early Discontinuation Visit

The assessments performed in women who discontinued the study prior to Visit 4 are listed in Table 3-4.

Follow-up Phase (Visit 6 within 7 days after Delivery)

The Follow-up Visit was performed within 7 days after delivery, for follow-up of any unresolved adverse event and documentation of new adverse events.

The list of assessments performed is available in Table 3-3.

Infants' bone density was assessed within 10 days after Visit 5.

Table 1-3 Schedule of procedures

	Visit 1 Screening	**Visit 2 Baseline**	**Visit 3**	**Visit 4 (or Early Discontinuation Visit)**	**Visit 5 Delivery**	**Visit 6 Follow-up**
Day / Week	**GA week 11/14**	**GA week 13/15**	**GA week 24/26**	**GA week 34/36 (or early discontinuation)**		**Within 7 days after Delivery**
Subject informed consent	X					
Inclusion/exclusion criteria	X	X				
Medical/surgical history	X					
Demographics	X					
History of previous birth outcomes	X					
History of folate and iron/multi-micronutrient intake	X					
History and review of drug, alcohol and nicotine use	X	X	X	X	X	
Assessment of pregnancy	X					
Assessment if elective Cesarean section is planned or recommended	X					
Medication history over past 30 days	X					
Physical examination including gynecological examination	X	X	X	X	X	X
Vital signs (sitting blood pressure, pulse rate)	X	X	X	X	X	X

	Visit 1 Screening	Visit 2 Baseline	Visit 3	Visit 4 (or Early Discontinuation Visit)	Visit 5 Delivery	Visit 6 Follow-up
Day / Week	**GA week 11/14**	**GA week 13/15**	**GA week 24/26**	**GA week 34/36 (or early discontinuation)**		**Within 7 days after Delivery**
Height, weight and body mass index (BMI)	X			X		
Type of delivery					X	
Gestational length					X	
Induced labor (need for urgent delivery)					X	
Delivery complications					X	
Ferritin	X			X		
C-reactive protein (CRP)	X	X	X	X		
Blood sampling for RBC fatty acid parameters (TFA, DHA wt% TFA, EPA wt% TFA, DHA/TFA ratio, Omega 3 index)	X		X	X	X[f]	
25-hydroxyvitamin D	X		X	X	X[f]	
Oxidative status: GSH/GSSG ratio, plasma lipid hydroperoxides (ROMs), 8-isoprostane	X		X	X	X[f]	
Randomization of supplementation kit to subject		X				
Check for supplementation compliance			X	X	X	
Supplementation	X[b]					
Dispensing of study treatment		X	X	X		
Return of study treatment			X	X	X	
Review of concomitant medications		X	X	X	X	
Food consumption assessment by using semi-quantitative Food Frequency Questionnaire (FFQ)		X	X	X		
Infant assessments [c]					X	

	Visit 1 Screening	Visit 2 Baseline	Visit 3	Visit 4 (or Early Discontinuation Visit)	Visit 5 Delivery	Visit 6 Follow-up
Day / Week	**GA week 11/14**	**GA week 13/15**	**GA week 24/26**	**GA week 34/36 (or early discontinuation)**		**Within 7 days after Delivery**
Cord blood evaluations [d, f]					X	
Placental evaluations [e, f]					X	
Assessment of Adverse Events (AEs)		X	X	X	X	X
Safety Laboratory	X[a]	X[g]	X[g]	X		
Subject ID assignment	X					
Discontinuation of subject's participation				(X)		

[a] At screening additional samples for Hepatitis B and C and Human Immunodeficiency Virus (HIV I and II) serology screening;

[b] Once daily administration of one tablet.

[c] Infant assessments include infant sex, gestational age, head circumference, weight and length measurements, ponderal index, infant skinfold thickness, Apgar score. Bone density can be assessed within 10 days after Visit 5.

[d] Cord blood evaluations include RBC fatty acid parameters, 25-hydroxyvitamin D, oxidative status (GSH/GSSG ratio, plasma lipid hydroperoxides (ROMs), 8-isoprostane), umbilical cord blood gas and PH analysis and metabolomics.

[e] Placental evaluations include placental weight and biometric parameters, fatty acid parameters, oxidative status (8-isoprostane, mtDNA) and inflammatory status evaluation (IL-6, IL-10 and TNF-a and metabolomics).

[f] Only in a subset of women (approximately 10 per study group) undergoing elective Caesarean section.

[g] At Visits 2 and 3 only complete blood count (CBC) was performed as safety assessment.

3.4.2 Population characteristics

Demographics and baseline

The following information were collected:

- Age
- Ethnicity
- Weight
- Height
- Body mass index (BMI).
- Planned delivery procedure.
- History of previous birth outcomes
- Smoking history
- Alcohol consumption history
- Changes in smoking, alcohol and drugs habits.
- Medical and surgical history
- Prior and concomitant therapy
- Physical and gynecological examinations data collected at Screening and Baseline.
- Vital signs data collected at Screening and Baseline.

3.4.3 EFFICACY VARIABLES

3.4.3.1 Primary efficacy variables

The primary efficacy variable was maternal RBC DHA (wt% total fatty acids) measured at Visit 1 (screening) and at Visit 4; the primary efficacy endpoint was the change of maternal RBC DHA (wt% total fatty acids) from Visit 1 to Visit 4.

3.4.3.2 Secondary efficacy variables

The secondary efficacy variables included the following:

Maternal variables (change from Visit 1 to Visit4)

- RBC FA parameters (TFA, EPA wt% TFA, DHA/TFA ratio, Omega 3 index);
- 25-hydroxyvitamin D;
- Oxidative status [i.e. reduced Glutathione (GSH)/oxidized Glutathione (GSSG) ratio, plasma lipid hydroperoxides (reactive oxygen metabolites (ROMs)), 8-isoprostane].

The **exploratory** efficacy variables included the following:

Infant Variables (Visit 5; Delivery)

- Gestational age;
- Head circumference, weight and length measurements;
- Weight index as a proxy of body composition;
- Skinfold thickness;
- Apgar score (breathing effort, heart rate, muscle tone, reflexes, skin color);
- Bone density

Maternal variables at Visit 5 (delivery) in a subset of women undergoing elective Caesarean section (10 per study group):

- Cord blood RBC FA parameters (TFA, DHA wt% TFA, EPA wt% TFA, DHA/TFA ratio, Omega 3 index);
- Cord blood 25-hydroxyvitamin D;
- Cord blood oxidative status [i.e. GSH/GSSG ratio, plasma lipid hydro peroxides (ROMs), 8-isoprostane];
- Umbilical cord blood gas and pH analysis (i.e pH, CO_2 partial pressure, O_2 partial pressure, lactate, hemoglobin);
- Placental weight and biometric parameters: chorionic elliptical disc diameters, feto/placental weight (F/P) ratio;
- Placental FA parameters (TFA, DHA wt% TFA, EPA wt% TFA, DHA/TFA ratio, Omega 3 index);
- Oxidative status in placental tissue and isolated trophoblast cells (8-isoprostane); Mitochondrial DNA (mtDNA) content and gene expression of IL6 (interleukin 6), IL10 (interleukin 10), TNFα (tumor necrosis factor alpha) in placental tissue and isolated trophoblast cells to assess oxidative stress and inflammatory status;
- Cord blood and placental tissue untargeted metabolomics analysis.

Moreover, also maternal blood RBC FA parameters (i.e. TFA, DHA wt% TFA, EPA wt% TFA, DHA/TFA ratio, Omega 3 index), maternal blood oxidative status (i.e. GSH/GSSG ratio, plasma lipid hydro peroxides (ROMs), 8-isoprostane) and 25-hydroxyvitamin D were considered for this subset of women undergoing elective Caesarean section.

Food consumption was assessed during the course of study after Visit 2 by using a semi-quantitative Food Frequency Questionnaire (FFQ) for evaluation of maternal intake habits of energy, macro- and micro-nutrients.

3.4.4 SAFETY VARIABLES

Safety and tolerability were assessed by evaluating the incidence and severity of AEs and their relationship to trial treatment.
The evaluation of abnormal findings in the measurement of objective tolerability through vital signs (blood pressure, pulse rate) and physical examinations were performed at all visits during the treatment period.

Clinical laboratory safety findings, measured at Visit 1 and 4, are as follows:

- Complete blood count (CBC) (measured also at Visits 2 and 3): hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBCs) count, white blood cells (WBCs)

count with differential count (neutrophils, eosinophils, basophils, lymphocytes, monocytes), and platelet count;

- C-reactive protein (CRP) (measured also at Visits 2 and 3);
- Ferritin;
- Kidney function tests: electrolytes (i.e. sodium, potassium, chloride, calcium), serum creatinine, urine analyses, albumin/creatinine ratio in urine;
- Liver function tests: albumin (ALB), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin;
- Blood coagulation parameters: activated partial thromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR);
- Hepatitis (B and C) and Human Immunodeficiency Virus (HIV I + II) screening serology (measured only at Visit 1).

3.4.4.1 Adverse events

Any adverse events (AEs) intended as any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) was reported. All AEs were assessed and documented according to seriousness, intensity, causal relationship between the AE and the treatment, causal relationship between the AE and protocol-required procedures, actions taken with study treatment, Other specific treatments of the AE, outcome.

3.4.5 Other safety variables or evaluations

Physical examination

Abnormal findings in the measurement of objective tolerability through physical examinations were recorded.

Complete physical examination, including gynecological examination, was performed at all study Visits.

Height, body weight, BMI calculation were assessed at Visits 1 and 4.

Vital signs

Abnormal findings in the measurement of objective tolerability through vital signs were recorded. Sitting blood pressure (systolic and diastolic blood pressure) monitored in supine position with an automatic non-invasive device after a resting period of 5 minutes, pulse rate after at least 5 minutes rest at sitting position, were assessed at all study Visits.

3.4.6 Other variables and evaluations

Nutrient intake, diet habits and physical activity (FFQ)

Food consumption, diet habits and physical activity were assessed at Visits 2, 3 and 4 until delivery using a semi-quantitative Food Frequency Questionnaire (FFQ). We used a semi-quantitative FFQ of five food categories to assess the usual daily intake of foods and nutrients. The FFQ is a slightly modified version from an FFQ validated and found reproducible by Vioque et al (2013) in pregnant women (Vioque 2013)

Delivery information
Delivery information (type of delivery, delivery complications, induced labor and infant sex) were also collected at Visit 5.

3.5 Data quality assurance

3.5.1 Data recording

The data collection tool for this study was a paper case report form (CRF). Subjects data necessary for analysis and reporting were entered/transmitted into a validated database or data system. Source documentation for the key data entered into the CRF was available at the sites

3.5.2 Quality management

Study management
The following steps were taken to ensure accurate, consistent, and complete data:

- An investigator meeting was conducted by the sponsor where principal investigators and sub-investigators discussed and developed a common understanding of the clinical study protocol, case report form, and study procedures.
- Study initiation meetings were conducted at each study center before subject enrollment to discuss the protocol and review data collection (including identification and documentation of source data items), adverse events monitoring and reporting procedures, and regulatory requirements. The investigators were provided with instructions regarding specific procedures and terminology required by the CSP.
- Monitoring visits were conducted by the CRO for the purpose of reviewing the data for (i) completeness, accuracy and authenticity, (ii) adherence to GCP and the protocol, (iii) cross-checking with source documents and (iv) verification of protection of safety and rights of subjects and of verifying that safety and rights of subjects were being protected. Newsletters were sent by the sponsor to the study centers to provide information to the study center personnel and to update the study centers on subjects' enrollment.
- The study product at the investigational site was inaccessible to unauthorized personnel. Product management was properly documented, according to the sponsor's procedures. Compliance to study product was verified by evaluating any discrepancies between actual and expected amount of returned study product.
- Clinical laboratory tests were performed by certified laboratories
- Essential documents were archived safely and securely in such way that ensures that they were readily available upon authorities' and Sponsor's request. Subject (hospital) files were archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice. Where the archiving procedures did not meet the minimum timelines required by the sponsor, alternative arrangements were made to ensure the availability of the source documents for the required period.

3.5.3 Data processing

Data management was performed by a selected CRO (OPIS s.r.l.) who is qualified and have demonstrated the ability to implement quality assurance and quality control. The sponsor ensured oversight of trial-related duties and functions carried out by the CRO. Any trial-related duties and functions not specifically transferred to and assumed by the CRO were retained by the sponsor.
Clinical data management was performed in accordance with applicable sponsor's/CRO's standards and data cleaning procedures. This was applicable for data recorded on CRF as well as for data from other sources (e.g. laboratories).

For data coding (e.g. AEs, medication), internationally recognized and accepted dictionaries were used.

3.6 STATISTICAL METHODS

All statistical tables, listings and analyses were performed and generated using SAS® release 9.4 or later (SAS Institute, Inc., Cary, NC, USA).
Continuous data were summarized by means of common descriptive statistics: mean, standard deviation (SD), median, minimum, maximum and first and third quartile. Categorical data were presented by absolute and relative frequencies (n and %) or contingency tables.
Unless otherwise stated, two-sided p-values <0.05 were considered statistically significant.

3.6.1 Analysis sets

The following analysis populations were defined:

- Safety Population (SAF): all subjects who were randomized into the study and took at least one dose of the IP for those randomized to the treatment group.
- Intent-to-Treat (ITT) Population: all subjects who belonged to the Safety Population and who underwent at least one post-baseline measurement of efficacy.
- Per-protocol (PP) Population: all ITT subjects who had the efficacy data for the primary efficacy endpoint collected at Visit 4 and did not have protocol violations.

Moreover, the subgroup of women who underwent elective Caesarean section was defined as subjects belonging to the PP population for whom an elective Caesarean section was planned or recommended at screening (and confirmed at randomization) and/or at Visit 4 and then actually had caesarean delivery.

3.6.2 Demographics and baseline characteristics

Demographics and baseline characteristics were summarized both on the PP and ITT population by study groups and overall by means of descriptive statistics.

3.6.3 Efficacy variables

All the efficacy analyses were performed on the PP population. The efficacy analyses conducted on ITT population were considered as supportive and were conducted to corroborate the results from the PP population. The exploratory efficacy analyses for the subgroup of women who underwent elective Caesarean section were conducted only on the PP population.

Primary maternal efficacy endpoint

Descriptive statistics for continuous variables was applied to maternal RBC DHA (wt% total fatty acids) at each visit as well as on the absolute change from baseline by study groups, both according to the observed data and the last observation carried forward (LOCF) approach.
Analysis of the primary endpoint was performed using the analysis of covariance (ANCOVA) model with treatment and center as fixed effects and baseline RBC DHA value as covariate.

Secondary maternal efficacy endpoints

All the maternal secondary endpoints were analyzed similarly to the primary endpoint.

Exploratory infant efficacy endpoints

The secondary infant efficacy endpoints were analyzed descriptively. The difference between study groups was tested by ANOVA model considering the study group and center as fixed effect.

Exploratory maternal efficacy endpoints

All the exploratory variables (were analyzed descriptively. The difference between study groups was planned to be tested by ANOVA model considering the study group and center as fixed effect.

3.6.4 Safety variables

Safety analyses were conducted on the Safety Population.
Safety and tolerability were assessed by evaluating incidence and severity of AEs, their relationship to trial treatment as well as the incidence of abnormal findings in measurement of objective tolerability through vital signs (blood pressure, pulse rate), physical examination, and clinical laboratory findings.
Laboratory data were summarized for the change from baseline and tabulated by study group. Abnormal findings were flagged in the data listings.
Vital signs and physical examinations were summarized for the change from baseline and tabulated by study group. Abnormal findings were flagged in the data listings

3.6.5 Other variables and evaluations

Nutrient intake, diet habits and physical activity (FFQ)

Food consumption was assessed during the study at Visit 2, Visit 3 and Visit 4 by using the semi-quantitative Food Frequency Questionnaire (FFQ) for evaluation of maternal intake habits of energy, macro- and micro-nutrients. For each food belonging to the following macro groups: dairy products; eggs, meat, fish, vegetables, pulses, fruit, bread, cereals, oils, fats and sweets, drinks and miscellaneous, frequency was collected as score from 0 to 8 where 0 = never or < 1/month; 1 = 1-3 a month; 2= 1 a week; 3 = 2-4 a week; 4 = 5-6 a week; 5 = 1 a day; 6 = 2-3 a day; 7 = 4-5 a day; 8 = 6+ a day.
Calculations for nutrient intake were performed by the Biomolecular Research laboratory for the Study of Pregnancy and Reproduction and Biological Clinical Laboratory and Biochemical Nutrition of the University of Milano, multiplying the reported frequency of each food by the amount of nutrient in a portion of that food.
Data of food-amount (g/day) were calculated by referring to average portion sizes. To estimate the daily levels of energy and nutrients for each woman, food composition tables by the European Institute of Oncology (http://www.bda-ieo.it/wordpress/en/) and the Centro di Ricerca per gli Alimenti e la Nutrizione (http://nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html)) were used to obtain the composition in energy and nutrients for each food. For iron and folic acid from other supplements eventually taken during pregnancy, the total daily nutrient intake was estimated by adding the average daily intake from supplements and the usual daily nutrient intake from the FFQ. To convert folic acid intake from supplements to dietary folate, we used the equivalence of 1 mcg of folate in the diet equals to 0.6 mcg of folic acid from supplements
The estimated nutrient intakes were analyzed descriptively by study groups and visit by means of descriptive statistics for continuous variables according to the following nutrient intakes:

- Calories (Kcal)
- Alcohol (g)
- Protein (g)
- Lipids (g)
- Carbohydrates (g)
- Sugars (g)
- Dietary Fibre (g)
- Cholesterol (g)
- Saturated fatty acids (g)
- Polyunsaturated fatty acids (g)

- Monounsaturated fatty acids (g)
- Calcium (mg)
- Sodium (mg)
- Potassium (mg)
- Phosphorus (mg)
- Iron (mg)
- Zinc (mg)
- Folic acid (µg)
- C20 5 EPA (g)
- C22 6 DHA (g)
- Animal proteins (g)
- Vegetable proteins (g)
- Drinking water (ml)

Consumption of dietary supplements (vitamins, minerals), diet, if any with reason, and physical activities and exercise are summarized descriptively by study group and visit by means of descriptive statistics.

Delivery information

Delivery information collected at Visit 5 was analyzed descriptively by study group and visit.

3.6.6 Determination of sample size

Assuming the treatment difference of 1.6 with standard deviation of 3.4 observed by Bergmann in 2008 (Bergmann et al., 2008), 70 subjects per arm were needed to achieve 80% power with 0.05 of alpha in order to detect the treatment difference between two treatment groups. To account for a drop-out rate of 15%, approximately 164 subjects (82 per treatment group) were planned to be randomized.

A subset of subjects (approximately 10 subjects per study group) undergoing elective Caesarean section was also planned to be studied at Caesarean section.

3.7 Ethics, investigators and study administrative structure

Independent Ethics Committee or Institutional Review Board

The protocol and protocol amendment were reviewed and approved by a single Independent Ethics Committee (IEC) for both sites before the start of the study.

Ethical conduct of the study

The conduct of this clinical study met all local legal and regulatory requirements. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and the International Council for Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP).

Subject information and consent

An informed consent form explaining the procedures of the study including the potential hazards was reviewed and approved by the IEC before its use.

Only after the subject voluntarily signed the informed consent form, was she able to enter the study.

Investigators and study administrative structure

The study was conducted at two study centers in Milan, Italy:

- Department of Biomedical and Clinical Sciences "L. Sacco", Obstetrics Gynecology - Operating Unit, ASST Fatebenefratelli Sacco, Hospital L. Sacco, Milan
- Woman, Mother, Newborn Department, Obstetrics Gynecology Unit, ASST Fatebenefratelli Sacco, Hospital V.Buzzi, Milan

Laboratory determinations were performed by three different laboratories:

- The laboratory of Biomolecular Research of Pregnancy and Reproduction (*Laboratorio di Ricerca Biomolecolare per lo Studio della Gravidanza e della Riproduzione*) and the Laboratory of Clinical Biology and Biochemistry of Nutrition (*Laboratorio di Clinica Biologica e BIochimica della Nutrizione*) of the Sacco Hospital (Milano, Italy) were responsible for evaluation of efficacy variables.
- The laboratory of Clinical Pathology (*Laboratorio di Patologia Clinica*) of the Sacco Hospital (Milano, Italy) was responsible for safety and vitamin D analyses.

Competent authorities
Once this study was approved by the IEC, the Italian “Ministry of Health” was notified.

4. STUDY SUBJECTS

4.1 Disposition of subjects

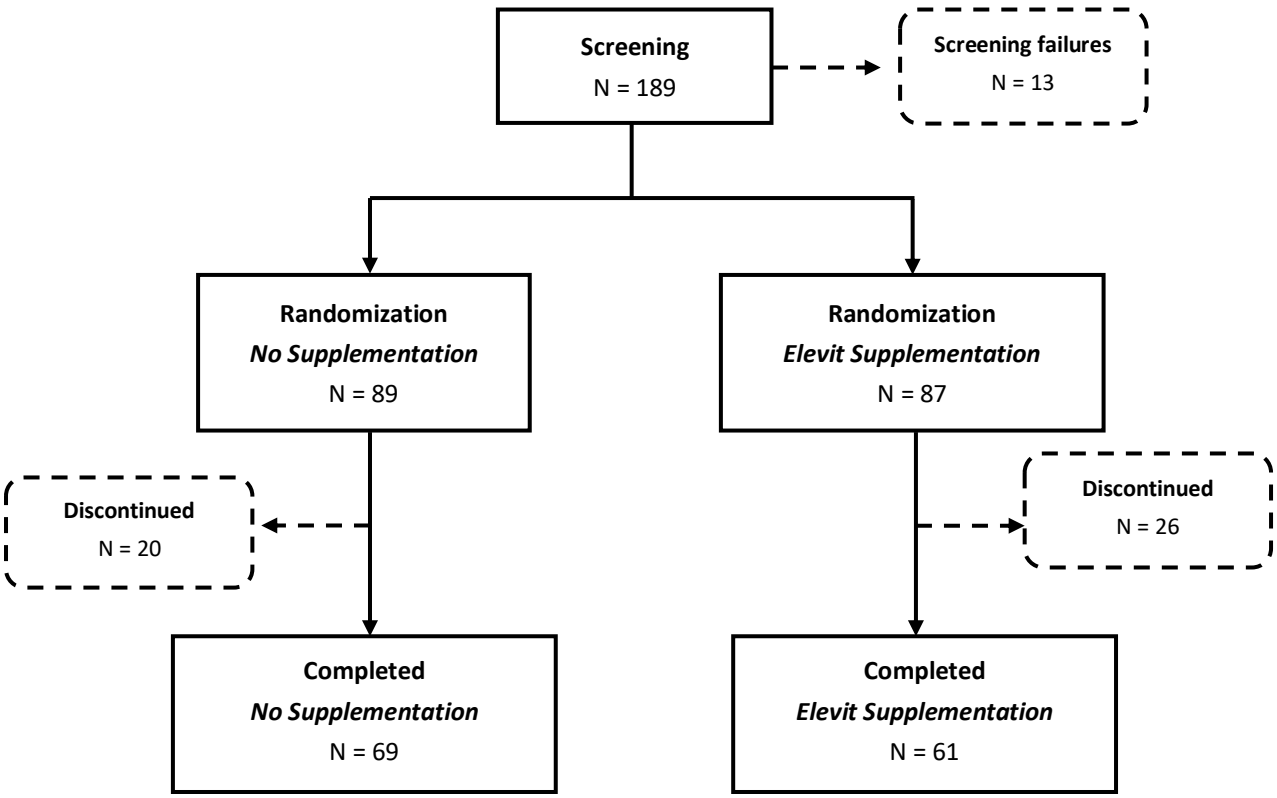
As shown in Table 4-1 and in figure 4-1, a total of one hundred eighty-nine (189) subjects were screened for enrollment. One hundred seventy-six (176, 93.12%) subjects were randomized, while thirteen (13, 6.88%) were excluded from the study because of screening failures.

Table 4-1 Subjects' enrollment; Screened subjects

	(N=189) n (%)
Screened subjects	189 (100.00)
Screening failures	13 (6.88)
Randomized subjects	176 (93.12)

Screened subjects: pregnant subjects who signed a valid Informed Consent Form.
Screening failures: pregnant subjects who consent to participate in the clinical trial but were not randomly assigned to one of the study group for any reason.
Randomized subjects: subjects that were randomly assigned to one of the study group.

Figure 4-1 Disposition chart



As displayed in Table 4-2, all (176, 100.00%) randomized subjects performed Visit 1 (Screening) and Visit 2 (Baseline), one hundred sixty-two (162, 92.05%) and one hundred forty-two (142, 80.68%) performed Visit 3 (GA Week 24/26) and Visit 4 (GA Week 34/36), respectively.
Twenty-four (24, 13.64%) subjects performed the Early Discontinuation Visit (EDV). Visit 5 (Delivery) was done by one hundred thirty-one (131, 74.43%) women, while Visit 6 (Follow-Up) by one hundred fifty-one (151, 85.80%). The difference between the number of subjects performing Delivery Visit and those performing the Follow-Up Visit is explained by the fact that the Follow-Up Visit was either performed after delivery or after EDV.
One hundred thirty (130, 73.86%) subjects completed the study and forty-six (46, 26.14%) discontinued. “Completed” refers to women who performed all the visits from screening to follow-up. Reasons for study discontinuation were mainly adverse events (32, 69.57%) (Table 4-2).
Among the subjects in the “Elevit supplementation” arm, sixty-two (62, 71.26%) completed the entire course of product intake and twenty-five (25, 28.74%) discontinued the supplementation. Reasons for product discontinuation were mainly adverse events (20, 80.00%), non-compliance with the study product (1, 4.00%) and protocol deviations (1, 4.00%).

Table 4-2 Subjects' disposition; Randomized subjects

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of subjects per visit			
Visit 1 - Screening (GA Week 11/14)	89 (100.00)	87 (100.00)	176 (100.00)
Visit 2 - Baseline (GA Week 13/15)	89 (100.00)	87 (100.00)	176 (100.00)
Visit 3 (GA Week 24/26)	84 (94.38)	78 (89.66)	162 (92.05)
Visit 4 (GA Week 34/36)	76 (85.39)	66 (75.86)	142 (80.68)
Early Discontinuation Visit	9 (10.11)	15 (17.24)	24 (13.64)
Visit 5 - Delivery	69 (77.53)	62 (71.26)	131 (74.43)
Visit 6 - Follow-up [a]	79 (88.76)	72 (82.76)	151 (85.80)
Study disposition			
Subject study status			
Completed [b]	69 (77.53)	61 (70.11)	130 (73.86)
Discontinued	20 (22.47)	26 (29.89)	46 (26.14)
Reason for study discontinuation [d]			
Adverse Event	11 (55.00)	21 (80.77)	32 (69.57)
Non-compliance with study drug	0	1 (3.85)	1 (2.17)
Protocol deviation	1 (5.00)	1 (3.85)	2 (4.35)
Lost to follow-up	1 (5.00)	0	1 (2.17)
Withdrawal of consent	2 (10.00)	1 (3.85)	3 (6.52)
Other	5 (25.00)	2 (7.69)	7 (15.22)
Treatment disposition [c]			
Subject treatment status			
Completed	NA	62 (71.26)	62 (35.23)
Discontinued	NA	25 (28.74)	25 (14.20)
Reason for treatment discontinuation [d]			
Adverse Event	NA	20 (80.00)	20 (80.00)
Non-compliance with study drug	NA	1 (4.00)	1 (4.00)
Protocol deviation	NA	1 (4.00)	1 (4.00)
Other	NA	3 (12.00)	3 (12.00)

GA=Gestational Age, NA=Not Applicable.

Some subjects did not perform either Visit 4 and Early discontinuation visit.

[a] Follow-up visit included both subjects who completed the study and subjects who discontinued but performed follow-up visit. The follow-up visit was performed after delivery or after early discontinuation visit.

[b] Completed refers to subjects who completed both treatment and study (i.e. performed all visits from the screening to the follow-up visit).

[c] Treatment disposition was provided only for subjects randomly assigned to Elevit supplementation.

[d] Percentages were computed on discontinued subjects.

4.2 Protocol deviations

Overall, ninety-seven (97, 55.11%) subjects had at least one protocol deviation: fifty-two (52, 58.43%) in the "No supplementation" group and forty-five (45, 51.72%) in the "Elevit supplementation" one. The main protocol deviations were protocol deviations related to procedures (62, 35.23%), such as the lack of at least one assessment of infants' anthropometric parameters at delivery (34, 19.32%).

Overall, thirty-four (34, 19.32%) women had at least one non-protocol deviation: thirteen (13, 14.61%) in the control group and twenty-one (21, 24.14%) in the supplementation group.

4.3 Analysis sets

As shown in Table 4-3, all randomized subjects for both study groups took at least one dose of the investigational product (IP) and therefore no subjects were excluded from the safety (SAF) population. Eighty-eight (88, 98.88%) subjects belonging to the control arm and eighty-two (82, 94.25%) belonging to the Elevit arm underwent at least one post-baseline measurement of the primary efficacy variable and thus were included in the Intent-To-Treat (ITT) population.

Furthermore, seventy-six (76, 85.39%) women of the non-supplemented group and sixty-five (65, 74.71%) of the supplemented group underwent assessment of the primary efficacy variable at Visit 4 without reporting protocol deviations and were hence included in the Per Protocol (PP) population. Overall, there was a total of twelve (12) subjects in the subgroup of women undergoing elective Caesarean section: six (6, 6.74%) in the control arm and six (6, 6.90%) in the "Elevit supplementation" arm.

Table 4-3 Analysis population; Randomized subjects

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Safety (SAF) population	89 (100.00)	87 (100.00)	176 (100.00)
Intent-To-Treat (ITT) population	88 (98.88)	82 (94.25)	170 (96.59)
Per Protocol (PP) population	76 (85.39)	65 (74.71)	141 (80.11)
Subgroup who underwent elective Caesarean Section	6 (6.74)	6 (6.90)	12 (6.82)

Safety (SAF) population: all subjects who were randomized into the study and took at least one dose of the Investigational Product (IP) if they were randomized to Elevit supplementation.

Intent-To-Treat (ITT) population: all subjects belonging to safety population that had at least one post-baseline measurements of efficacy data (i.e. maternal RBC DHA measurement after screening visit).

Per Protocol (PP) population: all ITT subjects who had the efficacy data for the primary efficacy endpoint at Visit 4 (i.e. maternal RBC DHA measurement at Visit 4) and did not have protocol violations.

Subgroup who underwent elective Caesarean Section: subjects belonging to PP population for whom the elective Caesarean section was planned or recommended at screening (and confirmed at randomization) and/or at Visit 4, and then had caesarean delivery. Subjects with PD IC000e were also excluded from this subgroup.

4.4 Demographics and baseline characteristics

As shown in Table 4-4, subjects were 31.9 ± 4.64 years on average (range: 18 - 41 years) and all women were white (141, 100,00%). Demographics characteristics were similar in both study arms.

Table 4-4 Demographics characteristics; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)	Total (N=141)
Age (years)			
n	76	65	141
Mean (SD)	32.3 (4.72)	31.4 (4.52)	31.9 (4.64)
Median	33.0	32.0	32.0
Q1; Q3	29.0; 36.0	29.0; 35.0	29.0; 35.0
Range	18; 41	20; 40	18; 41
Race, n (%)			
White	76 (100.00)	65 (100.00)	141 (100.00)

PP=Per Protocol.

Overall, women were 62.29 ± 9.743 kg (range: 45.0 - 95.0 kg) and 165.0 ± 6.48 cm high (range: 147 - 184 cm). Mean BMI was 22.86 ± 3.163 kg/m^2 (range: 18.0 - 29.9 kg/m^2). Similar mean weight, height and BMI were described for both study groups.

Table 4-5 Weight, Height and Body Mass index; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)	Total (N=141)
Weight (kg)			
n	76	65	141
Mean (SD)	61.54 (9.963)	63.16 (9.480)	62.29 (9.743)
Median	59.00	63.00	60.50
Q1; Q3	54.25; 70.00	56.50; 66.00	55.00; 68.20
Range	45.0; 87.0	47.0; 95.0	45.0; 95.0
Height (cm)			
n	76	65	141
Mean (SD)	164.1 (7.08)	165.9 (5.60)	165.0 (6.48)
Median	165.0	165.0	165.0
Q1; Q3	160.0; 168.5	163.0; 169.0	160.0; 169.0
Range	147; 184	150; 178	147; 184
Body Mass Index (kg/m^2)			
n	76	65	141
Mean (SD)	22.81 (3.235)	22.93 (3.100)	22.86 (3.163)
Median	21.70	22.00	21.90
Q1; Q3	20.25; 25.25	20.80; 24.50	20.50; 25.20
Range	18.0; 29.7	18.1; 29.9	18.0; 29.9

PP=Per Protocol.
Weight, Height and Body Mass index were assessed at screening visit.

Results of pregnancy assessments (gestational week 11-13/14, singleton pregnancy and planned or recommended Caesarean section) were similar for both study groups.
Eighty-one (81, 57.45%) subjects had at least one previous pregnancy while sixty (60, 42.55%) did not. Similar percentages were reported for the two study arms. A total of twenty (20, 14.18%) women had previous spontaneous abortions.
Smoking, alcohol and drugs habits were similar in the two groups. There were no differences between supplemented group and control group regarding medical and surgical history and prior and concomitant therapy.

5. EFFICACY EVALUATION

5.1 Treatment compliance

A summary of compliance for subjects belonging to the SAF population is shown in Table 5-1. Compliance was computed as the proportion between the number of capsules taken and the expected number of days under supplementation. Compliance between Visit 2 and Visit 4 (or early discontinuation) was ≥80% for seventy-seven (77, 88.51%) women of the Elevit group and not evaluable for three (3, 3.45%) women. Compliance levels for the total duration of the study was ≥80% for sixty-three (63, 72.41%) subjects in the "Elevit supplementation" group and not evaluable for twenty (20, 22.99%) women. Compliance was not evaluable for those women who did not return the IP at each visit.

Table 5-3 Summary of compliance; Safety population

	Elevit supplementation (N=87) n (%)
Compliance levels between Visit 2 and Visit 4 or early discontinuation	
< 80%	7 (8.05)
>= 80%	77 (88.51)
Not evaluable	3 (3.45)
Compliance levels for total duration of the study	
< 80%	4 (4.60)
>= 80%	63 (72.41)
Not evaluable	20 (22.99)

Compliance was computed as the proportion between the number of capsules taken and the expected number of days under treatment (i.e. days between the start date of treatment (date of Visit 2) and the last known date in which subject took the treatment collected in the End of treatment page).
The compliance was not evaluable for subjects who did not return the investigational product at each visit during the period of interest (i.e. between Visit 2 and 4 or between Visit 2 and 5).

5.2 Analysis of the efficacy variables

5.2.1 Primary maternal efficacy variable

Maternal RBC DHA (wt% total fatty acids)

The DHA status of the mother in blood was evaluated at each visit (except for Visit 2) by measuring DHA (wt% total fatty acids) in red blood cells (RBCs) and summarizing the results both according to the observed data and the last observation carried forward (LOCF) approach (Table 5-2). Similar results were reported with both approaches. As shown in Table 5-2 and Figure 5-1, mean DHA (wt% TFA) in RBCs according to the LOCF approach was similar in both groups at baseline (Visit 1). Mean DHA (wt% TFA) in RBCs slightly increased at Visit 3 and 4 in the control group and to a greater extent in the Elevit one.

Mean DHA (wt% TFA) in RBCs was 6.13 ± 1.230 at Visit 1, 6.56 ± 1.297 at Visit 3 and 6.65 ± 1.335 at Visit 4 in the control group and 6.05 ± 1.259 at Visit 1, 6.99 ± 1.301 at Visit 3 and 7.54 ± 1.484 at Visit 4 in the Elevit group. The ANCOVA model also shows an increase in RBCs DHA (wt% TFA) in both arms at Visit 4 (Table 5-3): absolute change from baseline (Visit 1) was 0.52 [95% CI: 0.28 to 0.76] in the "No supplementation" arm and 1.48 [95% CI: 1.22 to 1.74] in the "Elevit supplementation" one. Difference in absolute change between the two groups (Elevit supplementation – No supplementation) was 0.96 [95% CI: 0.61 to 1.31] and this difference was highly significant (p-value <0.0001) (Table 5-3 and Figure 5-2). The same analyses were performed in the ITT population and confirmed the results reported for the PP population.

Table 5-2 Descriptive statistics of RBC DHA (wt% total fatty acid) at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Baseline n	n	Mean	SD	Q1	Median	Q3	Min	Max
RBC DHA (wt% TFA)																	
No supplementation																	
Visit 1	76	6.13	1.230	5.22	6.07	7.05	3.8	9.3	-	-	-	-	-	-	-	-	-
Visit 3	76	6.56	1.297	5.64	6.49	7.55	4.0	10.4	6.13	76	0.44	0.913	-0.14	0.46	0.79	-1.3	3.6
Visit 4	76	6.65	1.335	5.62	6.66	7.76	4.3	9.6	6.13	76	0.52	0.933	-0.09	0.56	1.16	-2.4	2.8
Elevit supplementation																	
Visit 1	65	6.05	1.259	5.09	5.93	6.97	3.4	10.2	-	-	-	-	-	-	-	-	-
Visit 3	65	6.99	1.301	6.16	7.01	7.85	4.5	10.5	6.05	65	0.95	0.937	0.31	0.92	1.58	-1.4	2.8
Visit 4	65	7.54	1.484	6.48	7.45	8.39	5.0	13.0	6.05	65	1.50	1.218	0.67	1.38	1.98	-1.6	6.0

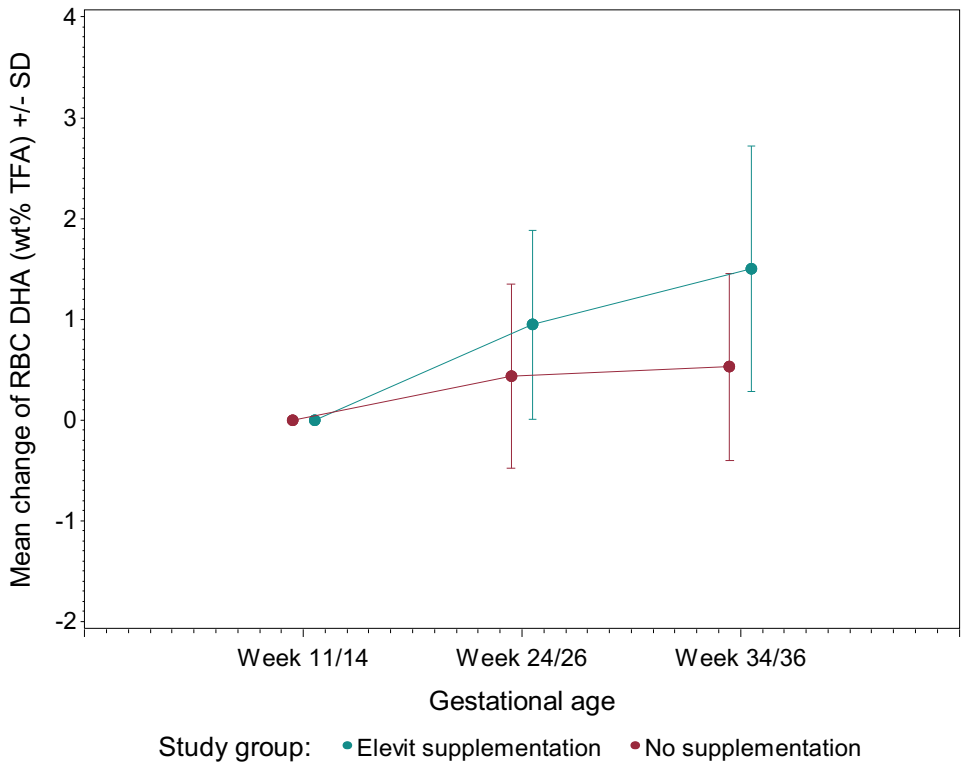
PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, DHA=docosahexaenoic acid, wt%=weight percent, TFA=Total Fatty Acids, GA=Gestational Age.

Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.

n refers to the number of subjects on Per Protocol population.

Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Figure 5-1 **Change from baseline (Visit 1) in RBC DHA (wt% total fatty acid) – LOCF approach; PP population**



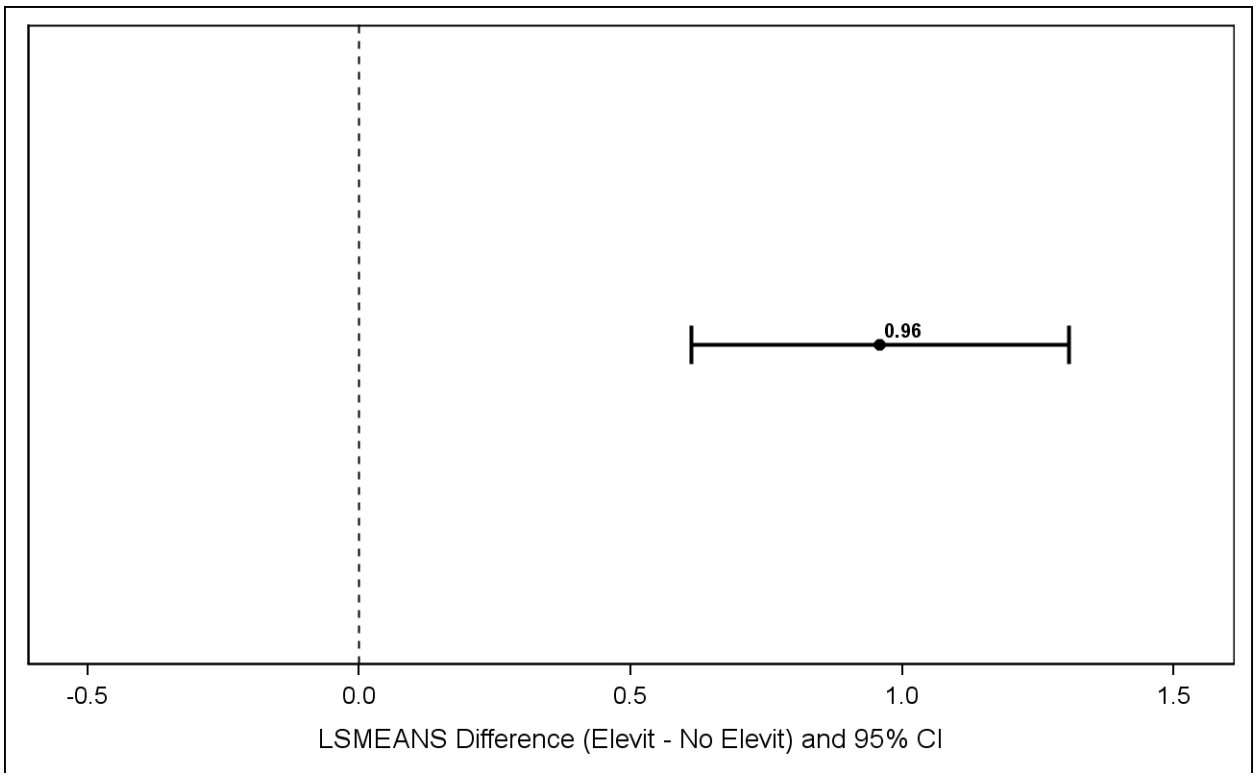
PP=Per Protocol, LOCF=Last Observation Carrie

Table 5-3 ANCOVA model on change from baseline (Visit 1) in RBC DHA (wt% total fatty acid); PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
RBC DHA (wt% TFA)						
Visit 4 (GA Week 34/36)	76	0.52 (0.28, 0.76)	65	1.48 (1.22, 1.74)	0.96 (0.61, 1.31)	<.0001

PP=Per Protocol, RBC=Red Blood Cell, DHA=docosahexaenoic acid, wt%=weight percent, TFA=Total Fatty Acids, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in
RBC DHA wt% TFA, and independent variables are RBC DHA wt% TFA at baseline, study group and center as covariates.

Figure 5-2 ANCOVA model on change from baseline (Visit 1) in RBC DHA (wt% total fatty acid) – LOCF approach; PP population



PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, DHA=docosahexaenoic acid, wt%=weight percent, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in RBC DHA wt% TFA, and independent variables are RBC DHA wt% TFA at baseline, study group and center as covariates.

5.2.2 Secondary maternal efficacy variables

Secondary maternal efficacy variables evaluated at Visit 1, Visit 3 and Visit 4 in the PP population and summarized according both to the observed data and the LOCF approach are presented below. Similar results were reported with both approaches. The same analyses were performed on the ITT population.

Maternal RBC total fatty acids

Status of TFA in RBCs by study visit summarized according to the LOCF approach in Table 5-4. Maternal RBC TFA at baseline (Visit 1) was slightly higher in the "No supplementation" group than in the "Elevit supplementation" one: 4662.2 ± 2650.40 and 4255.9 ± 2326.45 respectively. Based on the ANCOVA model on change from baseline in RBC TFA, absolute change from baseline was -190.0 [95% CI: -433.2 to 53.2] in the "No supplementation" group and 35.7 [95% CI: -221.5 to 293.0] in the "Elevit supplementation" one (Table 5-5). The difference between the two groups (Elevit supplementation – No supplementation) was not significant (p-value 0.2014). These results were confirmed in the ITT population.

Maternal RBC EPA (wt% total fatty acids)

Levels of EPA (wt% TFA) in RBCs by visit according to the LOCF approach in Table 5-6. Maternal EPA in RBCs slightly decreased at subsequent visits from Visit 1 in the non-supplemented group and remained almost unvaried in the supplemented one. The ANCOVA model indicates an absolute change from baseline of -0.04 [95% CI: -0.10 to 0.01] for the "No supplementation" group and -0.01 [95% CI: -0.07 to 0.05] for the "Elevit supplementation" one, with no significant difference between them (p-value 0.4291) (Table 5-7). Similar results were reported for the ITT population

Maternal RBC DHA/TFA ratio

Mean DHA/TFA ratio in RBCs according to the LOCF approach was similar at baseline and slightly increased at subsequent study visits in both groups (Table 5-8 and

Figure 1-3). The ANCOVA model also shows an increase in RBC DHA/TFA ratio in both arms at Visit 4 (Table 5-9): absolute change from baseline was 0.005 [95% CI: 0.003 to 0.008] in the "No supplementation" arm and 0.015 [95% CI: 0.012 to 0.017] in the "Elevit supplementation" one. Difference in absolute change between the two groups was 0.010 [95% CI: 0.006 to 0.013] and this difference was highly significant (p-value <0.0001) (Table 5-9 and Figure 5-4). Similar results were reported for the ITT population.

Maternal Omega 3 index

Mean Omega 3 index according to the LOCF approach was comparable at Visit 1 and increased at subsequent visits in both groups (Table 5-10 and Figure 5-5). The ANCOVA model also shows an increase in Omega 3 index at Visit 4 in both arms (Table 5-11): absolute change from baseline was 0.47 [95% CI: 0.21 to 0.72] in the "No supplementation" arm and 1.47 [95% CI: 1.20 to 1.74] in the "Elevit supplementation" one. Difference in absolute change between the two groups was 1.00 [95% CI: 0.64 to 1.37] and this difference was highly significant (p-value <0.0001) (Table 5-11 and Figure 5-6). These results were confirmed in the ITT population.

Maternal 25-hydroxyvitamin D

Levels of 25-hydroxyvitamin D in blood described according to the LOCF approach in Table 5-12. There was a decrease in mean 25-hydroxyvitamin D levels at Visit 3 and 4 in the control arm. In contrast, there was a slight increase in mean 25-hydroxyvitamin D levels at Visit 3 and 4 in the supplementation one (Table 5-12 and Figure 5-7). According to the ANCOVA model, absolute change from baseline in 25-hydroxyvitamin D levels at Visit 4 was -3.48 µg/L [95% CI: -5.62 to -1.33] in the "No supplementation" group and 0.48 µg/L [95% CI: -1.81 to 2.77] in the "Elevit supplementation" one (Table 5-13). Difference in absolute change between the two arms was 3.96 [95% CI: 0.88 to 7.04], with a p-value of 0.0122, which suggests a statistically significant difference between them (Table 5-13 and Figure 5-8). Comparable results were reported for the ITT population.

Maternal GSH/GSSG ratio

The ratio between GSH and GSSG (reduced to oxidized glutathione ratio) in blood, as a general marker of oxidative stress, presented according to the LOCF approach are shown in Table 5-14. Mean GSH/GSSG ratio slightly increased by visit in both study groups. The ANCOVA model shows an absolute change from baseline of 0.46 [95% CI: 0.00 to 0.92] in the non-supplemented arm and of 0.50 [95% CI: 0.01 to 0.99] in the supplemented one (Table 5-15). There was no significant difference between the two groups (p-value 0.9037). Similar results were reported for the ITT population.

Maternal ROMs

Levels of ROMs (mg H2O2 /dL) in blood, as a marker of oxidative stress according to the LOCF approach in Table 5-16. Mean ROMs slightly increased from baseline at each visit in both study arms. Based on the ANCOVA model (Table 5-17) absolute change from baseline in levels of ROMs was 1.48 mg H_2O_2 /dL [95% CI: 0.25 to 2.71] in the "No supplementation" arm and 2.26 mg H_2O_2 /dL [95% CI: 0.95 to 3.57] in the "Elevit supplementation" one. There was no difference between the two groups (p-value 0.3831). Similar results were reported for the ITT population.

Maternal 8-isoprostane

Concentration of 8-isoprostane in blood, as a marker of oxidative stress, described according to LOCF approach in Table 5-18. As shown in the table, mean 8-isoprostane increased by visit in both study groups. The ANCOVA model shows an absolute change from baseline at Visit 4 of 61.26 pg/mL [95% CI: 43.99 to 78.52] in the control group and of 44.82 pg/mL [95% CI: 26.42 to 63.22] in the Elevit one (Table 5-19). There was no significant difference between study arms (p-value 0.1905). Similar results were reported for the ITT population.

Maternal C-reactive protein

Concentration of C-reactive protein (CRP) in blood, as a marker of inflammation, are summarized according to the LOCF approach in Table 5-20. There was a decrease in mean CRP at Visit 4 compared with Visit 1 in the control group and a slight increase in the Elevit one. The ANCOVA model on change from baseline in CRP shows an absolute change from baseline of -15.22 nmol/L [95% CI: -31.26 to 10.92] in the control arm and 3.94 nmol/L [95% CI: -13.42 to 21.30] in the supplementation one (Table 5-21). Difference in absolute change between the two groups was 19.16 nmol/L [95% CI: -4.08 to 42.39] and this difference not significant (p-value 0.1053). The same analyses were performed in the ITT population and confirmed the results reported for the PP population.

Maternal ferritin

Concentration of ferritin in maternal blood was assessed at Visit 1 and Visit 4. Results summarized according to the LOCF approach in Table 5-22. Ferritin decreased at Visit 4 compared with Visit 1 in both groups: mean ferritin was 48.66 ± 39.105 µg/L at Visit 1 and 19.59 ± 12.908 µg/L at Visit 4 in the "No supplementation" arm, and 49.88 ± 34.417 µg/L at Visit 1 and 18.83 ± 10.881 µg/L at Visit 4 in the "Elevit supplementation" one. The ANCOVA model on change from baseline in ferritin shows an absolute change from baseline at Visit 4 of -30.49 µg/L [95% CI: -33.17 to -27.80] in the control group and of -31.05 µg/L [95% CI: -33.94 to -28.16] in the supplementation one (Table 5-23). There was no difference between the two groups (p-value 0.7729). Comparable results were reported for the ITT population.

Table 5-4 Descriptive statistics of RBC TFA at each visit – LOCF approach; PP population

										Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
RBC TFA																	
No supplementation																	
Visit 1	76	4662.2	2650.40	3116.9	3735.7	5338.1	338	15266	-	-	-	-	-	-	-	-	-
Visit 3	76	4377.4	2419.84	3044.7	3395.8	5539.5	335	14707	4662.2	76	-284.8	703.65	-661.7	-187.1	135.1	-2068	1833
Visit 4	76	4517.4	2761.49	3047.0	3459.9	5486.5	400	16014	4662.2	76	-144.8	1071.24	-604.9	-1.8	268.4	-4890	2696
Elevit supplementation																	
Visit 1	65	4255.9	2326.45	3018.8	3430.4	4353.7	406	11082	-	-	-	-	-	-	-	-	-
Visit 3	65	4136.5	2251.43	2982.3	3280.0	4491.9	371	11422	4255.9	65	-119.4	1008.73	-469.2	-160.7	89.0	-3154	4694
Visit 4	65	4332.3	2461.20	3055.7	3334.6	4266.6	390	11964	4255.9	65	76.4	1006.45	-213.0	-16.1	275.5	-2335	4096

PP=Per Protocol, RBC=Red Blood Cell, TFA=Total Fatty Acids, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-5 ANCOVA model on change from baseline (Visit 1) in RBC TFA – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
RBC TFA						
Visit 4 (GA Week 34/36)	76	-190.0 (-433.2, 53.2)	65	35.7 (-221.5, 293.0)	225.7 (-122.0, 573.4)	0.2014

PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, TFA=Total Fatty Acids, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in
TFA, and independent variables are TFA at baseline, study group and center as covariates.

Table 5-6 Descriptive statistics of RBC EPA (wt% total fatty acid) at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
RBC EPA (wt% TFA)																	
No supplementation																	
Visit 1	76	0.54	0.242	0.38	0.46	0.70	0.1	1.6	-	-	-	-	-	-	-	-	-
Visit 3	76	0.45	0.229	0.31	0.41	0.58	0.1	1.4	0.54	76	-0.09	0.162	-0.17	-0.06	0.01	-0.9	0.2
Visit 4	76	0.49	0.323	0.30	0.39	0.63	0.1	2.4	0.54	76	-0.06	0.308	-0.16	-0.08	-0.01	-0.8	2.0
Elevit supplementation																	
Visit 1	65	0.49	0.222	0.31	0.45	0.66	0.1	1.0	-	-	-	-	-	-	-	-	-
Visit 3	65	0.52	0.232	0.39	0.46	0.65	0.1	1.3	0.49	65	0.03	0.174	-0.06	0.02	0.12	-0.4	0.4
Visit 4	65	0.49	0.194	0.36	0.47	0.61	0.1	1.1	0.49	65	-0.00	0.171	-0.09	0.03	0.10	-0.6	0.4

PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, EPA=eicosapentaenoic acid, wt%=weight percent, TFA=Total Fatty Acids, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-7 ANCOVA model on change from baseline (Visit 1) in RBC EPA (wt% total fatty acid) – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
RBC EPA (wt% TFA)						
Visit 4 (GA Week 34/36)	76	-0.04 (-0.10, 0.01)	65	-0.01 (-0.07, 0.05)	0.03 (-0.05, 0.11)	0.4291

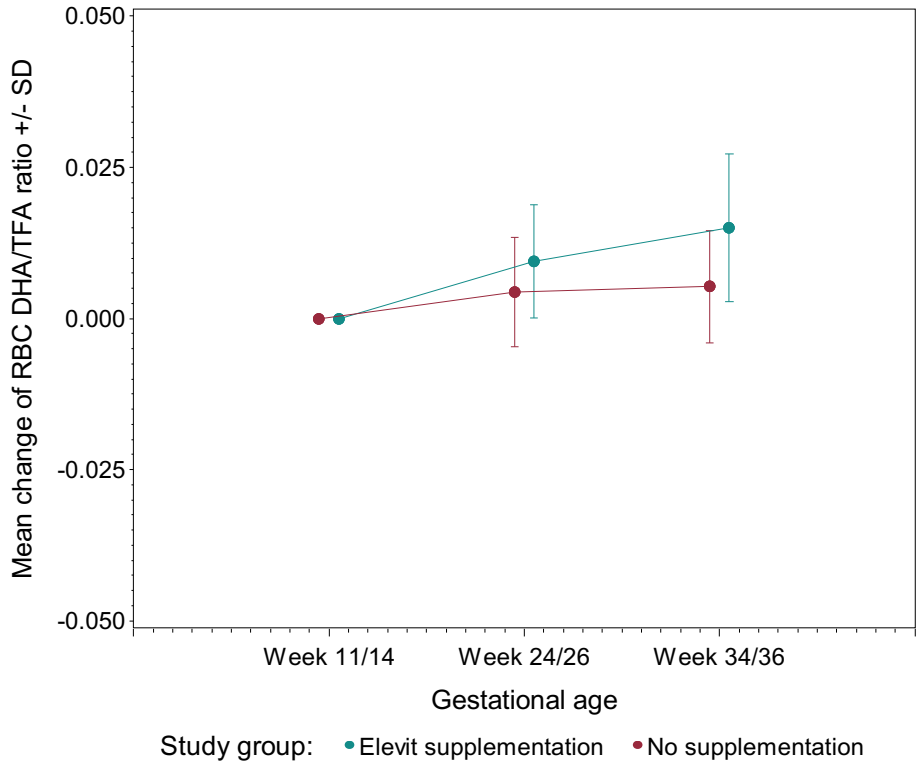
PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, EPA=eicosapentaenoic acid, wt%=weight percent, TFA=Total Fatty Acids, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in RBC EPA wt% TFA, and independent variables are RBC EPA wt% TFA at baseline, study group and center as covariates.

Table 5-8 Descriptive statistics of RBC DHA/TFA ratio at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
RBC DHA/TFA ratio																	
No supplementation																	
Visit 1	76	0.061	0.0123	0.052	0.061	0.071	0.04	0.09	-	-	-	-	-	-	-	-	-
Visit 3	76	0.066	0.0130	0.056	0.065	0.075	0.04	0.10	0.061	76	0.004	0.0091	-0.001	0.005	0.008	-0.01	0.04
Visit 4	76	0.067	0.0133	0.056	0.067	0.078	0.04	0.10	0.061	76	0.005	0.0093	-0.001	0.006	0.012	-0.02	0.03
Elevit supplementation																	
Visit 1	65	0.060	0.0126	0.051	0.059	0.070	0.03	0.10	-	-	-	-	-	-	-	-	-
Visit 3	65	0.070	0.0130	0.062	0.070	0.079	0.04	0.11	0.060	65	0.009	0.0094	0.003	0.009	0.016	-0.01	0.03
Visit 4	65	0.075	0.0148	0.065	0.074	0.084	0.05	0.13	0.060	65	0.015	0.0122	0.007	0.014	0.020	-0.02	0.06

PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, DHA=docosahexaenoic acid, TFA=Total Fatty Acids, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Figure 1-3 Change from baseline (Visit 1) in RBC DHA/TFA ratio – LOCF approach; PP population



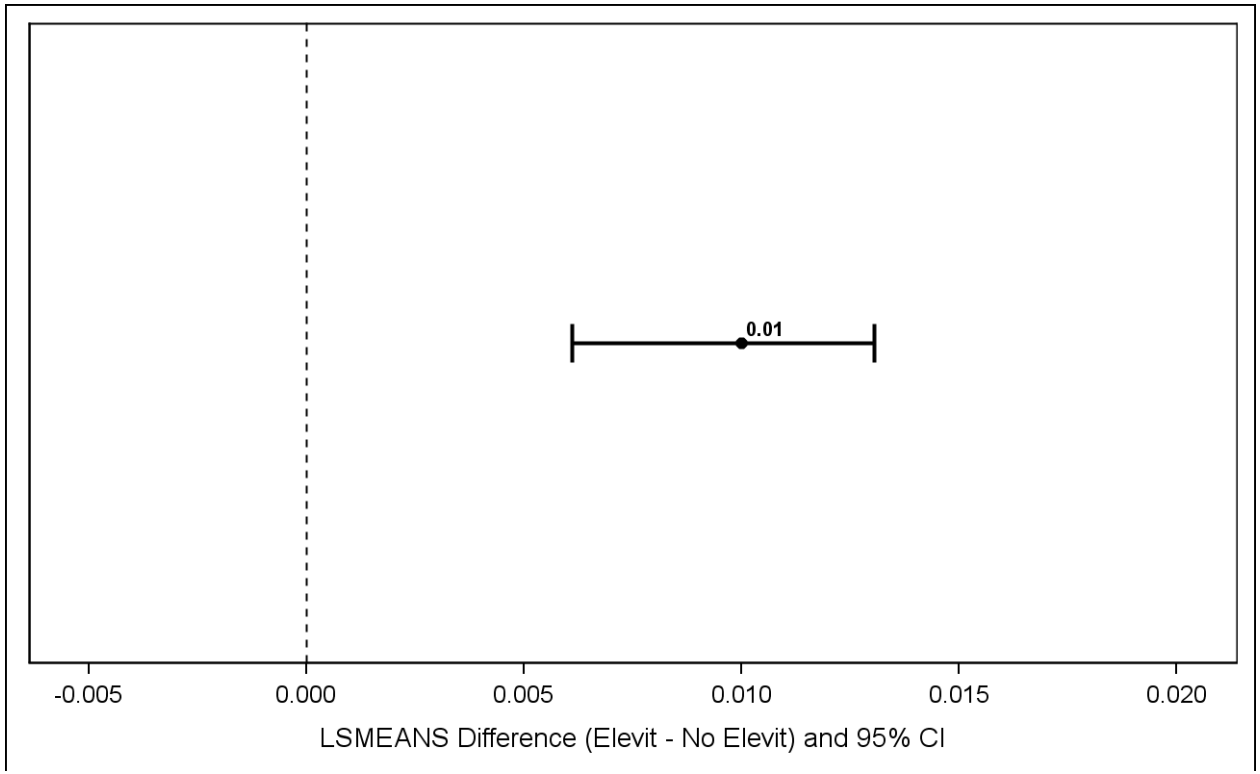
PP=Per Protocol, LOCF=Last Observation Carried Forward, RBC=Red Blood Cell, DHA=docosahexaenoic acid, TFA=Total Fatty Acids.

Table 5-9 ANCOVA model on change from baseline (Visit 1) in RBC DHA/TFA ratio – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
RBC DHA/TFA ratio						
Visit 4 (GA Week 34/36)	76	0.005 (0.003, 0.008)	65	0.015 (0.012, 0.017)	0.010 (0.006, 0.013)	<.0001

PP=Per Protocol, RBC=Red Blood Cell, DHA=docosahexaenoic acid, TFA=Total Fatty Acids, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in RBC DHA/TFA ratio, and independent variables are RBC DHA/TFA ratio at baseline, study group and center as covariates.

Figure 5-4 ANCOVA model on change from baseline (Visit 1) in RBC DHA/TFA ratio – LOCF approach; PP population



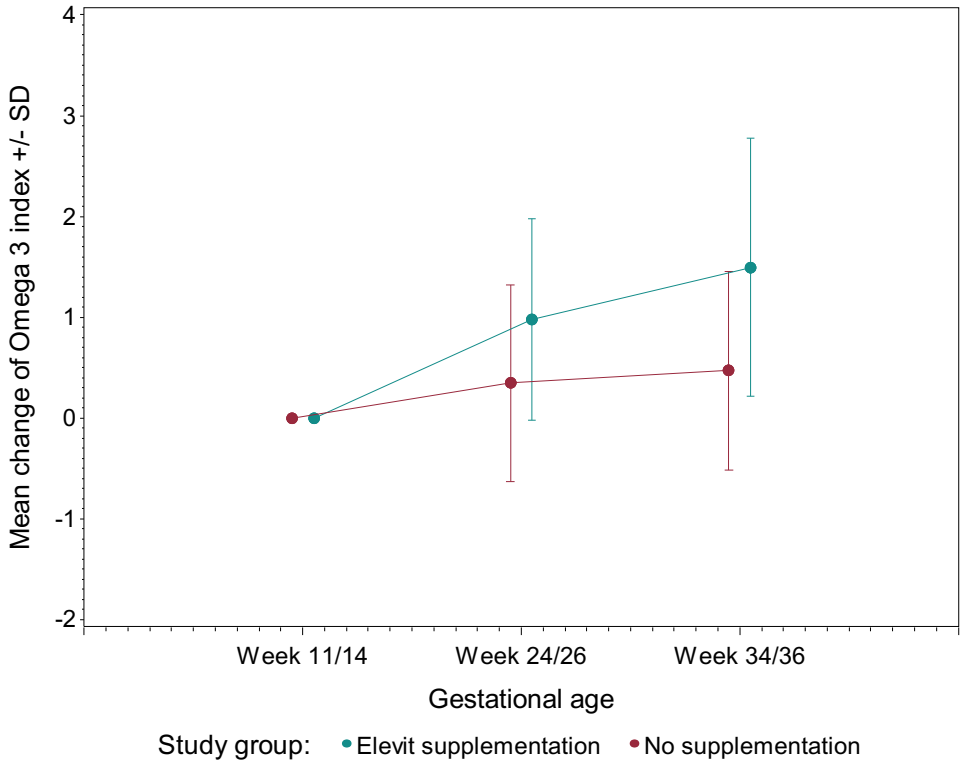
PP=Per Protocol, RBC=Red Blood Cell, DHA=docosahexaenoic acid, TFA=Total Fatty Acids, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in RBC DHA/TFA ratio, and independent variables are RBC DHA/TFA ratio at baseline, study group and center as covariates.

Table 5-10 Descriptive statistics of Omega 3 index at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
Omega 3 index																	
No supplementation																	
Visit 1	76	6.67	1.375	5.63	6.62	7.81	4.2	10.1	-	-	-	-	-	-	-	-	-
Visit 3	76	7.02	1.425	5.90	6.93	8.08	4.2	10.7	6.67	76	0.35	0.977	-0.25	0.32	0.77	-1.7	3.6
Visit 4	76	7.14	1.453	6.02	6.94	8.28	4.5	10.0	6.67	76	0.47	0.982	-0.25	0.50	1.07	-2.6	2.8
Elevit supplementation																	
Visit 1	65	6.54	1.395	5.57	6.45	7.50	3.7	10.9	-	-	-	-	-	-	-	-	-
Visit 3	65	7.52	1.428	6.51	7.47	8.49	4.7	11.1	6.54	65	0.98	1.004	0.25	0.95	1.67	-1.0	3.0
Visit 4	65	8.03	1.594	6.95	7.81	8.84	5.3	13.6	6.54	65	1.50	1.281	0.59	1.37	2.13	-1.8	6.2

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Figure 5-5 Change from baseline (Visit 1) in Omega 3 index – LOCF approach; PP population



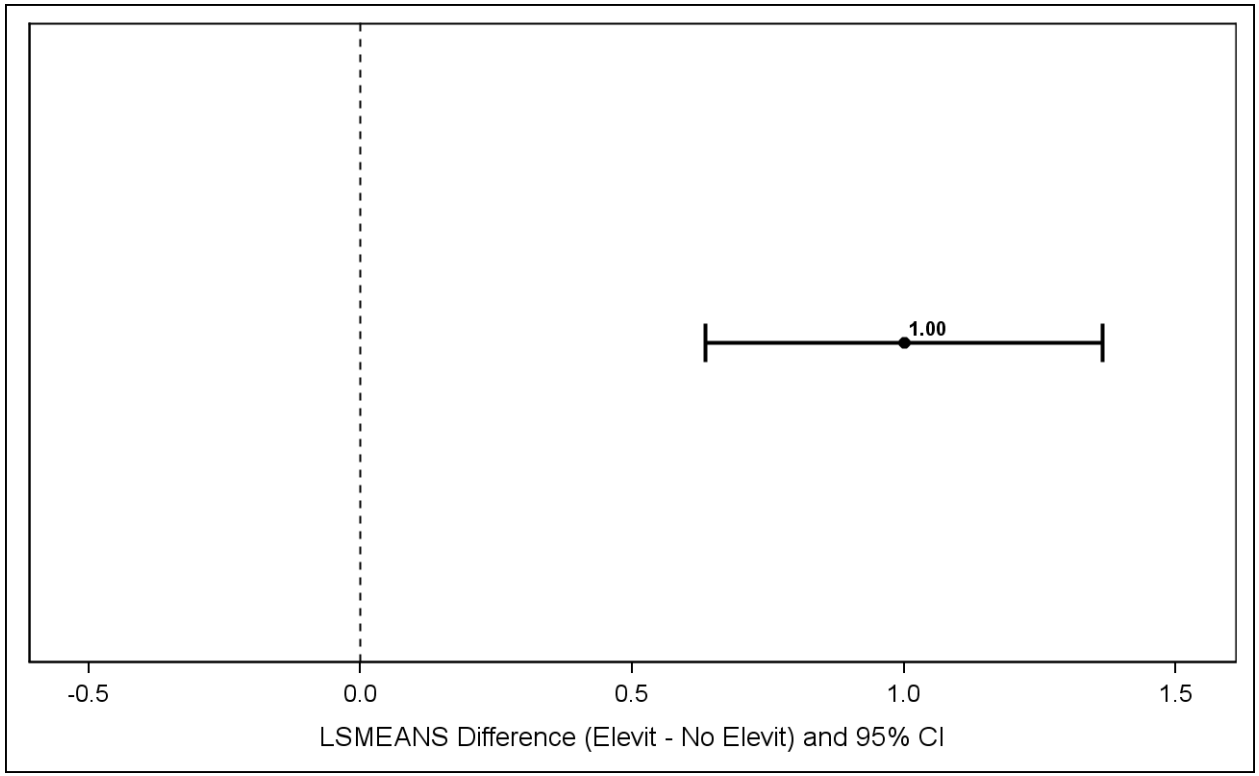
PP=Per Protocol, LOCF=Last Observation Carried Forward

Table 5-11 ANCOVA model on change from baseline (Visit 1) in Omega 3 index – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
Omega 3 index						
Visit 4 (GA Week 34/36)	76	0.47 (0.21, 0.72)	65	1.47 (1.20, 1.74)	1.00 (0.64, 1.37)	<.0001

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in Omega 3 index, and independent variables are Omega 3 index at baseline, study group and center as covariates.

Figure 5-6 ANCOVA model on change from baseline (Visit 1) in Omega 3 index – LOCF approach; PP population



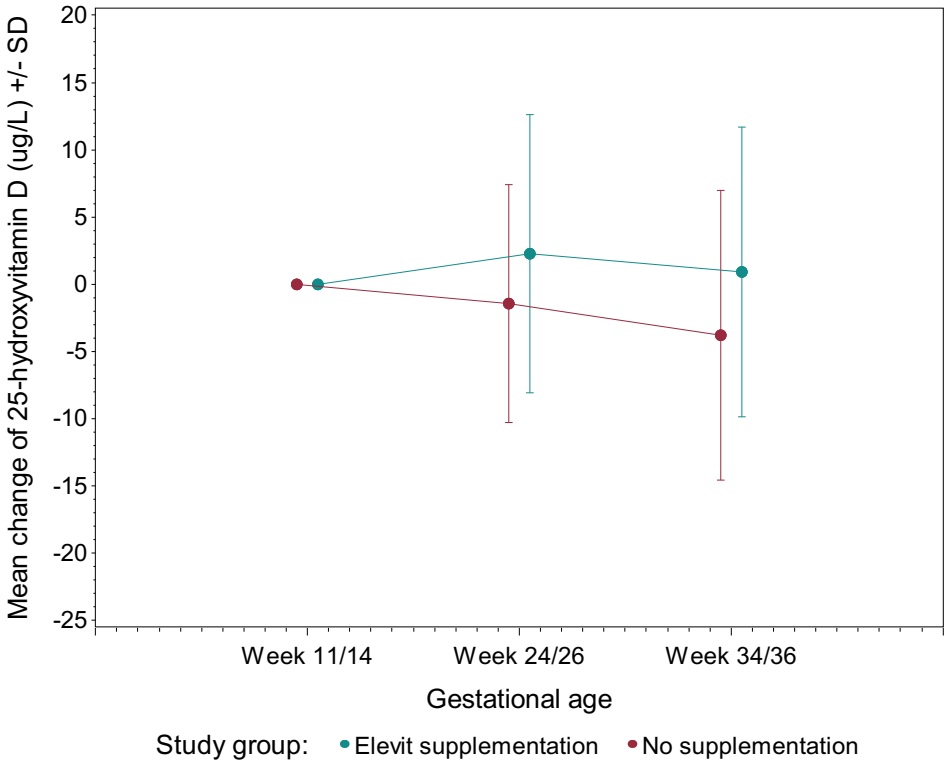
PP=Per protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in Omega 3 index, and independent variables are Omega 3 index at baseline, study group and center as covariates.

Table 5-12 Descriptive statistics of 25-hydroxyvitamin D at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
25-hydroxyvitamin D (ug/L)																	
No supplementation																	
Visit 1	76	21.55	8.937	15.15	20.60	28.20	5.5	48.8	-	-	-	-	-	-	-	-	-
Visit 3	76	20.07	9.911	12.75	19.00	26.40	4.6	64.1	21.55	76	-1.47	8.860	-8.35	-0.65	4.75	-19.7	19.0
Visit 4	76	17.75	9.717	10.15	15.30	24.45	4.0	45.0	21.55	76	-3.79	10.775	-12.10	-5.40	3.80	-28.9	19.0
Elevit supplementation																	
Visit 1	64	20.49	7.536	15.05	19.35	26.75	4.4	36.5	-	-	-	-	-	-	-	-	-
Visit 3	64	22.71	8.923	15.40	22.00	27.25	4.0	48.6	20.49	64	2.22	10.355	-6.50	1.70	10.65	-16.9	26.8
Visit 4	64	21.38	9.073	13.65	20.40	28.35	5.5	42.7	20.49	64	0.90	10.771	-6.30	1.35	9.35	-24.3	22.0

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Figure 5-7 Change from baseline (Visit 1) in 25-hydroxyvitamin D – LOCF approach; PP population



PP=Per Protocol, LOCF=Last Observation Carried Forward

Table 5-13 ANCOVA model on change from baseline (Visit 1) in 25-hydroxyvitamin D – LOCF approach; PP population

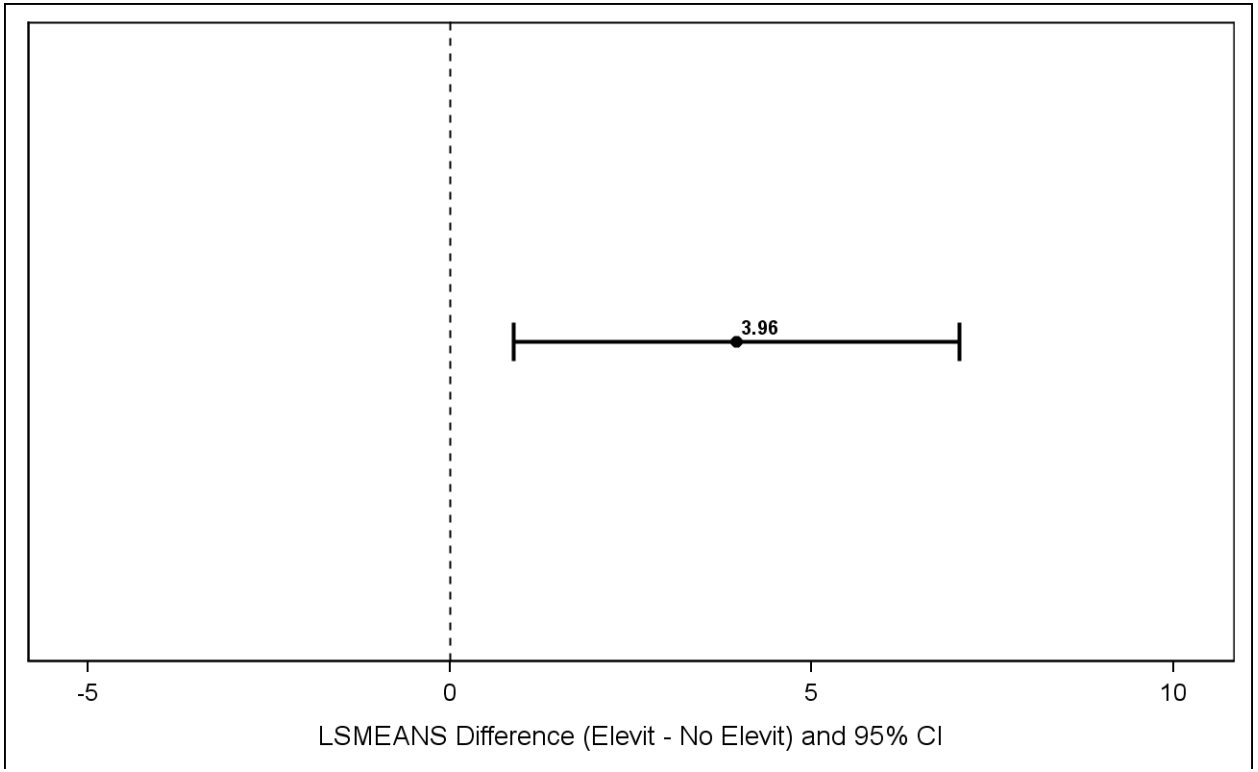
	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
25-hydroxyvitamin D (ug/L)						
Visit 4 (GA Week 34/36)	76	-3.48 (-5.62, -1.33)	64	0.48 (-1.81, 2.77)	3.96 (0.88, 7.04)	0.0122

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in 25-hydroxyvitamin D, and independent variables are 25-hydroxyvitamin D at baseline, study group and center as covariates.

Figure 5-8 ANCOVA model on change from baseline (Visit 1) in 25-hydroxyvitamin D – LOCF approach; PP population



PP=Per protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in 25-hydroxyvitamin D, and independent variables are 25-hydroxyvitamin D at baseline, study group and center as covariates

Table 5-14 Descriptive statistics of GSH/GSSG ratio at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
GSH/GSSG ratio																	
No supplementation																	
Visit 1	76	7.08	2.430	5.16	6.48	8.37	3.4	15.7	-	-	-	-	-	-	-	-	-
Visit 3	76	7.24	2.445	5.42	6.56	8.81	3.0	13.8	7.08	76	0.16	2.039	-1.09	-0.15	1.33	-5.3	5.9
Visit 4	76	7.53	2.431	5.72	7.08	8.97	3.3	13.6	7.08	76	0.44	2.177	-0.81	0.44	1.69	-4.4	9.0
Elevit supplementation																	
Visit 1	65	7.11	2.245	5.43	7.08	8.56	3.1	14.6	-	-	-	-	-	-	-	-	-
Visit 3	65	7.58	2.483	6.00	7.44	9.15	3.1	16.6	7.11	65	0.48	2.268	-0.91	0.46	1.75	-4.5	7.9
Visit 4	65	7.59	2.600	5.62	6.89	9.28	3.0	16.9	7.11	65	0.48	2.035	-1.01	0.39	1.72	-4.4	5.3

PP=Per Protocol, LOCF=Last Observation Carried Forward, GSH=reduced glutathione, GSSG=oxidized glutathione, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-15 ANCOVA model on change from baseline (Visit 1) in GSH/GSSG ratio – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
GSH/GSSG ratio						
Visit 4 (GA Week 34/36)	76	0.46 (0.00, 0.92)	65	0.50 (0.01, 0.99)	0.04 (-0.62, 0.70)	0.9037

PP=Per Protocol, LOCF=Last Observation Carried Forward, GSH=reduced glutathione, GSSG=oxidized glutathione, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in GSH/GSSG ratio, and independent variables are GSH/GSSG ratio at baseline, study group and center as covariates.

Table 5-16 Descriptive statistics of ROMs at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
ROMs (mg H2O2/dL)																	
No supplementation																	
Visit 1	76	34.14	4.405	31.16	33.31	36.55	24.6	44.9	-	-	-	-	-	-	-	-	-
Visit 3	76	35.92	4.805	33.04	35.31	38.73	25.7	51.1	34.14	76	1.78	3.968	-0.56	1.00	3.89	-11.1	12.7
Visit 4	76	35.48	5.278	32.85	35.39	38.49	21.0	55.2	34.14	76	1.34	5.450	-2.31	0.80	4.12	-13.1	27.0
Elevit supplementation																	
Visit 1	65	33.33	5.308	29.66	33.37	36.92	21.9	45.8	-	-	-	-	-	-	-	-	-
Visit 3	65	35.92	5.901	32.29	35.24	39.16	24.2	57.8	33.33	65	2.59	5.653	-0.43	1.96	4.10	-7.2	33.3
Visit 4	65	35.94	5.765	32.22	36.50	39.02	21.4	49.9	33.33	65	2.61	6.736	-1.06	1.78	5.12	-13.0	24.4

PP=Per Protocol, LOCF=Last Observation Carried Forwars, ROMs=reactive oxygen metabolites, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-17 ANCOVA model on change from baseline (Visit 1) in ROMs – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
ROMs (mg H2O2/dL)						
Visit 4 (GA Week 34/36)	76	1.48 (0.25, 2.71)	65	2.26 (0.95, 3.57)	0.78 (-0.98, 2.54)	0.3831

PP=Per Protocol, LOCF=Last Observation Carried Forwars, ROMs=reactive oxygen metabolites, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in ROMs, and independent variables are ROMs at baseline, study group and center as covariates.

Table 5-18 Descriptive statistics of 8-isoprostane at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
8-isoprostane (pg/mL)																	
No supplementation																	
Visit 1	76	108.53	66.976	57.05	79.25	140.45	26.0	299.6	-	-	-	-	-	-	-	-	-
Visit 3	76	142.63	98.297	71.35	113.75	211.15	29.3	496.2	108.53	76	34.10	71.433	1.25	17.35	52.45	-70.5	396.1
Visit 4	76	163.27	103.526	86.80	129.15	201.95	29.3	486.4	108.53	76	54.74	83.699	6.00	26.65	70.85	-58.3	309.6
Elevit supplementation																	
Visit 1	65	111.57	63.729	65.10	86.50	163.50	17.9	316.9	-	-	-	-	-	-	-	-	-
Visit 3	65	141.79	100.244	73.40	101.60	178.50	18.6	505.0	111.57	65	30.21	68.006	-13.90	15.60	47.20	-63.6	260.6
Visit 4	65	151.34	94.317	86.20	128.10	175.70	36.2	494.2	111.57	65	39.76	65.630	8.30	34.10	62.40	-97.5	361.9

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-19 ANCOVA model on change from baseline (Visit 1) in 8-isoprostane – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
8-isoprostane (pg/mL)						
Visit 4 (GA Week 34/36)	76	61.26 (43.99, 78.52)	65	44.82 (26.42, 63.22)	-16.44 (-41.14, 8.27)	0.1905

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in 8-isoprostane, and independent variables are 8-isoprostane at baseline, study group and center as covariates.

Table 5-20 Descriptive statistics of C-reactive protein at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
CRP - C Reactive Protein (nmol/L)																	
No supplementation																	
Visit 1	76	54.67	76.338	20.00	35.71	66.67	1.9	618.1	-	-	-	-	-	-	-	-	-
Visit 2	76	49.80	48.029	22.38	35.24	65.71	3.8	323.8	54.67	76	-4.87	77.175	-5.71	2.38	9.52	-553.3	299.0
Visit 3	76	54.97	78.987	19.52	34.29	52.86	3.8	579.0	54.67	76	0.30	102.367	-10.95	-0.95	7.62	-608.6	504.8
Visit 4	76	38.53	27.177	20.00	30.95	52.38	1.9	125.7	54.67	76	-16.14	75.236	-20.95	-0.95	10.48	-599.0	41.0
Elevit supplementation																	
Visit 1	64	45.97	68.034	11.43	29.05	58.57	1.9	515.2	-	-	-	-	-	-	-	-	-
Visit 2	65	40.94	30.458	15.24	33.33	61.90	2.9	130.5	45.97	64	-4.81	65.227	-3.81	2.38	8.10	-488.6	71.4
Visit 3	65	50.97	69.947	12.38	29.52	67.62	1.9	519.0	45.97	64	5.39	89.762	-8.10	1.43	10.48	-491.4	466.7
Visit 4	65	55.93	97.616	14.29	33.33	54.29	2.9	637.1	45.97	64	10.36	115.036	-10.48	1.43	20.00	-486.7	561.9

PP=Per Protocol, LOCF=Last Observation Carried Forwars, CRP=C-reactive protein, GA=Gestational Age.

Visit 1: Screening (GA Week 11/14); Visit 2: Baseline (GA Week 13/15); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36; EDV=Early Discontinuation Visit.

n refers to the number of subjects on Per Protocol population.

Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-21 ANCOVA model on change from baseline (Visit 1) in C-reactive protein – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
CRP - C Reactive Protein (nmol/L)						
Visit 4 (GA Week 34/36)	76	-15.22 (-31.36, 0.92)	64	3.94 (-13.42, 21.30)	19.16 (-4.08, 42.39)	0.1053

PP=Per Protocol, LOCF=Last Observation Carried Forwars, CRP=C-reactive protein, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in CRP, and independent variables are CRP at baseline, study group and center as covariates.

Table 5-22 Descriptive statistics of ferritin at each visit – LOCF approach; PP population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
Ferritin (ug/L)																	
No supplementation																	
Visit 1	76	48.66	39.105	23.00	35.50	59.00	7.0	171.0	-	-	-	-	-	-	-	-	-
Visit 4	76	19.59	12.908	8.50	17.00	25.00	4.0	58.0	48.66	76	-29.07	38.716	-45.50	-18.00	-4.50	-153.0	35.0
Elevit supplementation																	
Visit 1	64	49.88	34.417	24.50	44.00	64.00	6.0	138.0	-	-	-	-	-	-	-	-	-
Visit 4	65	18.83	10.881	12.00	16.00	24.00	3.0	55.0	49.88	64	-30.95	33.291	-45.50	-27.50	-5.00	-116.0	17.0

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 4: GA Week 34/36.
n refers to the number of subjects on Per Protocol population.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 5-23 ANCOVA model on change from baseline (Visit 1) in ferritin – LOCF approach; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS of change from baseline (95% CI)	n	LSMEANS of change from baseline (95% CI)	LSMEANS [a] of change from baseline (95% CI)	p-value
Ferritin (ug/L)						
Visit 4 (GA Week 34/36)	76	-30.49 (-33.17, -27.80)	64	-31.05 (-33.94, -28.16)	-0.57 (-4.43, 3.30)	0.7729

PP=Per Protocol, LOCF=Last Observation Carried Forward, GA=Gestational Age, LSMEANS=Least Squares Means, CI=Confidence Interval.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANCOVA model, in which the dependent variable is change at Visit 4 (GA week 34/36) from baseline (Visit 1 - Screening (GA Week 11/14)) in ferritin, and independent variables are ferritin at baseline, study group and center as covariates.

5.2.2 Exploratory infant efficacy variables

Infants' efficacy variables were assessed at Visit 5 (Delivery). The Delivery Visit was performed by 69 subjects in the "No supplementation" group and by 62 in the "Elevit supplementation" one in the PP population. Overall, the reported infants' variables were similar in both groups. Results below are summarized for the PP population.

Infants' gestational age

As shown in Table 5-24, no difference was observed in gestational age (GA) at delivery between the two arms: mean GA was 39.92 ± 1.203 weeks in the control arm and 39.86 ± 1.121 weeks in the Elevit one. Results from the ANOVA model also show no significant difference between the study groups (p-value 0.8019) (Table 5-25). Same results were reported for the ITT population.

Table 5-24 Descriptive statistics of infants' gestational age; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Gestational age (weeks)		
n	69	62
Mean (SD)	39.92 (1.203)	39.86 (1.121)
Median	40.00	39.86
Q1; Q3	39.14; 40.57	39.14; 40.57
Range	36.1; 42.0	35.9; 41.9

PP=Per Protocol.

n refers to the number of subjects who performed the assessment.

Table 5-25 ANOVA model on infants' gestational age; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Gestational age (weeks)						
Visit 5 - Delivery	69	39.91 (39.62, 40.19)	62	39.86 (39.56, 40.15)	-0.05 (-0.46, 0.35)	0.8019

PP=Per Protocol, LSMEANS=Least Squares Means.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is gestational age, and independent variables are study group and center as covariates.

Infants' head circumference

Table 5-26 shows results of the measurements of infants' head circumference at delivery: mean head circumference was similar in the "No supplementation" and "Elevit supplementation" groups (34.41 ± 1.081 cm vs 34.36 ± 1.139 cm, respectively). The ANOVA model displayed in Table 5-27 also shows no difference between the two arms (p-value 0.8250). Same results were reported for the ITT population.

Table 5-26 Descriptive statistics of infants' head circumference; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Head circumference (cm)		
n	66	58
Mean (SD)	34.41 (1.081)	34.36 (1.139)
Median	34.50	34.00
Q1; Q3	34.00; 35.00	34.00; 35.00
Range	32.0; 36.5	32.0; 37.0

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Table 5-27 ANOVA model on infants' head circumference; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Head circumference (cm)						
Visit 5 - Delivery	66	34.39 (34.11, 34.67)	58	34.35 (34.05, 34.64)	-0.04 (-0.44, 0.35)	0.8250

PP=Per Protocol, LSMEANS=Least Squares Means.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is head circumference, and independent variables are study group and center as covariates.

Infants' weight

As shown in Table 5-28 no relevant difference in infants' weight between the two groups was reported: mean weight was 3.43 ± 0.430 kg in the control group and 3.42 ± 0.411 kg in the Elevit one. No difference was reported also according to the ANOVA model (p-value 0.9542) (Table 5-29). Same results were reported for the ITT population

Table 5-28 Descriptive statistics of infants' weight; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Weight (Kg)		
n	69	61
Mean (SD)	3.43 (0.430)	3.42 (0.411)
Median	3.47	3.48
Q1; Q3	3.14; 3.65	3.17; 3.71
Range	2.4; 4.7	2.7; 4.6

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Table 5-29 ANOVA model on infants' weight; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Weight (Kg)						
Visit 5 - Delivery	69	3.42 (3.32, 3.53)	61	3.42 (3.31, 3.53)	-0.00 (-0.15, 0.14)	0.9542

PP=Per Protocol, LSMEANS=Least Squares Means.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is weight, and independent variables are stud y group and center as covariates.

Infants' length

Infants' length, reported in Table 5-30 was comparable in both arms: mean length was 50.17 ± 2.039 cm in the control arm and 50.25 ± 1.792 cm in the supplementation one. Results from the ANOVA model also reported no difference between the two groups (p-value 0.8346) (Table 5-31). Same results were reported for the ITT population.

Table 5-30 Descriptive statistics of infants' length; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Length (cm)		
n	67	58
Mean (SD)	50.17 (2.039)	50.25 (1.792)
Median	50.20	50.00
Q1; Q3	49.00; 51.50	49.00; 51.50
Range	45.0; 56.0	45.7; 54.3

PP=Per Protocol.

n refers to the number of subjects who performed the assessment.

Table 5-31 ANOVA model on infants' length; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Length (cm)						
Visit 5 - Delivery	67	50.20 (49.72, 50.69)	58	50.27 (49.76, 50.79)	0.07 (-0.61, 0.76)	0.8346

PP=Per Protocol, LSMEANS=Least Squares Means.

n refers to the number of observations used in the model.

[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is length, and independent variables are stud y group and center as covariates.

Infants' ponderal index

Mean ponderal index was comparable in both the control and Elevit arms (2.73 ± 0.230 g/cm^3 vs 2.70 ± 0.210 g/cm^3, respectively) (Table 5-32). No difference was reported also according to the ANOVA model (p-value 0.4643) (Table 5-33). Same results were described for the ITT population.

Table 5-32 Descriptive statistics of infants' ponderal index; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Ponderal index (g/cm3)		
n	66	58
Mean (SD)	2.73 (0.230)	2.70 (0.210)
Median	2.69	2.70
Q1; Q3	2.59; 2.90	2.56; 2.82
Range	2.1; 3.5	2.0; 3.1

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Table 5-33 ANOVA model on infant ponderal index; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Ponderal index (g/cm3)						
Visit 5 - Delivery	66	2.73 (2.67, 2.78)	58	2.70 (2.64, 2.76)	-0.03 (-0.11, 0.05)	0.4643

PP=Per Protocol, LSMEANS=Least Squares Means.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is ponderal index, and independent variables are study group and center as covariates.

Infants' skinfold thickness

Table 5-34 reports infants' skinfold thickness by site at delivery. Skinfold thickness was slightly higher in the supplementation group compared with the control group at triceps (4.40 ± 0.862 mm in vs 4.20 ± 0.803 mm, respectively) (Table 5-34). According to the ANOVA model, the difference between the two study arms was not significant (p-value 0.2422) (Table 5-35). Furthermore, biceps skinfold thickness was comparable between the study groups (3.50 ± 0.663 mm in the control group vs 3.54 ± 0.704 mm in the Elevit one) (Table 5-34) and there was no significant difference (p-value 0.8313) (Table 5-35). Suprailiac skinfold thickness was higher in the supplementation arm than in the control arm (4.55 ± 0.918 mm vs 4.36 ± 0.942 mm, respectively) (Table 5-34). The difference between the two groups was not significant (p-value 0.3074) (Table 5-35). Moreover, subscapular skinfold thickness was higher in the supplemented arm compared with the non-supplemented one (4.74 ± 1.007 mm vs 4.33 ± 0.894 mm, respectively) (Table 5-34). According to the ANOVA model, the difference between the two groups was significant with a p-value of 0.0292, which indicates higher subscapular skinfold thickness in the Elevit group (Table 5-35). Same results were reported for the ITT population.

Table 5-34 Descriptive statistics of infants' skinfold thickness by site; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Triceps skinfold average (mm)		
n	59	50
Mean (SD)	4.20 (0.803)	4.40 (0.862)
Median	4.13	4.25
Q1; Q3	3.67; 4.73	3.67; 5.20
Range	2.7; 6.2	3.0; 6.5
Biceps skinfold average (mm)		
n	59	50
Mean (SD)	3.50 (0.663)	3.54 (0.704)
Median	3.33	3.42
Q1; Q3	3.07; 4.00	3.07; 3.93
Range	2.2; 5.3	2.3; 5.4
Suprailiac skinfold average (mm)		
n	59	50
Mean (SD)	4.36 (0.942)	4.55 (0.918)
Median	4.20	4.30
Q1; Q3	3.63; 4.93	3.97; 5.00
Range	2.9; 6.5	3.0; 7.3
Subscapular skinfold average (mm)		
n	59	50
Mean (SD)	4.33 (0.894)	4.74 (1.007)
Median	4.20	4.68
Q1; Q3	3.73; 4.97	4.07; 5.27
Range	2.6; 6.7	3.0; 8.4

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.
For each site, the average of the three skinfold measurements collected was considered.

Table 5-35 ANOVA model on infants' skinfold thickness by site; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Triceps skinfold average (mm)						
Visit 5 - Delivery	59	4.23 (4.01, 4.45)	50	4.42 (4.18, 4.66)	0.19 (-0.13, 0.51)	0.2422
Biceps skinfold average (mm)						
Visit 5 - Delivery	59	3.53 (3.35, 3.72)	50	3.56 (3.37, 3.76)	0.03 (-0.23, 0.29)	0.8313
Suprailiac skinfold average (mm)						
Visit 5 - Delivery	59	4.42 (4.17, 4.67)	50	4.60 (4.34, 4.86)	0.18 (-0.17, 0.53)	0.3074
Subscapular skinfold average (mm)						
Visit 5 - Delivery	59	4.38 (4.12, 4.63)	50	4.78 (4.51, 5.05)	0.40 (0.04, 0.76)	0.0292

PP=Per Protocol, LSMEANS=Least Squares Means.

n refers to the number of observations used in the model.

For each site, the average of the three skinfold measurements collected was considered.

[a] For each site, LSMEANS are derived based on the ANOVA model, in which the dependent variables is the average of skinfold measurements, and independent variables are study group and center as covariates.

Infants' APGAR score

APGAR score (assessing newborn's color, heart rate, reflex response, muscle tone and respiratory effort) at 1 and 5 minutes is shown in Table 5-36. No differences in mean APGAR score between the two groups were reported. APGAR score at 1 minute was normal for most subjects, except for 1 subject in both arms that presented a moderately abnormal score at 1 minute. Mean APGAR score at 1 minute was 9.46 ± 0.905 in the "No supplementation" group and 9.51 ± 0.924 in the "Elevit supplementation" one. APGAR score at 5 minutes was normal for all subjects. Mean APGAR score at 5 minutes was 9.90 ± 0.352 in the "No supplementation" arm and 9.89 ± 0.370 in the "Elevit supplementation" one, respectively. Results from the ANOVA model also reported no significant difference between the study groups (p-value for APGAR score at 1 minute was 0.5596 and at 5 minutes was 0.9113) (Table 5-37).

Table 5-36 Descriptive statistics of infants' APGAR score – one minute and five minutes; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
APGAR score at 1 minute		
n	68	61
Mean (SD)	9.46 (0.905)	9.51 (0.924)
Median	10.00	10.00
Q1; Q3	9.00; 10.00	9.00; 10.00
Range	5.0; 10.0	5.0; 10.0
Classification, n (%) [a,b]		
Moderately abnormal	1 (1.47)	1 (1.64)
Normal	67 (98.53)	60 (98.36)
APGAR score at 5 minutes		
n	68	61
Mean (SD)	9.90 (0.352)	9.89 (0.370)
Median	10.00	10.00
Q1; Q3	10.00; 10.00	10.00; 10.00
Range	8.0; 10.0	8.0; 10.0
Classification, n (%) [a,b]		
Normal	68 (100.00)	61 (100.00)

PP=Per Protocol. APGAR=Appearance, Pulse, Grimace, Activity, and Respiration.
n refers to the number of subjects who performed the assessment.
[a] Low: Apgar scores between 0 and 3; Moderately abnormal: Apgar scores between 4 and 6; Normal: Apgar scores greater or equal to 7.
[b] Percentages were computed on subjects who performed the assessment.

Table 5-37 ANOVA model on infants' APGAR score – one minute and five minutes; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
APGAR score at 1 minute						
Visit 5 - Delivery	68	9.36 (9.14, 9.58)	61	9.45 (9.22, 9.67)	0.09 (-0.22, 0.40)	0.5596
APGAR score at 5 minutes						
Visit 5 - Delivery	68	9.89 (9.80, 9.97)	61	9.88 (9.79, 9.97)	-0.01 (-0.13, 0.12)	0.9113

PP=Per Protocol, LSMEANS=Least Squares Means. APGAR=Appearance, Pulse, Grimace, Activity, and Respiration.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is Apgar score (at 1 minute and at 5 minutes respectively), and independent variables are study group and center as covariates.

Infants' bone density

Infants' bone density by unit of measure was assessed within 10 days after delivery. As shown in Table 5-38, bone density in m^2 was slightly higher in the control group than in the Elevit group (2990.93 ± 161.616 m^2 vs 2929.46 ± 168.879 m^2). According to the ANOVA model this difference was significant (p-value 0.0486, which is borderline) (Table 5-39). Bone density in % was 24.76 ± 25.031 % in the "No supplementation" arm and 20.25 ± 21.221 % in the "Elevit supplementation" arm, and bone density by z-score was -0.89 ± 0.985 and -1.15 ± 1.405, respectively (Table 5-38).

The ANOVA model shows no significant difference between study groups in both bone density in % and by z-score (p-value 0.3518 and 0.2936, respectively) (Table 5-39). Same results were reported for the ITT population.

Table 5-38 Descriptive statistics of infants' bone density by unit of measures; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Subjects performing Delivery Visit	69	62
Bone density (m2)		
n	59	50
Mean (SD)	2990.93 (161.616)	2929.46 (168.879)
Median	2972.00	2952.50
Q1; Q3	2928.00; 3069.00	2879.00; 3029.00
Range	2688.0; 3839.0	2325.0; 3215.0
Bone density (%)		
n	53	44
Mean (SD)	24.76 (25.031)	20.25 (21.221)
Median	13.00	10.50
Q1; Q3	5.10; 35.00	4.00; 32.00
Range	0.0; 92.0	0.0; 86.0
Bone density (z-score)		
n	53	44
Mean (SD)	-0.89 (0.985)	-1.15 (1.405)
Median	-1.00	-1.15
Q1; Q3	-1.40; -0.20	-1.75; -0.40
Range	-3.7; 1.4	-6.4; 2.4

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Table 5-39 ANOVA model on infants' bone density by unit of measures; PP population

	No supplementation (N=76)		Elevit supplementation (N=65)		Difference (Elevit - No Elevit)	
	n	LSMEANS (95% CI)	n	LSMEANS (95% CI)	LSMEANS [a] (95% CI)	p-value
Bone density (m2)						
Visit 5 - Delivery	59	2998.66 (2954.62, 3042.70)	50	2935.54 (2888.52, 2982.57)	-63.12 (-125.84, -0.40)	0.0486
Bone density (%)						
Visit 5 - Delivery	53	24.22 (17.43, 31.01)	44	19.74 (12.39, 27.09)	-4.48 (-13.99, 5.03)	0.3518
Bone density (z-score)						
Visit 5 - Delivery	53	-0.88 (-1.23, -0.54)	44	-1.14 (-1.52, -0.77)	-0.26 (-0.74, 0.23)	0.2936

PP=Per Protocol, LSMEANS=Least Squares Means.
n refers to the number of observations used in the model.
[a] LSMEANS are derived based on the ANOVA model, in which the dependent variable is bone density, and independent variables ar e study group and center as covariates.

5.2.3 Exploratory maternal efficacy variables

A total of 20 subjects were planned to be included in the subgroup of women undergoing elective Caesarean section. However, during the study, only 12 women were included in this subgroup. Samples were not collected at Delivery Visit for 1 subject in the "No supplementation" group and 2 subjects in the "Elevit supplementation" one. Therefore, each parameter was evaluated in 5 subjects of the control arm and 4 of the Elevit arm in the PP population, unless otherwise stated. Due to the small number of women in this subgroup, the ANOVA model that was planned in order to compare results between the two arms could not be performed. Therefore, only descriptive statistics are provided, and comparison described on median values (Q1; Q3).

Medians of most cord blood fatty acids parameters in RBCs were slightly higher in the "Elevit supplementation" arm compared with the "No supplementation" arm, except for cord blood TFA. Furthermore, the median of all placental RBC FA parameters was also slightly higher in the "Elevit supplementation" group than in the "No supplementation" group. Details for each parameter are presented below.

Cord blood TFA

Median cord blood TFA was 337.5 (Q1; Q3: 302.9; 342.9) in the control arm and 311.7 (Q1; Q3: 277.2; 357.6) in the Elevit one (Table 5-40).

Table 5-40 Descriptive statistics of cord blood TFA; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood TFA		
n	5	4
Mean (SD)	322.5 (42.52)	317.4 (55.10)
Median	337.5	311.7
Q1; Q3	302.9; 342.9	277.2; 357.6
Range	259; 370	258; 388

PP=Per Protocol, TFA=Total Fatty Acids.
n refers to the number of subjects who performed the assessment.

Cord blood DHA (wt% TFA)

As displayed in Table 5-41, median cord blood DHA (wt% TFA) was higher in the supplementation group than in the control one: median cord blood DHA (wt% TFA) was 8.05 (Q1; Q3: 7.27; 8.99) and 6.94 (Q1; Q3: 6.45; 7.46), respectively.

Table 5-41 Descriptive statistics of cord blood DHA (wt% total fatty acid); PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood DHA (wt% TFA)		
n	5	4
Mean (SD)	6.91 (0.628)	8.13 (1.368)
Median	6.94	8.05
Q1; Q3	6.45; 7.46	7.27; 8.99
Range	6.1; 7.6	6.5; 9.9

PP=Per Protocol, DHA=docosahexaenoic acid, wt%=weight percent, TFA=Total Fatty Acids.
n refers to the number of subjects who performed the assessment.

Cord blood EPA (wt% TFA)

As shown in Table 5-42 median cord blood EPA (wt% TFA) was slightly higher in the Elevit group compared with the control one: 2.92 (Q1; Q3: 2.40; 3.68) and 2.46 (Q1; Q3: 2.19; 2.98), respectively.

Table 5-41 Descriptive statistics of cord blood EPA (wt% total fatty acid); PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood EPA (wt% TFA)		
n	5	4
Mean (SD)	2.72 (0.928)	3.04 (0.764)
Median	2.46	2.92
Q1; Q3	2.19; 2.98	2.40; 3.68
Range	1.8; 4.2	2.4; 3.9

PP=Per Protocol, EPA=eicosapentaenoic acid, wt%=weight percent, TFA=Total Fatty Acids.
n refers to the number of subjects who performed the assessment.

Cord blood DHA/TFA ratio

Median DHA/TFA ratio in cord blood was also slightly greater in the Elevit group than in the control one (Table 5-42): 0.080 (Q1; Q3: 0.073; 0.090) vs 0.069 (Q1; Q3: 0.064; 0.075).

Table 5-42 Descriptive statistics of cord blood DHA/TFA ratio; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood DHA/TFA ratio		
n	5	4
Mean (SD)	0.069 (0.0063)	0.081 (0.0137)
Median	0.069	0.080
Q1; Q3	0.064; 0.075	0.073; 0.090
Range	0.06; 0.08	0.07; 0.10

PP=Per Protocol, DHA=docosahexaenoic acid, TFA=Total Fatty Acids.
n refers to the number of subjects who performed the assessment.

Cord blood omega 3 index

Median cord blood omega 3 index was also higher in the Elevit group compared with the "No supplementation" group (Table 5-44): 8.96 (Q1; Q3: 6.16; 10.10) and 7.78 (Q1; Q3: 7.67; 8.17), respectively.

Table 5-43 Descriptive statistics of cord blood omega 3 index; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood Omega 3 index		
n	5	4
Mean (SD)	7.75 (0.560)	8.13 (3.335)
Median	7.78	8.96
Q1; Q3	7.67; 8.17	6.16; 10.10
Range	6.9; 8.3	3.4; 11.2

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Cord blood 25-hydroxyvitamin D

Median cord blood 25-hydroxyvitamin D was markedly greater in the supplementation arm compared with the control one (Table 5-45) 23.60 µg/L (Q1; Q3: 17.80; 25.00) and 13.10 µg/L (Q1; Q3: 10.10; 18.30), respectively.

Table 5-44 Descriptive statistics of cord blood 25-hydroxyvitamin D; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood 25-hydroxyvitamin D (ug/L)		
n	5	4
Mean (SD)	13.04 (6.996)	21.40 (5.722)
Median	13.10	23.60
Q1; Q3	10.10; 18.30	17.80; 25.00
Range	3.0; 20.7	13.0; 25.4

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Cord blood GSH/GSSG ratio

The GSH/GSSG ratio in cord blood was determined in 4 subjects of the control arm and 1 of the Elevit arm. Results are shown in Table 5-46.

Table 5-45 Descriptive statistics of cord blood GSH/GSSG ratio; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood GSH/GSSG ratio		
n	4	1
Mean (SD)	4.96 (0.626)	4.68
Median	5.14	4.68
Q1; Q3	4.50; 5.41	4.68; 4.68
Range	4.1; 5.4	4.7; 4.7

PP=Per Protocol, GSH=reduced glutathione, GSSG=oxidized glutathione.
n refers to the number of subjects who performed the assessment.

Cord blood ROMs

As shown in Table 5-47, levels of cord blood ROMs were higher in the supplementation arm compared with the control one. Median cord blood ROMs was 9.32 mg H202/dL (Q1; Q3: 7.11; 10.90) in the "No supplementation" arm and 12.29 mg H202/dL (Q1; Q3: 6.83; 13.19) in the "Elevit supplementation" one.

Table 5-46 Descriptive statistics of cord blood ROMs; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood ROMs (mg H2O2/dL)		
n	5	4
Mean (SD)	9.11 (2.749)	10.01 (5.392)
Median	9.32	12.29
Q1; Q3	7.11; 10.90	6.83; 13.19
Range	5.7; 12.5	2.0; 13.5

PP=Per Protocol, ROMs=reactive oxygen metabolites.
n refers to the number of subjects who performed the assessment.

Cord blood 8-isoprostane

Median cord blood 8-isoprostane was similar in both groups (Table 5-48).

Table 5-47 Descriptive statistics of cord blood 8-isoprostane; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Cord blood 8-isoprostane (pg/mL)		
n	5	4
Mean (SD)	155.24 (76.487)	131.15 (22.297)
Median	125.30	124.45
Q1; Q3	113.50; 212.20	115.35; 146.95
Range	69.3; 255.9	113.2; 162.5

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Umbilical cord blood gas and pH analysis

As shown in Table 5-49, all parameters (pH, CO_2 partial pressure, O_2 partial pressure, lactate and hemoglobin) were comparable in both groups.

Table 5-48 Descriptive statistics of umbilical cord blood gas and pH analysis; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
pH		
n	5	4
Mean (SD)	7.37 (0.039)	7.36 (0.036)
Median	7.38	7.36
Q1; Q3	7.33; 7.38	7.33; 7.39
Range	7.3; 7.4	7.3; 7.4
CO2 partial pressure		
n	5	4
Mean (SD)	43.66 (5.067)	43.60 (6.119)
Median	45.00	44.00
Q1; Q3	44.00; 46.30	39.00; 48.20
Range	35.0; 48.0	36.0; 50.4
O2 partial pressure		
n	5	4
Mean (SD)	21.86 (8.646)	21.30 (2.568)
Median	22.30	20.55
Q1; Q3	17.00; 25.00	19.70; 22.90
Range	11.0; 34.0	19.1; 25.0
Lactate (mmol/L)		
n	5	4
Mean (SD)	1.80 (0.200)	1.70 (0.294)
Median	1.70	1.70
Q1; Q3	1.70; 1.90	1.45; 1.95
Range	1.6; 2.1	1.4; 2.0
Hemoglobin (g/L)		
n	5	4
Mean (SD)	155.40 (11.459)	156.75 (16.460)
Median	155.00	157.00
Q1; Q3	152.00; 161.00	143.50; 170.00
Range	139.0; 170.0	138.0; 175.0

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Placental weight and biometric parameters

Placental weight and biometric parameters, such as chorionic elliptical disc larger diameter, chorionic elliptical disc smaller diameter and fetal/placental weight ratio, were similar in both groups. Refer to Table 5-50 for details.

Table 5-49 Descriptive statistics of placental weight and biometric parameters; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Chorionic elliptical disc larger (D) diameter (cm)		
n	5	4
Mean (SD)	21.8 (1.75)	20.5 (2.65)
Median	21.0	20.0
Q1; Q3	21.0; 22.5	18.5; 22.5
Range	20; 25	18; 24
Chorionic elliptical disc smaller (D) diameter (cm)		
n	5	4
Mean (SD)	16.8 (2.28)	18.5 (2.38)
Median	16.0	17.5
Q1; Q3	16.0; 18.0	17.0; 20.0
Range	14; 20	17; 22
Fetal/placental weight (F/P ratio)		
n	5	4
Mean (SD)	7.2 (1.19)	7.5 (1.90)
Median	6.9	7.0
Q1; Q3	6.3; 7.9	6.0; 8.9
Range	6; 9	6; 10

PP=Per Protocol.
n refers to the number of subjects who performed the assessment.

Placental TFA

Median placental TFA was markedly higher in the Elevit arm compared with the control one (Table 5-51): 510.3 (Q1; Q3: 436.3; 540.4) and 364.4 (Q1; Q3: 362.0; 480.2), respectively.

Table 5-50 Descriptive statistics of placental TFA; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental TFA		
n	5	4
Mean (SD)	411.5 (88.45)	488.3 (84.93)
Median	364.4	510.3
Q1; Q3	362.0; 480.2	436.3; 540.4
Range	322; 529	367; 566

PP=Per Protocol, TFA=Total Fatty Acids.

Placental DHA (wt% TFA)

As shown in Table 5-52, placental DHA (wt% TFA) was higher in the supplementation group than in the control one. Median DHA (wt% TFA) was 3.96 (Q1; Q3: 3.95; 4.06) in the control group and 4.90 (Q1; Q3: 4.58; 5.57) in the Elevit one.

Table 5-51 Descriptive statistics of placental DHA (wt% total fatty acid); PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental DHA (wt% TFA)		
n	5	4
Mean (SD)	4.14 (0.504)	5.08 (0.732)
Median	3.96	4.90
Q1; Q3	3.95; 4.06	4.58; 5.57
Range	3.7; 5.0	4.4; 6.1

PP=Per Protocol, DHA=docosahexaenoic acid, wt%=weight percent, TFA=Total Fatty Acids.

Placental EPA (wt% TFA)

Median placental EPA (wt% TFA) was also greater in the supplementation arm compared with the control one (Table 5:53): 1.28 (Q1; Q3: 1.01; 1.48) and 0.78 (Q1; Q3: 0.58; 0.86), respectively.

Table 5-52 Descriptive statistics of placental EPA (wt% total fatty acid); PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental EPA (wt% TFA)		
n	5	4
Mean (SD)	0.80 (0.272)	1.24 (0.291)
Median	0.78	1.28
Q1; Q3	0.58; 0.86	1.01; 1.48
Range	0.6; 1.2	0.9; 1.5

PP=Per Protocol, EPA=eicosapentaenoic acid, wt%=weight percent, TFA=Total Fatty Acids.

Placental DHA/TFA ratio

As displayed in Table 5-54 median placental DHA/TFA ratio was higher in the Elevit group than in the control one: 0.049 (Q1; Q3: 0.046; 0.056) vs 0.040 (Q1; Q3: 0.040; 0.041).

Table 5-53 Descriptive statistics of placental DHA/TFA ratio; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental DHA/TFA ratio		
n	5	4
Mean (SD)	0.041 (0.0050)	0.051 (0.0073)
Median	0.040	0.049
Q1; Q3	0.040; 0.041	0.046; 0.056
Range	0.04; 0.05	0.04; 0.06

PP=Per Protocol, DHA=docosahexaenoic acid, TFA=Total Fatty Acids.

Placental omega 3 index

Median omega 3 index in the placenta was also greater in the supplementation group compared with the control one (Table 5-55): 5.14 (Q1; Q3: 4.82; 5.85) vs 4.13 (Q1; Q3: 4.11; 4.33).

Table 5-54 Descriptive statistics of placental omega 3 index; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental Omega 3 index		
n	5	4
Mean (SD)	4.34 (0.534)	5.34 (0.730)
Median	4.13	5.14
Q1; Q3	4.11; 4.33	4.82; 5.85
Range	3.9; 5.2	4.7; 6.4

PP=Per Protocol.

Placental 8-isoprostane

Median placental 8-isoprostane was higher in the Elevit group than in the control group (Table 5-56) 91.33 (Q1; Q3: 81.73; 111.27) vs 73.29 (Q1; Q3: 70.87; 83.86).

Table 0-55 Descriptive statistics of placental 8-isoprostane; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental 8-isoprostane (pg/mL)		
n	5	4
Mean (SD)	76.44 (10.120)	96.50 (22.639)
Median	73.29	91.33
Q1; Q3	70.87; 83.86	81.73; 111.27
Range	64.6; 89.6	75.1; 128.2

PP=Per Protocol.

Placental mitochondrial DNA levels

Median placental mitochondrial DNA levels, as a marker of oxidative stress, was lower in the supplementation group compared with the control one: 154.36 (Q1; Q3: 116.06; 270.65) and 169.91 (Q1; Q3: 72.80; 203.84), respectively (Table 5-57).

Table 5-56 Descriptive statistics of placental mitochondrial DNA levels; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Placental mtDNA levels		
n	5	4
Mean (SD)	184.67 (140.628)	193.36 (114.464)
Median	169.91	154.36
Q1; Q3	72.80; 203.84	116.06; 270.65
Range	64.9; 411.9	106.8; 357.9

PP=Per Protocol, mtDNA= mitochondrial DNA.

Mitochondrial DNA levels in placental cytotrophoblast cells

Mitochondrial DNA levels in placental cytotrophoblast cells were also lower in the Elevit group than in the control group: 320.81 (Q1; Q3: 202.82; 413.26) and 333.05 (Q1; Q3: 166.51; 356.11), respectively (Table 5-58).

Table 5-57 Descriptive statistics of mitochondrial DNA levels in placental cytotrophoblast cells; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
mtDNA levels in placental cytotrophoblast cells		
n	5	4
Mean (SD)	301.25 (144.144)	308.04 (161.169)
Median	333.05	320.81
Q1; Q3	166.51; 356.11	202.82; 413.26
Range	152.4; 498.2	99.2; 491.3

PP=Per Protocol, mtDNA= mitochondrial DNA.

Placental interleukin-6 gene expression

Expression of the proinflammatory cytokine interleukin-6 (*IL6*) gene was higher in the Elevit group compared with the control one (Table 5-59) median was 0.38 (Q1; Q3: 0.22; 1.01) and 0.25 (Q1; Q3: 0.21; 0.72), respectively.

Table 5-58 Descriptive statistics of placental interleukin 6 (*IL6*) gene expression; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
IL-6 gene expression placental		
n	5	4
Mean (SD)	0.42 (0.287)	0.62 (0.616)
Median	0.25	0.38
Q1; Q3	0.21; 0.72	0.22; 1.01
Range	0.2; 0.8	0.2; 1.5

PP=Per Protocol.

Interleukin-6 gene expression in placental cytotrophoblast cells

Median expression of *IL6* gene in placental cytotrophoblast cells was 0.13 (Q1; Q3: 0.06; 0.20) in the control group and 0.19 (Q1; Q3: 0.10; 0.28) in the Elevit group (Table 5-60).

Table 5-59 Descriptive statistics of interleukin 6 (*IL6*) gene expression in placental cytotrophoblast cells; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
IL-6 gene expression placental cytotrophoblast cells		
n	5	4
Mean (SD)	0.32 (0.479)	0.19 (0.119)
Median	0.13	0.19
Q1; Q3	0.06; 0.20	0.10; 0.28
Range	0.1; 1.2	0.0; 0.3

PP=Per Protocol.

Placental interleukin-10 gene expression

Expression of the anti-inflammatory cytokine *IL10* gene in the placenta was similar between the two study arms (Table 5-61).

Table 5-60 Descriptive statistics of placental interleukin 10 (*IL10*) gene expression; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
IL-10 gene expression placental		
n	5	4
Mean (SD)	0.35 (0.133)	0.36 (0.188)
Median	0.31	0.31
Q1; Q3	0.25; 0.38	0.25; 0.48
Range	0.2; 0.6	0.2; 0.6

PP=Per Protocol.

Interleukin-10 gene expression in placental cytotrophoblast cells

Median *IL10* gene expression in placental cytotrophoblast cells was higher in the "No supplementation" arm compared with the "Elevit supplementation" one: 0.13 (Q1; Q3: 0.07; 0.16) vs 0.05 (Q1; Q3: 0.03; 0,67) (Table 5-62).

Table 5-61 Descriptive statistics of interleukin 10 (*IL10*) gene expression in p**lacental cytotrophoblast cells; PP population – Caesarean section**

	No supplementation (N=6)	Elevit supplementation (N=6)
IL-10 gene expression placental cytotrophoblast cells		
n	5	4
Mean (SD)	0.12 (0.078)	0.35 (0.628)
Median	0.13	0.05
Q1; Q3	0.07; 0.16	0.03; 0.67
Range	0.0; 0.2	0.0; 1.3

PP=Per Protocol.

Placental tumor necrosis factor-alpha gene expression

Gene expression of the proinflammatory tumor necrosis factor-alpha (*TNFα*) gene in the placenta was lower in the Elevit group compared with the control one: 0.18 (Q1; Q3: 0.18; 0.35) and 0.21 (Q1; Q3: 0.21; 0.25), respectively (Table 5-63).

Table 5-62 Descriptive statistics of placental tumor necrosis factor-alpha (*TNFα*) gene expression; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
TNF-alpha gene expression placental		
n	5	4
Mean (SD)	0.27 (0.164)	0.26 (0.162)
Median	0.21	0.18
Q1; Q3	0.21; 0.25	0.18; 0.35
Range	0.1; 0.6	0.2; 0.5

PP=Per Protocol.

Tumor necrosis factor-alpha gene expression in placental cytotrophoblast cells

Median TNF-α gene expression in placental cytotrophoblast cells was similar in both study arms (Table 5-64).

Table 5-63 Descriptive statistics of tumor necrosis factor-alpha (*TNFα*) gene expression in placental cytotrophoblast cells; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
TNF-alpha gene expression placental cytotrophoblast cells		
n	5	4
Mean (SD)	0.11 (0.179)	0.34 (0.639)
Median	0.02	0.02
Q1; Q3	0.02; 0.06	0.01; 0.66
Range	0.0; 0.4	0.0; 1.3

PP=Per Protocol.

Maternal blood parameters in women undergoing elective Caesarean section

Descriptive statistics of blood parameters in subjects of the PP population undergoing elective Caesarean section is presented in the following tables (Table 5-65, Table 5-66 and Table 5-67).

Variables were assessed in 6 subjects of the control group and 6 of the Elevit group at Visit 1, 3 and 4, and in 5 and 4 women, respectively, at Visit 5.

Descriptive statistics of fatty acids parameters in RBCs in maternal blood of subjects undergoing elective Caesarean section is presented in Table 5-65. These results were comparable with those of the PP population.

Table 5-64 Descriptive statistics of maternal blood RBC FA parameters at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
RBC DHA (wt% TFA)		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	6.12 (1.118)	5.67 (1.229)
Median	5.77	5.37
Q1; Q3	5.35; 7.12	4.84; 6.76
Range	4.9; 7.8	4.2; 7.5
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	6.79 (1.246)	6.19 (0.932)
Median	6.23	6.10
Q1; Q3	6.15; 7.55	5.72; 6.91
Range	5.6; 9.0	4.8; 7.5
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	6.48 (1.327)	6.58 (1.025)
Median	6.74	6.70
Q1; Q3	5.14; 7.30	5.53; 7.45
Range	4.7; 8.2	5.3; 7.8
Visit 5 - Delivery		
n	5	4
Mean (SD)	6.06 (1.078)	6.52 (0.980)
Median	6.01	6.77
Q1; Q3	5.19; 6.36	5.86; 7.19
Range	5.0; 7.7	5.1; 7.4
RBC TFA		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	6004.5 (3570.61)	5239.4 (2245.72)
Median	5085.6	4529.6
Q1; Q3	3444.6; 7160.5	3440.5; 7056.9
Range	2830; 12421	3250; 8630
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	5832.2 (3099.17)	5466.5 (2617.10)
Median	4871.5	5007.7
Q1; Q3	3216.0; 8127.3	3094.8; 7714.3
Range	3014; 10893	3025; 8949
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	6108.1 (4044.71)	5383.0 (2686.52)
Median	4700.8	4779.9
Q1; Q3	3553.4; 6325.1	3200.8; 6819.5
Range	3390; 13979	3039; 9679
Visit 5 - Delivery		
n	5	4
Mean (SD)	335.3 (53.57)	426.5 (49.11)
Median	343.3	440.4
Q1; Q3	282.6; 362.1	396.3; 456.7
Range	282; 406	356; 469
RBC EPA (wt% TFA)		

Table 5-64 Descriptive statistics of maternal blood RBC FA parameters at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	0.46 (0.176)	0.47 (0.203)
Median	0.38	0.42
Q1; Q3	0.33; 0.62	0.29; 0.60
Range	0.3; 0.7	0.3; 0.8
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	0.38 (0.182)	0.48 (0.257)
Median	0.31	0.36
Q1; Q3	0.23; 0.58	0.32; 0.71
Range	0.2; 0.6	0.3; 0.9
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	0.40 (0.242)	0.49 (0.253)
Median	0.29	0.40
Q1; Q3	0.24; 0.60	0.29; 0.72
Range	0.2; 0.8	0.3; 0.9
Visit 5 - Delivery		
n	5	4
Mean (SD)	1.05 (0.348)	2.47 (1.283)
Median	1.00	2.23
Q1; Q3	0.91; 1.33	1.49; 3.45
Range	0.6; 1.4	1.3; 4.2
RBC DHA/TFA ratio		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	0.061 (0.0112)	0.057 (0.0123)
Median	0.058	0.054
Q1; Q3	0.053; 0.071	0.048; 0.068
Range	0.05; 0.08	0.04; 0.07
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	0.068 (0.0125)	0.062 (0.0093)
Median	0.062	0.061
Q1; Q3	0.062; 0.075	0.057; 0.069
Range	0.06; 0.09	0.05; 0.07
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	0.065 (0.0133)	0.066 (0.0102)
Median	0.067	0.067
Q1; Q3	0.051; 0.073	0.055; 0.074
Range	0.05; 0.08	0.05; 0.08
Visit 5 - Delivery		
n	5	4
Mean (SD)	0.061 (0.0108)	0.065 (0.0098)
Median	0.060	0.068
Q1; Q3	0.052; 0.064	0.059; 0.072
Range	0.05; 0.08	0.05; 0.07
Omega 3 index		
Visit 1 - Screening (GA Week 11/14)		

Table 5-64 Descriptive statistics of maternal blood RBC FA parameters at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
n	6	6
Mean (SD)	6.58 (1.276)	6.14 (1.265)
Median	6.13	6.01
Q1; Q3	5.73; 7.85	5.21; 7.37
Range	5.2; 8.4	4.5; 7.7
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	7.17 (1.406)	6.67 (1.032)
Median	6.53	6.69
Q1; Q3	6.38; 8.16	6.12; 7.23
Range	5.8; 9.6	5.1; 8.2
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	6.88 (1.519)	7.08 (1.232)
Median	7.16	7.26
Q1; Q3	5.42; 7.61	5.80; 7.82
Range	4.9; 9.0	5.6; 8.7
Visit 5 - Delivery		
n	5	4
Mean (SD)	6.38 (1.188)	14.16 (13.045)
Median	6.28	7.94
Q1; Q3	5.39; 6.69	7.32; 21.01
Range	5.3; 8.2	7.0; 33.7

PP=Per Protocol, DHA=docosahexaenoic acid, wt%=weight percent, TFA=Total Fatty Acids, EPA=eicosapentaenoic acid, GA=Gestational Age.
n refers to the number of subjects who performed the assessment.

Maternal blood 25-hydroxyvitamin D in the subgroup of women who underwent Caesarean section decreased by visit in the non-supplemented group, whereas it increased in the supplemented one. Details are presented in Table 5-66.

Table 5-65 Descriptive statistics of maternal blood 25-hydroxyvitamin D at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
25-hydroxyvitamin D (ug/L)		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	25.12 (10.151)	22.83 (5.569)
Median	26.40	22.60
Q1; Q3	15.70; 34.20	19.40; 28.40
Range	11.2; 36.8	14.6; 29.4
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	19.55 (10.291)	25.10 (13.157)
Median	20.95	21.75
Q1; Q3	8.40; 26.50	13.20; 39.00
Range	8.2; 32.3	11.7; 43.2
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	17.65 (9.025)	23.55 (9.833)
Median	15.90	21.65
Q1; Q3	12.80; 17.70	16.70; 31.90
Range	8.8; 34.8	11.5; 37.9
Visit 5 - Delivery		
n	5	4
Mean (SD)	18.60 (10.868)	21.42 (10.232)
Median	14.80	17.95
Q1; Q3	12.80; 15.30	14.20; 28.65
Range	12.2; 37.9	13.9; 35.9

PP=Per Protocol.

n refers to the number of subjects who performed the assessment.

Oxidative status in maternal blood at Visit 1, 3, 4 and 5, in subjects undergoing elective Caesarean section is presented in Table 5-67. Median GSH/GSSH ratio and ROMs at Visit 5 were lower in the “Elevit supplementation” arm than in the “No supplementation” arm, while median 8-isoprostane levels were higher in the “Elevit supplementation” group.

Table 5-66 Descriptive of maternal blood oxidative status at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
GSH/GSSG ratio		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	6.13 (2.815)	5.69 (2.221)
Median	4.95	5.57
Q1; Q3	4.13; 8.98	3.72; 6.75
Range	3.4; 10.3	3.2; 9.3
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	6.06 (2.987)	6.56 (2.036)
Median	5.24	6.36
Q1; Q3	4.20; 7.32	4.66; 8.45
Range	3.0; 11.4	4.2; 9.3
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	6.07 (1.815)	7.23 (1.372)
Median	5.57	6.79
Q1; Q3	5.38; 7.06	6.46; 8.44
Range	3.8; 9.1	5.6; 9.3
Visit 5 - Delivery		
n	5	4
Mean (SD)	6.70 (0.683)	5.63 (0.349)
Median	6.72	5.62
Q1; Q3	6.13; 6.98	5.34; 5.92
Range	6.0; 7.7	5.3; 6.0
ROMs (mg H2O2/dL)		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	33.23 (4.884)	33.38 (7.975)
Median	32.10	30.69
Q1; Q3	30.02; 38.57	27.11; 40.10
Range	27.1; 39.5	25.9; 45.8
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	34.66 (3.558)	35.08 (10.114)
Median	34.27	34.52
Q1; Q3	31.89; 37.33	25.23; 45.91
Range	30.7; 39.5	24.2; 46.1
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	36.82 (4.820)	34.66 (10.934)
Median	36.50	35.61
Q1; Q3	33.32; 40.31	23.79; 42.77
Range	30.7; 43.6	21.4; 48.8
Visit 5 - Delivery		
n	5	4
Mean (SD)	36.12 (3.174)	36.89 (4.878)
Median	36.79	35.88
Q1; Q3	35.88; 38.20	33.35; 40.42

Table 5-66 Descriptive of maternal blood oxidative status at each visit; PP population – Caesarean section

	No supplementation (N=6)	Elevit supplementation (N=6)
Range	30.8; 38.9	32.3; 43.5
8-isoprostane (pg/mL)		
Visit 1 - Screening (GA Week 11/14)		
n	6	6
Mean (SD)	82.87 (42.615)	133.38 (70.991)
Median	73.60	108.90
Q1; Q3	54.70; 88.70	93.20; 158.70
Range	43.3; 163.3	66.8; 263.8
Visit 3 (GA Week 24/26)		
n	6	6
Mean (SD)	147.28 (84.362)	222.87 (174.130)
Median	110.80	193.05
Q1; Q3	86.60; 213.40	60.90; 327.40
Range	77.1; 285.0	57.8; 505.0
Visit 4 (GA Week 34/36)		
n	6	6
Mean (SD)	190.75 (100.754)	201.07 (116.484)
Median	184.50	169.55
Q1; Q3	112.90; 301.50	119.70; 252.10
Range	54.8; 306.3	86.2; 409.3
Visit 5 - Delivery		
n	5	4
Mean (SD)	441.52 (152.471)	499.93 (171.434)
Median	362.40	570.95
Q1; Q3	345.30; 518.90	395.55; 604.30
Range	307.9; 673.1	246.4; 611.4

PP=Per Protocol, GSH=reduced glutathione, GSSG=oxidized glutathione, ROMs=reactive oxygen metabolites, GA=Gestational Age.
n refers to the number of subjects who performed the assessment.

5.3 EFFICACY CONCLUSIONS

Women in the "Elevit supplementation" group showed a good overall compliance to the study product: ≥80% of the supplementation was taken by sixty-three (63, 72.41%) subjects.

Analysis of the primary maternal variable indicated an increase in the levels of DHA (wt% TFA) in RBCs in both groups during pregnancy and to a greater extent in the Elevit arm: absolute change from baseline was 0.52 [95% CI: 0.28 to 0.76] in the "No supplementation" arm and 1.48 [95% CI: 1.22 to 1.74] in the "Elevit supplementation" one. The difference in absolute change between the two groups (Elevit supplementation – No supplementation) was 0.96 [95% CI: 0.61 to 1.31] and this difference was highly significant (p-value <0.0001), demonstrating a benefit of supplementation with Elevit soft gel capsules during 2nd and 3rd trimesters of pregnancy on the DHA status of the mother.

Furthermore, among the secondary maternal efficacy variables analyzed, a similar trend as for the primary endpoint was observed for the DHA/TFA ratio and the Omega-3 index in RBCs. In contrast, supplementation with Elevit had no effect on the levels of maternal TFA and EPA (wt% TFA) in RBCs in the 3rd trimester. Furthermore, levels of 25-hydroxyvitamin D in blood in the Elevit arm remained stable in contrast to the "No supplementation" arm, where there was a decrease. Intake of the study product did not influence maternal oxidative status, as reflected by the lack of modulation of the GSH/GSSG ratio, of the levels of ROMs and of 8-isoprostane in blood.

Supplementation with Elevit did not affect any parameters of the infant analyzed. Overall, there was no difference in gestational age, head circumference, weight, length, ponderal index, skinfold thickness, APGAR score and bone density between the two groups.

Due to the small number of subjects included in the subgroup of women undergoing elective Caesarean section, no comparison between study groups concerning the exploratory variables could be performed. However, a trend could be observed describing higher medians of most cord blood and placental RBC fatty acids parameters in the supplementation group compared with the control group. These results suggest a positive effect of "Elevit supplementation" on fetal PUFAs status. On the other hand, no clear trend could be described for the oxidative and inflammatory parameters in the placenta and in placental cytotrophoblast cells. Analyses of the presented exploratory variables in a larger group of patients is required in order to draw conclusions about a possible impact of supplementation with Elevit on the explored variables.

Overall, supplementation with Elevit during 2nd and 3rd trimesters of pregnancy was beneficial for the mother and did not show a negative effect neither on mother status nor on neonatal health.

6. SAFETY EVALUATION

All the safety analyses presented in this section were performed on the safety (SAF) population.

6.1 EXTENT OF EXPOSURE

As presented in Table 6-1, the overall mean study duration was 24.46 ± 6.489 weeks (range: 1.0 – 30.9). Similar results were reported for both the control and supplementation group.

Table 6-1 Summary of study duration; Safety population

	No supplementation (N=89)	Elevit supplementation (N=87)	Total (N=176)
Study duration (weeks)			
n	89	87	176
Mean (SD)	25.05 (5.768)	23.85 (7.134)	24.46 (6.489)
Median	26.86	26.86	26.86
Q1; Q3	25.43; 28.29	22.57; 28.14	24.21; 28.29
Range	2.0; 30.9	1.0; 30.1	1.0; 30.9

Study duration was computed as the time (in weeks) elapsed from the date of the Screening Visit and the date of the last performed visit, excluding Follow-up Visit, plus one day.

Mean duration of exposure in the Elevit group was 22.06 ± 6.669 weeks (range: 0.4 – 30.6) (Table 6-2).

Table 6-2 Summary of exposure; Safety population

	Elevit supplementation (N=87)
Duration of exposure (weeks)	
n	87
Mean (SD)	22.06 (6.669)
Median	24.57
Q1; Q3	20.71; 26.14
Range	0.4; 30.6

Duration of exposure was computed for Elevit supplementation as the time (in weeks) elapsed from the start date of first treatment dispensing (i.e. date of Visit 2) to the last consumption collected in the 'End of treatment' page as last known date in which subject took the investigational product plus one day.

6.2 ADVERSE EVENTS

6.2.1 Brief summary of adverse events

One hundred-twenty-five (125, 71.02%) subjects reported at least one treatment-emergent adverse event (TEAE) pertinent to the mother, for a total of two hundred thirty-two (232) TEAEs pertinent to the mother reported (Table 6-3): one hundred fourteen (114) TEAEs in the control group and one hundred eighteen (118) in the Elevit one. Frequencies of subjects affected by at least one TEAE pertinent to the mother ranged from 71.91% (64 subjects, "No supplementation" arm) to 70.11% (61 subjects, "Elevit supplementation" arm). Three (3, 3.45%) subjects of the Elevit group had at least one TEAE suspected of being related to the study product. Twenty-three (23, 13.07%) women reported at least one serious TEAE (TESAE). Frequencies were similar across both groups: eleven (11, 12.36%) subjects in the control group and twelve (12, 13.79%) subjects in the supplementation one reported at least one TESAE pertinent to the mother. One (1, 1.15%) subject in the Elevit group experienced a TEAE leading to temporary interruption of supplements intake and nineteen (19, 21.84%) women had at least one TEAE leading to permanent treatment discontinuation. No TEAE pertinent to the mother had a fatal outcome.

Table 6-3 Summary of subjects with treatment emergent Adverse Events pertinent to the mother; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of TEAEs pertinent to the mother	114	118	232
Any TEAEs pertinent to the mother	64 (71.91)	61 (70.11)	125 (71.02)
At least one suspected related [a]	NA	3 (3.45)	3 (1.70)
At least one serious TEAE	11 (12.36)	12 (13.79)	23 (13.07)
At least one leading to temporary treatment interruption [b]	NA	1 (1.15)	1 (0.57)
At least one leading to permanent treatment discontinuation [c]	NA	19 (21.84)	19 (10.80)
Fatal outcome	0	0	0

NA=Not Applicable.

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.

[a] Suspected related adverse events were those events with causal relationship equal to related.

[b] Adverse events leading to temporary treatment interruption were those events with action taken equal to drug interrupted.

[c] Adverse events leading to permanent treatment discontinuation were those events with action taken equal to drug withdrawn.

Ten (10, 5.68%) subjects experienced at least one TEAE pertinent to the fetus/child, for a total of thirteen (13) TEAEs reported (Table 6-4): four (4) TEAEs in the control group and nine (9) in the supplementation group. Frequencies of subjects affected by at least one TEAE pertinent to the fetus/child ranged from 3.37% (3 women, "No supplementation" group) to 8.05% (7 women, "Elevit supplementation" group). No TEAE was suspected of being related to the study supplement. A total of five (5, 2.84%) women reported at least one TESAE pertinent to the fetus/child and frequencies were similar between both groups: two (2, 2.25%) subjects in the control arm and three (3, 3.45%) in the Elevit arm. One (1, 1.15%) subject in the Elevit group experienced a TEAE leading to temporary treatment interruption and one (1, 1.15%) subject had a TEAE leading to permanent treatment discontinuation. One (1, 1.15%) subject in the supplementation group experienced TEAEs pertinent to the fetus/child with fatal outcome.

Table 6-4 Summary of subjects with treatment emergent Adverse Events pertinent to the fetus/child; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of TEAEs pertinent to fetus/child	4	9	13
Any TEAEs pertinent to fetus/child	3 (3.37)	7 (8.05)	10 (5.68)
At least one suspected related [a]	NA	0	0
At least one serious TEAE	2 (2.25)	3 (3.45)	5 (2.84)
At least one leading to temporary treatment interruption [b]	NA	1 (1.15)	1 (0.57)
At least one leading to permanent treatment discontinuation [c]	NA	1 (1.15)	1 (0.57)
Fatal outcome	0	1 (1.15)	1 (0.57)

NA=Not Applicable.

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.

[a] Suspected related adverse events were those events with causal relationship equal to related.

[b] Adverse events leading to temporary treatment interruption were those events with action taken equal to drug interrupted.

[c] Adverse events leading to permanent treatment discontinuation were those events with action taken equal to drug withdrawn.

Twenty-three (23, 13.07%) subjects reported at least one TESAE pertinent to the mother, for a total of twenty-four (24) TESAEs pertinent to the mother reported: eleven (11) TESAEs in the "No supplementation" group and thirteen (13) in the "Elevit supplementation" one (Table 6-5). Among the twenty-three (23) subjects that reported at least one TESAEs pertinent to the mother, eleven (11, 12.36%) belonged to the control group and twelve (12, 13.79%) to the Elevit one. None of the subjects reported a TESAE pertinent to the mother suspected of being related to the study product nor a TESAE leading to temporary interruption of supplements intake. Four (4, 4.60%) subjects in the Elevit group had at least one TESAE leading to permanent treatment discontinuation. No TESAE had a fatal outcome.

Table 6-5 Summary of subjects with treatment emergent Serious Adverse Events pertinent to the mother; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of serious TEAEs pertinent to the mother	11	13	24
Any serious TEAEs pertinent to the mother	11 (12.36)	12 (13.79)	23 (13.07)
At least one suspected related [a]	NA	0	0
At least one leading to temporary treatment interruption [b]	NA	0	0
At least one leading to permanent treatment discontinuation [c]	NA	4 (4.60)	4 (2.27)
Fatal outcome	0	0	0

NA=Not Applicable.

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.

[a] Suspected related adverse events were those events with causal relationship equal to related.

[b] Adverse events leading to temporary treatment interruption were those events with action taken equal to drug interrupted.

[c] Adverse events leading to permanent treatment discontinuation were those events with action taken equal to drug withdrawn.

Five (5, 2.84%) subjects reported at least one TESAE pertinent to the fetus/child, for a total of seven (7) TESAEs pertinent to the fetus/child reported: two (2) TESAEs in the control group and five (5) in the supplementation group (Table 6-6). Among the five (5) subjects that experienced at least one TESAE pertinent to the fetus/child, two (2, 2.25%) belonged to the control group and three (3, 3.45%) to the supplementation one. None of the women reported a TESAE pertinent to the fetus/child suspected of being related to the study product nor a TESAE leading to temporary interruption of supplements intake by the mother. One (1, 1.15%) subject in the Elevit group had at least one TESAE pertinent to the fetus/child leading to permanent treatment discontinuation and one (1, 1.15%) subject in the same group had at least one TESAE with a fatal outcome.

Table 6-6 Summary of subjects with treatment emergent Serious Adverse Events pertinent to the fetus/child; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of serious TEAEs pertinent to fetus/child	2	5	7
Any serious TEAEs pertinent to fetus/child	2 (2.25)	3 (3.45)	5 (2.84)
At least one suspected related [a]	NA	0	0
At least one leading to temporary treatment interruption [b]	NA	0	0
At least one leading to permanent treatment discontinuation [c]	NA	1 (1.15)	1 (0.57)
Fatal outcome	0	1 (1.15)	1 (0.57)

NA=Not Applicable.

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.

[a] Suspected related adverse events were those events with causal relationship equal to related.

[b] Adverse events leading to temporary treatment interruption were those events with action taken equal to drug interrupted.

[c] Adverse events leading to permanent treatment discontinuation were those events with action taken equal to drug withdrawn.

6.2.2 Display and analysis of adverse events

Treatment-emergent adverse events

Treatment-emergent adverse events pertinent to the mother

In both groups, the most frequently recorded TEAEs pertinent to the mother fell in the following system organ class (SOCs):

- "Investigations"
  (32.58% of subjects [No supplementation] to 22.99% of subjects [Elevit supplementation])
- "Pregnancy, puerperium and perinatal conditions"
  (21.35% of subjects [No supplementation] to 26.44% of subjects [Elevit supplementation])
- "Blood and lymphatic system disorders"
  (8.99% of subjects [No supplementation] to 12.64% of subjects [Elevit supplementation])
- "Gastrointestinal disorders"
  (7.87% of subjects [No supplementation] to 13.79% of subjects [Elevit supplementation])
- "Infections and infestations"
  (7.87% of subjects [No supplementation] to 13.79% of subjects [Elevit supplementation])
- "Reproductive system and breast disorders"
  (11.24% of subjects [No supplementation] to 8.05% of subjects [Elevit supplementation]).

The frequencies of the preferred terms for the most frequently recorded TEAEs pertinent to the mother (>10% of any group) showed differences between the study groups but not relevant as none of the reported events were suspected of being related to the study product. These included:

- "Serum ferritin decreased" (SOC: "Investigations")
  (23.60% of subjects [No supplementation] to 17.24% of subjects [Elevit supplementation])
- "Gestational diabetes" (SOC: "Pregnancy, puerperium and perinatal conditions")
  (3.37% of subjects [No supplementation] to 11.49% of subjects [Elevit supplementation])
- "Anaemia" (SOC: "Blood and lymphatic system disorders")
  (8.99% of subjects [No supplementation] to 11.49% of subjects [Elevit supplementation]).

In the "No supplementation" group, the TEAEs classified as severe were "Postpartum hemorrhage" (1, 1.12%) and "Vaginal hemorrhage" (1, 1.12%), whereas in the "Elevit supplementation" group the TEAEs classified as severe were "Pre-eclampsia" (1, 1.15%) and "Premature separation of placenta" (1, 1.15%).

Overall, three (3) subjects (3.45%) in the Elevit group reported at least a TEAE judged by the investigator as related to the study product (Table 6-7). The recorded suspected related TEAE was "Vomiting" (3, 3.45%). All TEAEs were of mild intensity as judged by the investigator.

Table 6-7: Number of subjects with suspected related treatment emergent Adverse Events pertinent to the mother by System Organ Class and Preferred Term; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of subjects with any suspected related treatment-emergent adverse events pertinent to the mother	NA	3 (3.45)	3 (1.70)
MedDRA System organ class / Preferred term			
Gastrointestinal disorders	NA	3 (3.45)	3 (1.70)
Vomiting	NA	3 (3.45)	3 (1.70)

NA=Not Applicable.
Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.
Suspected related adverse events were those events with causal relationship equal to related.
A subject with multiple occurrences of TEAEs within a System Organ Class (SOC) or Preferred Term (PT), was counted only once in the SOC or PT category.
Terms are coded according to MedDRA dictionary, version 22.1.

Treatment-emergent adverse events pertinent to the fetus/child

In both groups, the most frequently recorded TEAEs pertinent to the fetus/child fell in the following SOCs:

- "Pregnancy, puerperium and perinatal conditions"
  (2.25% of subjects [No supplementation] to 4.60% of subjects [Elevit supplementation])
- "Investigations"
  (1.12% of subjects [No supplementation] to 1.15% of subjects [Elevit supplementation])

The frequencies of the PTs for the TEAEs pertinent to the fetus/child showed no relevant differences between the study groups. PTs frequencies of any group were all below 2%.

In the Elevit group, the TEAEs pertinent to the fetus/child classified as severe were "Duodenal atresia" (1, 1.15%), "Fetal compartment fluid collection "(1, 1.15%), "Fetal growth restriction" (1, 1.15%) and "Polyhydramnios" (1, 1.15%).

No TEAE pertinent to the fetus/child was suspected of being related to the study product.

Serious treatment-emergent adverse events pertinent to the mother

In both groups, the most frequently recorded TESAEs pertinent to the mother (>5% of any group) fell in the following SOC:

- "Pregnancy, puerperium and perinatal conditions"
  (10.11% of subjects [No supplementation] to 9.20% of subjects [Elevit supplementation]).

The frequencies of the PTs for the most frequently recorded TESAEs pertinent to the mother showed no relevant differences between the study groups. PTs frequencies were all below 5%. See Table 6-8 for details.

In the supplementation group, the TESAEs of severe intensity were "Pre-eclampsia" (1, 1.15%) and "Premature separation of the placenta" (1, 1.15%).

Table 6-8: Number of subjects with treatment emergent Serious Adverse Events pertinent to the mother by System Organ Class and Preferred Term; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of subjects with any serious treatment-emergent adverse events pertinent to the mother	11 (12.36)	12 (13.79)	23 (13.07)
MedDRA System organ class / Preferred term			
Hepatobiliary disorders	0	1 (1.15)	1 (0.57)
Cholestasis of pregnancy	0	1 (1.15)	1 (0.57)
Injury, poisoning and procedural complications	0	1 (1.15)	1 (0.57)
Abdominal injury	0	1 (1.15)	1 (0.57)
Pregnancy, puerperium and perinatal conditions	9 (10.11)	8 (9.20)	17 (9.66)
Arrested labour	0	1 (1.15)	1 (0.57)
Breech delivery	0	1 (1.15)	1 (0.57)
Breech presentation	1 (1.12)	0	1 (0.57)
Cervix dystocia	4 (4.49)	0	4 (2.27)
Gestational hypertension	2 (2.25)	2 (2.30)	4 (2.27)
Pre-eclampsia	0	1 (1.15)	1 (0.57)
Premature separation of placenta	0	2 (2.30)	2 (1.14)
Preterm premature rupture of membranes	0	1 (1.15)	1 (0.57)
Threatened labour	2 (2.25)	1 (1.15)	3 (1.70)
Renal and urinary disorders	0	1 (1.15)	1 (0.57)
Renal colic	0	1 (1.15)	1 (0.57)
Surgical and medical procedures	1 (1.12)	1 (1.15)	2 (1.14)
Caesarean section	1 (1.12)	1 (1.15)	2 (1.14)
Vascular disorders	1 (1.12)	0	1 (0.57)
Thrombophlebitis superficial	1 (1.12)	0	1 (0.57)

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.
A subject with multiple occurrences of TEAEs within a System Organ Class (SOC) or Preferred Term (PT), was counted only once in the SOC or PT category.
Terms are coded according to MedDRA dictionary, version 22.1.

<u>Serious treatment-emergent adverse events pertinent to the fetus/child</u>

In both groups, the most frequently recorded TESAEs pertinent to the fetus/child fell in the following SOCs:

- "Pregnancy, puerperium and perinatal conditions"
  (2.25% of subjects [No supplementation] to 2.30% of subjects [Elevit supplementation])

The frequencies of the PTs for the TESAEs pertinent to the fetus/child showed no relevant differences between the study groups. PTs frequencies of any group were all below 2%. For details on TESAEs pertinent to the fetus/child by SOC and PT refer to Table 6-9.

In the Elevit group, TESAEs pertinent to the fetus/child classified as severe were "Duodenal atresia" (1, 1.15%), "Fetal compartment fluid collection" (1, 1,15%), "Fetal growth restriction" (1, 1.15%) and "Polyhydramnios" (1, 1.15%).

Table 6-9: Number of subjects with treatment emergent Serious Adverse Events pertinent to the fetus/child by System Organ Class and Preferred Term; Safety population

	No supplementation (N=89) n (%)	Elevit supplementation (N=87) n (%)	Total (N=176) n (%)
Number of subjects with any serious treatment-emergent adverse events pertinent to the fetus/child	2 (2.25)	3 (3.45)	5 (2.84)
MedDRA System organ class / Preferred term			
Congenital, familial and genetic disorders	0	1 (1.15)	1 (0.57)
Duodenal atresia	0	1 (1.15)	1 (0.57)
Pregnancy, puerperium and perinatal conditions	2 (2.25)	2 (2.30)	4 (2.27)
Foetal compartment fluid collection	0	1 (1.15)	1 (0.57)
Foetal distress syndrome	1 (1.12)	0	1 (0.57)
Foetal growth restriction	1 (1.12)	1 (1.15)	2 (1.14)
Low birth weight baby	0	1 (1.15)	1 (0.57)
Polyhydramnios	0	1 (1.15)	1 (0.57)

Treatment-Emergent Adverse Event (TEAEs) were those events with an onset date after randomization.
A subject with multiple occurrences of TEAEs within a System Organ Class (SOC) or Preferred Term (PT), was counted only once in t he SOC or PT category.
Terms are coded according to MedDRA dictionary, version 22.1.

6.2.3 Listing of adverse events

Listing of deaths, serious adverse events and other significant adverse events

Deaths

Child death was reported in one subject (subject ID 22002-0068) of the "Elevit supplementation" group. This event was related to two (2) TESAEs pertinent to the fetus/child. The reported TESAEs were "Polyhydramnios" and "Fetal compartment fluid collection", that were judged not related to the study product.

Serious adverse events

A total of thirty-one (31) TESAEs were reported: two (2) were fatal and twenty-nine (29) non-fatal. Twenty-four (24) reported TESAEs were pertinent to the mother and seven (7) to the fetus/child. The two (2) fatal TESAEs were pertinent to the fetus/child. None of the TESAEs recorded was judged as being related to the study product.

Other significant adverse events

Three (3) women (Subject IDs: 22001-0086, 22001-0110 and 22002-0056) experienced TEAEs pertinent to the mother that were suspected of being related to the study product. The intensity of these TEAEs was mild and the PT was vomiting for all subjects. The duration of the event was one day for subject 22001-0086 and 22001-0110, and one week for subject 22002-0056.

Two (2) TEAEs leading to temporary product intake interruption were reported. The reported TEAEs were both not related to the study product. One event ("Breech presentation") was pertinent to the fetus/child, classified as not serious and judged as mild by the investigator (Subject ID: 22001-0007). The other event ("Gestational hypertension") was pertinent to the mother (Subject ID: 22001-0045), classified as not serious and judged as moderate by the investigator.

Twenty (20) TEAEs leading to permanent discontinuation were reported. Eighteen (18) recorded TEAEs were judged of being not related to the study product, while two (2) were. Nineteen (19)

events were pertinent to the mother and one (1) was pertinent to the fetus/child. Five subjects (5) reported serious events:

- Subject 22001-0011 reported “Preterm premature rupture of membranes” pertinent to the mother rated by the Investigator as being of moderate intensity. The outcome of the event was recovered/resolved. Remedial drug therapy consisted in treatment with 200 mg of progesterone four times a day. For details refer to Listing 16.2-5.2 of Section 16.2.
- Subject 22001-0020 reported “Premature separation of placenta” pertinent to the mother rated by the Investigator as being of severe intensity. The outcome of the event was recovered/resolved.
- Subject 22001-0045 reported “Fetal growth restriction” pertinent to the fetus/child rated by the Investigator as being of severe intensity. The outcome of the event was recovered/resolved.
- Subject 22001-0046 reported “Gestational hypertension” pertinent to the mother rated by the Investigator as being of moderate intensity. The outcome of the event was recovered/resolved with sequelae. Remedial drug therapy consisted in treatment with 20 mg nifedipine administered twice. For details refer to Listing 16.2-5.2 of Section 16.2.
- Subject 22002-0046 reported “Gestational hypertension” pertinent to the mother rated by the Investigator as being of moderate intensity. The outcome of the event was recovered/resolved. Remedial drug therapy consisted first in treatment with 500 mg of methyldopa four times a day and later with 20 mg nifedipine four times a day. For details refer to Listing 16.2-5.2 of Section 16.2.

6.3 CLINICAL LABORATORY EVALUATION

Hematology parameters

Hematology parameters, such as hemoglobin, hematocrit, mean MCV, MCH, MCHC, RBCs, WBCs with differential count (neutrophils, eosinophils, basophils, lymphocytes, monocytes) and platelets, were measured at Visit 1, 2, 3 and 4 (or Early discontinuation Visit -EDV). See most important results in Table 6-10.

Low levels of hemoglobin and hematocrit were reported for most subjects in both study groups at baseline and Visit 4 (or EDV)

Table 6-10: Hematology parameters at each visit; Safety population

									Change from baseline (Visit 1)							
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
Hemoglobin (g/L)																	
No supplementation																	
Visit 1	89	125.17	7.579	120.00	124.00	131.00	108.0	145.0	-	-	-	-	-	-	-	-	-
Visit 2	86	122.09	7.835	117.00	122.00	127.00	99.0	142.0	125.19	86	-3.09	4.963	-6.00	-3.00	0.00	-17.0	8.0
Visit 3	83	114.82	9.439	108.00	114.00	122.00	84.0	139.0	125.27	83	-10.45	8.138	-16.00	-10.00	-5.00	-29.0	10.0
Visit 4	76	117.00	11.755	109.00	116.00	124.50	80.0	152.0	125.04	76	-8.04	10.635	-16.00	-6.00	-1.00	-29.0	16.0
EDV	9	115.89	5.622	110.00	118.00	121.00	109.0	122.0	126.44	9	-10.56	6.635	-12.00	-10.00	-7.00	-24.0	0.0
Elevit supplementation																	
Visit 1	87	125.14	8.357	119.00	125.00	130.00	100.0	143.0	-	-	-	-	-	-	-	-	-
Visit 2	85	121.93	8.416	117.00	123.00	127.00	94.0	140.0	125.09	85	-3.16	5.661	-8.00	-4.00	1.00	-15.0	19.0
Visit 3	77	117.01	7.706	111.00	118.00	122.00	101.0	133.0	125.57	77	-8.56	6.153	-12.00	-10.00	-5.00	-22.0	18.0
Visit 4	66	118.82	9.946	114.00	120.00	126.00	88.0	141.0	125.88	66	-7.06	9.730	-12.00	-7.00	-2.00	-38.0	21.0
EDV	13	109.92	10.103	101.00	115.00	118.00	92.0	123.0	120.46	13	-10.54	9.107	-19.00	-11.00	-8.00	-20.0	8.0
Hematocrit (%)																	
No supplementation																	
Visit 1	89	36.20	2.238	35.00	36.00	38.00	31.0	41.0	-	-	-	-	-	-	-	-	-
Visit 2	86	35.36	2.315	34.00	35.00	37.00	30.0	41.0	36.22	86	-0.85	1.548	-2.00	-1.00	0.00	-5.0	4.0
Visit 3	83	33.51	2.676	32.00	34.00	35.20	25.0	40.0	36.21	83	-2.70	2.372	-4.00	-2.00	-1.00	-9.0	3.0
Visit 4	76	34.38	2.984	32.50	34.00	36.00	26.0	42.0	36.18	76	-1.80	2.777	-3.00	-2.00	0.00	-7.8	4.0
EDV	9	34.00	1.414	33.00	34.00	35.00	32.0	36.0	36.44	9	-2.44	1.424	-3.00	-3.00	-1.00	-4.0	0.0
Elevit supplementation																	
Visit 1	87	36.47	2.202	35.00	37.00	38.00	30.0	41.0	-	-	-	-	-	-	-	-	-
Visit 2	85	35.38	2.324	34.00	35.00	37.00	28.0	41.0	36.47	85	-1.08	1.774	-2.30	-1.00	0.00	-4.0	6.0
Visit 3	77	34.22	2.256	33.00	34.00	36.00	30.0	43.0	36.58	77	-2.35	1.977	-4.00	-2.30	-1.00	-6.0	7.0
Visit 4	66	35.00	2.572	33.00	35.00	37.00	28.0	40.0	36.67	66	-1.67	2.653	-3.00	-2.00	-0.30	-9.0	5.0

Table 6-10: Hematology parameters at each visit; Safety population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
EDV	13	32.38	2.902	30.00	33.00	34.00	27.0	36.0	34.95	13	-2.56	2.305	-4.00	-3.00	-2.00	-5.2	2.0
MCV - Mean Corpuscular Volume (fL)																	
No supplementation																	
Visit 1	89	86.51	3.428	84.70	86.50	88.30	77.3	94.9	-	-	-	-	-	-	-	-	-
Visit 2	86	86.80	3.854	84.50	86.80	89.30	74.1	95.5	86.53	86	0.27	1.449	-0.80	0.00	1.30	-3.2	3.2
Visit 3	83	89.02	3.449	86.40	89.10	91.30	79.0	97.0	86.53	83	2.48	2.620	0.90	2.40	4.00	-4.5	8.7
Visit 4	76	87.72	4.553	84.70	87.75	91.40	74.9	96.4	86.59	76	1.13	3.885	-1.00	1.85	3.30	-8.8	10.8
EDV	9	88.89	4.102	87.60	88.30	90.80	81.3	95.9	86.78	9	2.11	1.818	2.00	2.20	2.80	-2.2	4.5
Elevit supplementation																	
Visit 1	87	85.80	4.697	83.30	86.30	88.40	66.0	98.6	-	-	-	-	-	-	-	-	-
Visit 2	85	85.95	4.904	83.80	86.10	89.30	65.3	95.7	85.86	85	0.09	1.965	-0.90	-0.10	1.40	-9.6	3.8
Visit 3	77	88.97	4.367	86.20	89.50	91.70	77.6	99.5	86.13	77	2.84	2.484	1.20	2.90	3.90	-1.6	12.8
Visit 4	66	88.35	4.944	85.20	88.65	92.00	75.6	99.2	86.25	66	2.10	3.700	0.20	2.25	3.80	-10.0	16.6
EDV	13	88.05	7.431	87.40	89.10	91.50	65.3	95.0	84.38	13	3.67	3.277	0.80	3.70	6.20	-0.7	9.4
RBCs - Red Blood Cells (10^12/L)																	
No supplementation																	
Visit 1	89	4.19	0.296	3.93	4.17	4.43	3.6	5.0	-	-	-	-	-	-	-	-	-
Visit 2	86	4.08	0.305	3.84	4.06	4.31	3.6	4.9	4.19	86	-0.11	0.166	-0.22	-0.11	0.00	-0.6	0.3
Visit 3	83	3.77	0.301	3.56	3.79	4.01	3.0	4.4	4.19	83	-0.42	0.251	-0.58	-0.37	-0.23	-1.0	0.1
Visit 4	76	3.92	0.297	3.69	3.93	4.13	3.2	4.8	4.18	76	-0.26	0.272	-0.45	-0.25	-0.07	-1.0	0.3
EDV	9	3.81	0.227	3.77	3.84	3.91	3.4	4.2	4.21	9	-0.39	0.133	-0.44	-0.41	-0.28	-0.6	-0.2
Elevit supplementation																	
Visit 1	87	4.25	0.293	4.05	4.24	4.47	3.5	4.8	-	-	-	-	-	-	-	-	-
Visit 2	85	4.11	0.308	3.93	4.14	4.28	3.1	4.8	4.25	85	-0.14	0.225	-0.27	-0.15	-0.01	-1.1	0.6
Visit 3	77	3.84	0.250	3.66	3.85	4.00	3.2	4.5	4.25	77	-0.40	0.176	-0.53	-0.43	-0.31	-0.8	0.0
Visit 4	66	3.95	0.299	3.75	3.94	4.14	3.2	4.9	4.25	66	-0.30	0.261	-0.44	-0.32	-0.17	-1.1	0.4
EDV	13	3.70	0.358	3.44	3.75	3.92	3.0	4.3	4.15	13	-0.45	0.244	-0.67	-0.47	-0.40	-0.8	0.0
WBC - White Blood Cells (10^9/L)																	
No supplementation																	

Table 6-10: Hematology parameters at each visit; Safety population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
Visit 1	89	7.87	1.852	6.68	7.60	8.72	2.6	13.9	-	-	-	-	-	-	-	-	-
Visit 2	86	8.60	1.818	7.17	8.33	9.25	5.4	16.1	7.84	86	0.76	1.218	0.09	0.62	1.52	-3.7	6.1
Visit 3	83	9.25	2.076	7.71	8.93	10.15	5.8	17.6	7.91	83	1.35	1.513	0.32	1.20	2.46	-3.6	4.8
Visit 4	76	9.42	2.278	8.04	9.01	10.08	5.3	17.6	7.91	76	1.51	1.948	0.25	1.24	2.73	-3.6	8.4
EDV	9	8.73	1.089	7.92	8.70	9.73	7.1	10.1	7.71	9	1.03	1.736	0.78	1.25	1.49	-3.0	3.1
Elevit supplementation																	
Visit 1	87	8.25	1.726	7.03	7.96	9.47	4.9	14.3	-	-	-	-	-	-	-	-	-
Visit 2	85	8.77	1.848	7.29	8.76	10.14	5.5	13.9	8.27	85	0.50	1.273	-0.29	0.41	1.12	-3.2	4.6
Visit 3	77	9.69	2.069	8.32	9.20	10.78	5.1	15.4	8.18	77	1.51	1.658	0.38	1.20	2.22	-1.3	6.7
Visit 4	66	9.92	2.126	8.38	9.51	10.93	6.4	16.9	8.25	66	1.67	1.896	0.09	1.76	2.85	-2.0	7.1
EDV	13	9.24	2.248	7.73	9.31	11.59	6.1	12.3	7.95	13	1.29	2.107	-0.01	1.90	2.82	-3.9	4.3
EDV	13	1.87	0.483	1.52	1.79	2.09	1.2	2.8	1.72	13	0.15	0.362	-0.08	0.10	0.26	-0.2	1.0
Platelets (10^9/L)																	
No supplementation																	
Visit 1	89	236.57	46.113	204.00	231.00	260.00	157.0	383.0	-	-	-	-	-	-	-	-	-
Visit 2	86	237.06	48.656	205.00	234.50	266.00	100.0	399.0	235.79	86	1.27	25.669	-18.00	0.50	13.00	-64.0	90.0
Visit 3	83	227.98	52.106	194.00	219.00	255.00	147.0	400.0	238.67	83	-10.70	29.415	-30.00	-11.00	4.00	-65.0	117.0
Visit 4	76	217.43	49.496	186.50	206.50	242.00	123.0	396.0	237.74	76	-20.30	34.795	-46.50	-23.00	3.00	-94.0	56.0
EDV	9	215.00	55.944	177.00	217.00	245.00	124.0	291.0	236.44	9	-21.44	14.458	-27.00	-21.00	-10.00	-44.0	-2.0
Elevit supplementation																	
Visit 1	87	239.01	59.375	202.00	231.00	270.00	112.0	465.0	-	-	-	-	-	-	-	-	-
Visit 2	85	236.74	55.378	202.00	230.00	273.00	112.0	408.0	239.81	85	-3.07	19.458	-13.00	-2.00	7.00	-82.0	40.0
Visit 3	77	224.95	62.421	175.00	215.00	255.00	97.0	397.0	236.61	77	-11.66	27.303	-23.00	-11.00	1.00	-128.0	78.0
Visit 4	66	209.86	64.639	161.00	202.00	249.00	103.0	380.0	232.44	66	-22.58	32.134	-39.00	-26.00	-9.00	-110.0	59.0
EDV	13	247.38	73.942	201.00	249.00	293.00	125.0	365.0	259.15	13	-11.77	57.898	-43.00	-20.00	22.00	-128.0	90.0

GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 2: Baseline (GA Week 13/15); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36; EDV=Early Discontinuation Visit.
n refers to the number of subjects who performed the assessment at each visit.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Kidney function tests

Kidney function tests, such as electrolytes, serum creatinine, albumin/creatinine ratio in urine, specific gravity and pH, were performed at Visit 1 and 4 (or EDV). No relevant changes were described across study visits and most subjects had normal values for all kidney function tests both at baseline and at Visit 4 (or EDV) in both study groups. Urine analyses parameters, such as leukocyte esterase, protein, ketones, bilirubin, urobilinogen, glucose, blood and nitrite, were measured at Visit 1 and 4 (or EDV). Negative results for all parameters were reported for most subjects at each visit, except for leukocyte esterase. The proportion of subjects with a positive result for leukocyte esterase was higher at each visit in both groups than the proportion of subjects with a negative result. This means that most subjects had positive results or reported a concentration greater than zero for this parameter. This does not necessarily imply that the reported values were clinically significant: indeed, the majority of reported values was not considered clinically significant by the investigator, even if the concentration was out of normal range.

Liver function tests

Liver function tests, such as albumin, ALP, GGT, AST, ALT and total bilirubin, were performed at Visit 1 and 4 (or EDV). No relevant changes were observed throughout the visits except for albumin, which decreased from Visit 1 to Visit 4 (mean change from baseline at Visit 4 was -8.17 ± 2.627 g/L in the "No supplementation" group and -8.02 ± 3.110 g/L in the "Elevit supplementation" group) and ALP, which increased from Visit 1 to Visit 4 (mean change from baseline at Visit 4 was 85.28 ± 31.668 U/L in the "No supplementation" group and 93.72 ± 38.809 U/L in the "Elevit supplementation" group). Normal albumin values at baseline were described for most subjects. However, the majority of women had low values at Visit 4 (or EDV). Furthermore, normal values for ALP were reported for most subjects at baseline, but most women in both groups had high values for ALP at Visit 4. However, all albumin values reported as "Low" and all ALP values reported as "High" were not considered clinically significant by the investigator even if the concentration reported was out of normal range.

Blood coagulation parameters

Blood coagulation parameters, such as aPTT, PT and INR, were assessed at Visit 1 and Visit 4 (or EDV). No relevant changes were observed throughout the visits and normal values were observed for most subjects both at baseline and at Visit 4 (or EDV) in both study groups.

C-reactive protein

C-reactive protein (CRP), as a marker of inflammation, was measured at Visit 1, 2, 3 and 4 (or EDV). CRP decreased from Visit 1 to Visit 4 in the control group and slightly increased in the Elevit group (mean change from baseline at Visit 4 was -16.14 ± 75.236 nmol/L in the control group and 10.58 ± 114.148 nmol/L in the Elevit arm). Normal values were observed for most subjects both at baseline and at Visit 4 (or EDV) in both study groups.

Ferritin

Ferritin was measured at Visit 1 and 4 (or EDV). Results were summarized in the SAF population and are presented in Table 6-11. Mean values of ferritin decreased at Visit 4 in both study groups: mean change from baseline at Visit 4 was -29.07 ± 38.716 ug/L in the "No supplementation" group and -30.94 ± 33.030 ug/L in the "Elevit supplementation" group. Low ferritin values were reported for most subjects in both groups at baseline and at Visit 4 (or EDV) (Table 6-12).

Table 6-11: Ferritin at each visit; Safety population

									Change from baseline (Visit 1)								
	n	Mean	SD	Q1	Median	Q3	Min	Max	Base Mean	n	Mean	SD	Q1	Median	Q3	Min	Max
Ferritin (ug/L)																	
No supplementation																	
Visit 1	89	49.58	37.962	23.00	37.00	62.00	7.0	171.0	-	-	-	-	-	-	-	-	-
Visit 4	76	19.59	12.908	8.50	17.00	25.00	4.0	58.0	48.66	76	-29.07	38.716	-45.50	-18.00	-4.50	-153.0	35.0
EDV	9	20.78	21.194	10.00	13.00	22.00	7.0	75.0	57.00	9	-36.22	20.247	-50.00	-39.00	-31.00	-64.0	2.0
Elevit supplementation																	
Visit 1	86	49.40	34.288	24.00	44.00	64.00	6.0	154.0	-	-	-	-	-	-	-	-	-
Visit 4	66	18.86	10.800	12.00	16.00	24.00	3.0	55.0	49.89	65	-30.94	33.030	-45.00	-28.00	-6.00	-116.0	17.0
EDV	13	15.54	7.720	10.00	13.00	23.00	5.0	27.0	43.92	13	-28.38	28.660	-42.00	-24.00	-13.00	-95.0	12.0

GA=Gestational Age.
Visit 1: Screening (GA Week 11/14); Visit 4: GA Week 34/36; EDV=Early Discontinuation Visit.
n refers to the number of subjects who performed the assessment at each visit.
Baseline is defined as the Visit 1 - Screening (GA Week 11/14).

Table 6-12 Ferritin: shift table; Safety population

	Baseline (Visit 1)					
	No supplementation (N=89)			Elevit supplementation (N=87)		
	Low n (%)	Normal n (%)	Total n (%)	Low n (%)	Normal n (%)	Total n (%)
Ferritin (ug/L)						
Visit 4						
Low	44 (66.67)	22 (33.33)	66 (74.16)	35 (57.38)	26 (42.62)	61 (70.11)
Normal	4 (40.00)	6 (60.00)	10 (11.24)	1 (25.00)	3 (75.00)	4 (4.60)
Total	48 (63.16)	28 (36.84)	76 (85.39)	36 (55.38)	29 (44.62)	65 (74.71)
EDV						
Low	2 (28.57)	5 (71.43)	7 (7.87)	6 (54.55)	5 (45.45)	11 (12.64)
Normal	0	2 (100.00)	2 (2.25)	0	2 (100.00)	2 (2.30)
Total	2 (22.22)	7 (77.78)	9 (10.11)	6 (46.15)	7 (53.85)	13 (14.94)

GA=Gestational Age.
Visit 4: GA Week 34/36; EDV=Early Discontinuation Visit.
For each parameter, the total column includes all subjects with available data at both baseline (Visit 1 - Screening (GA Week 11/14)) and post baseline (Visit 4 (GA Week 34/36) or Early Discontinuation Visit).
Percentages for low/normal columns are calculated with the number of subjects in the total column as denominator.
Percentages in the total column are calculated with the number of subjects in each study group as denominator.

Serology parameters

Serology parameters, such as HBV, HCV, HIV-1 and HIV-2 antibodies, were tested at screening. Results were negative for all subjects of the SAF population.

6.4 OTHER SAFETY VARIABLES OR EVALUATIONS

Physical and gynecological examination

All women in both study groups had normal cervical examination, normal uterine volume correspondence and presence of fetal heartbeat at each study visit, except for 1 subject in the "Elevit supplementation" group and 1 subject in the "No supplementation" one that reported abnormal cervical examination at Visit 4 and at EDV, respectively.

Vital signs

Vital signs were measured and summarized at each visit, together with their change from baseline at each time point. No relevant changes were observed between study visits before delivery concerning both systolic and diastolic blood pressure, while at Visit 5 and at Visit 6 both of them were, on average, higher than at Visit 1. Heart rate was similar across all study visits. Moreover, the change from baseline (Visit 1) at Visit 4 for weight and BMI indicate an expected increase in weight of 11.08 ± 3.464 kg for subjects in the control arm and of 10.91 ± 3.553 gg in the Elevit one and, consequently, in BMI before delivery.

6.5 SAFETY CONCLUSIONS

One hundred twenty-five (125, 71.02%) subjects reported at least one TEAE pertinent to the mother, for a total of two hundred thirty-two (232) TEAEs. Ten (10, 5.68%) subjects reported at least one TEAE pertinent to the fetus/child, for a total of thirteen (13) TEAEs.

Overall, twenty-three (23, 13.07%) women reported at least one TESAE pertinent to the mother and five (5, 2.84%) reported at least one TESAE pertinent to the fetus/child. One (1, 0.57%) subject reported TESAEs pertinent to the fetus child with fatal outcome. Frequency of TESAEs was similar between both study groups and none of the TESAEs was suspected of being related to the study product.

Only three (3, 1.70%) subjects reported at least one TEAE pertinent to the mother that was suspected of being related to the study product. The reported TEAE was "Vomiting" with mild severity for all subjects and lasting from one day to maximum one week, in two (2) women and one (1), respectively.

Nineteen (19, 10.80%) women reported at least one TEAE pertinent to the mother leading to permanent treatment discontinuation and one (1, 0.57%) reported at least one TEAE pertinent to the mother leading to temporary treatment interruption. One (1, 0.57%) subject reported at least one TEAE pertinent to the fetus child leading to permanent treatment discontinuation and one (1, 0.57%) reported at least one TEAE pertinent to the fetus/child leading to temporary treatment interruption.

There were no relevant changes in clinical laboratory evaluation throughout the visits for most parameters (i.e. hematology, kidney function tests, liver function test, blood coagulation parameter and CRP), with the exception for ferritin, which decreased by visit in both study groups.

In conclusion, no major safety concerns related to intake of the study product during 2nd and 3rd trimesters of pregnancy were reported.

7. OTHER VARIABLES AND EVALUATIONS

7.1 Nutrient intake, diet habits and physical activity (FFQ)

Nutrient intake was measured from Visit 2 to Visit 4 (or EDV) by means of a food frequency questionnaire (FFQ). Details about nutrient intake in the PP population are presented in Table 7-1.

On average, the majority of nutrient intake was slightly higher in the “No supplementation” group compared with the “Elevit supplementation” group. This was the case for intake of calories, proteins, lipids, carbohydrates, dietary fibers, cholesterol, polyunsaturated fatty acids, calcium, sodium, potassium, phosphorus, iron, zinc, folic acid, EPA, DHA, animal proteins, vegetable proteins and drinking water.

Consumption of dietary supplements, diet followed with reasons and physical activity level were also assessed from Visit 2 to Visit 4 (or EDV) by means of the FFQ (Table 7-2).

Food supplements intake was reported for the majority of subjects throughout the study and most women did not follow a specific diet. The level of physical activity was similar in both study arms and varied at subsequent study visits compared with Visit 2, with a decrease in the frequencies of women reporting a fairly active and very active activity level.

Similar results concerning nutrient intake, consumption of dietary supplements, diet followed, and physical activity levels were reported for the ITT population.

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Calories (kcal)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	2074.0 (737.31)	2006.5 (737.67)
Median	1942.4	1853.1
Q1; Q3	1561.7; 2387.4	1524.0; 2383.7
Range	1093; 4684	986; 4623
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	1959.6 (784.09)	1907.3 (672.15)
Median	1806.6	1756.3
Q1; Q3	1407.0; 2366.6	1444.8; 2337.4
Range	785; 4743	757; 3746
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	1941.7 (679.30)	1871.2 (851.34)
Median	1791.9	1685.1
Q1; Q3	1475.0; 2212.0	1301.8; 2213.0
Range	819; 4217	731; 5869
Alcohol (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	0.4 (0.86)	0.7 (1.79)
Median	0.0	0.0
Q1; Q3	0.0; 0.4	0.0; 0.6
Range	0; 4	0; 13
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	0.6 (1.08)	1.0 (2.24)
Median	0.0	0.0
Q1; Q3	0.0; 0.9	0.0; 1.0
Range	0; 5	0; 15
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	0.8 (2.35)	1.1 (2.69)
Median	0.0	0.0
Q1; Q3	0.0; 0.8	0.0; 0.8
Range	0; 16	0; 18
Proteins (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	83.3 (33.59)	76.2 (29.49)
Median	76.5	71.5
Q1; Q3	60.9; 96.6	55.2; 88.0
Range	27; 234	31; 203
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	78.1 (29.97)	72.3 (25.41)
Median	72.8	68.7

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Q1; Q3	58.6; 95.7	56.3; 85.0
Range	25; 201	31; 170
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	80.0 (31.96)	74.1 (35.70)
Median	74.0	61.9
Q1; Q3	61.3; 95.7	53.9; 85.0
Range	32; 197	30; 213
Lipids (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	67.4 (25.68)	64.7 (23.41)
Median	62.9	63.4
Q1; Q3	49.5; 81.8	48.9; 76.0
Range	24; 181	28; 170
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	64.2 (26.35)	63.2 (23.83)
Median	57.2	58.3
Q1; Q3	45.5; 79.9	46.7; 76.3
Range	18; 137	27; 149
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	60.4 (21.55)	59.9 (33.75)
Median	58.9	50.5
Q1; Q3	43.7; 72.0	38.4; 68.6
Range	21; 115	26; 221
Carbohydrates (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	287.7 (117.87)	284.7 (123.66)
Median	255.0	245.0
Q1; Q3	206.8; 331.8	199.2; 326.8
Range	150; 675	124; 697
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	271.2 (125.82)	265.8 (110.70)
Median	245.0	247.7
Q1; Q3	189.6; 323.3	195.4; 312.5
Range	90; 699	73; 628
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	273.4 (111.85)	261.2 (122.69)
Median	246.0	237.4
Q1; Q3	189.1; 316.6	178.7; 310.7
Range	106; 689	59; 780
Sugars (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Mean (SD)	114.6 (56.80)	114.3 (52.79)
Median	103.1	102.6
Q1; Q3	76.9; 135.6	83.6; 130.6
Range	31; 348	52; 326
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	113.6 (67.69)	110.7 (53.03)
Median	90.8	97.8
Q1; Q3	73.5; 130.3	80.3; 132.0
Range	29; 374	25; 323
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	112.7 (57.27)	111.1 (60.79)
Median	94.2	91.6
Q1; Q3	77.6; 129.5	66.9; 138.2
Range	34; 341	28; 352
Dietary Fibre (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	25.6 (13.53)	23.1 (10.20)
Median	22.5	20.4
Q1; Q3	17.7; 29.9	15.8; 30.0
Range	9; 97	7; 53
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	23.0 (10.01)	21.9 (9.28)
Median	21.7	20.3
Q1; Q3	16.1; 27.3	15.8; 26.7
Range	8; 64	8; 46
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	23.3 (10.82)	21.3 (13.36)
Median	20.6	18.6
Q1; Q3	16.6; 26.4	12.8; 24.0
Range	8; 64	3; 79
Cholesterol (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	262.1 (139.37)	244.1 (91.00)
Median	243.0	235.6
Q1; Q3	183.8; 295.9	173.4; 300.3
Range	70; 1133	54; 573
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	249.8 (103.02)	235.3 (99.71)
Median	240.7	215.5
Q1; Q3	180.6; 309.7	174.2; 263.7
Range	50; 625	76; 585
Visit 4 (GA Week 34/36)		
n	76	65

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Mean (SD)	258.2 (118.06)	240.4 (125.24)
Median	244.0	205.2
Q1; Q3	178.0; 300.6	162.2; 268.1
Range	83; 808	91; 786
Saturated fatty acids (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	20.5 (6.86)	21.4 (8.69)
Median	19.7	20.8
Q1; Q3	15.9; 24.7	15.6; 25.1
Range	7; 40	9; 67
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	20.2 (8.72)	20.3 (8.17)
Median	18.4	18.6
Q1; Q3	14.0; 24.7	13.9; 24.8
Range	7; 52	9; 44
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	19.2 (7.37)	19.6 (11.61)
Median	17.2	16.6
Q1; Q3	13.4; 24.6	12.7; 22.6
Range	7; 37	7; 85
Polyunsaturated fatty acids (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	10.0 (4.58)	9.1 (3.62)
Median	9.0	8.9
Q1; Q3	6.8; 11.8	6.5; 10.6
Range	3; 33	4; 22
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	9.6 (4.74)	8.9 (3.74)
Median	8.6	7.5
Q1; Q3	5.7; 11.4	6.4; 10.8
Range	3; 30	4; 20
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	9.2 (4.19)	8.4 (4.76)
Median	8.4	7.2
Q1; Q3	6.1; 11.1	5.2; 9.7
Range	3; 21	3; 26
Monounsaturated fatty acids (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	29.0 (13.77)	27.0 (10.60)
Median	27.5	25.8
Q1; Q3	19.2; 35.5	19.4; 33.1
Range	10; 101	10; 64

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	26.7 (11.83)	26.5 (11.40)
Median	23.3	24.3
Q1; Q3	18.4; 32.9	18.9; 33.6
Range	6; 57	9; 72
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	24.4 (9.95)	24.6 (15.59)
Median	23.8	19.7
Q1; Q3	17.1; 31.8	14.5; 30.9
Range	6; 52	9; 89
Calcium (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	791.1 (320.55)	733.4 (351.14)
Median	768.3	711.4
Q1; Q3	602.6; 924.0	483.6; 886.3
Range	130; 2034	280; 2179
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	757.0 (322.64)	699.7 (283.29)
Median	737.8	654.7
Q1; Q3	550.5; 926.3	489.8; 881.6
Range	137; 1921	245; 1527
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	762.3 (353.96)	756.4 (415.07)
Median	702.9	677.2
Q1; Q3	533.7; 903.0	554.7; 869.0
Range	216; 1896	231; 3165
Sodium (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	2706.0 (1920.59)	3030.3 (1988.95)
Median	2196.8	2325.5
Q1; Q3	1486.8; 2895.0	1522.1; 3666.4
Range	539; 11426	930; 8602
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	2726.8 (1921.01)	2463.8 (1474.92)
Median	2088.4	2044.5
Q1; Q3	1509.3; 3250.3	1399.6; 3031.6
Range	631; 9070	499; 7216
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	2440.0 (1574.66)	2360.5 (1603.23)
Median	1824.2	1830.6
Q1; Q3	1306.1; 3207.1	1330.4; 3026.5
Range	819; 8558	458; 8329

Table 7-1: Descriptive statistics of nutrient intake; PP population		
	No supplementation (N=76)	Elevit supplementation (N=65)
Potassium (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	3779.2 (1630.14)	3450.1 (1253.07)
Median	3437.6	3240.2
Q1; Q3	2913.8; 4426.9	2392.6; 4395.9
Range	1280; 12344	1733; 7815
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	3606.6 (1400.84)	3479.7 (1223.98)
Median	3427.4	3172.7
Q1; Q3	2681.3; 4149.1	2637.2; 4098.1
Range	1271; 8510	1542; 6980
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	3822.7 (1669.35)	3396.5 (1717.38)
Median	3534.2	2985.2
Q1; Q3	2634.3; 4307.0	2279.0; 4034.9
Range	1505; 10638	1257; 9974
Phosphorus (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	1332.4 (572.82)	1186.1 (443.17)
Median	1218.5	1096.8
Q1; Q3	978.9; 1564.7	847.9; 1366.8
Range	477; 4219	560; 2850
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	1243.8 (464.38)	1146.2 (386.38)
Median	1186.8	1114.9
Q1; Q3	899.1; 1520.6	878.6; 1311.9
Range	382; 2879	504; 2549
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	1280.7 (521.48)	1192.7 (564.00)
Median	1209.2	1044.7
Q1; Q3	963.6; 1529.0	877.7; 1349.0
Range	496; 3311	493; 3643
Iron (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	13.4 (9.91)	10.9 (4.07)
Median	11.1	10.1
Q1; Q3	8.8; 13.8	7.8; 12.7
Range	6; 84	6; 27
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	11.7 (4.73)	11.0 (4.33)
Median	11.1	10.0

Table 7-1: Descriptive statistics of nutrient intake; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Q1; Q3	8.3; 14.1	8.2; 12.7
Range	4; 24	5; 28
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	12.5 (6.26)	11.0 (6.34)
Median	11.7	9.2
Q1; Q3	8.8; 14.5	6.9; 13.0
Range	5; 43	4; 39
Zinc (mg)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	13.7 (13.62)	12.0 (5.84)
Median	10.8	10.6
Q1; Q3	8.7; 14.2	7.6; 15.1
Range	6; 121	4; 37
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	12.4 (6.21)	11.1 (5.41)
Median	10.7	9.7
Q1; Q3	8.2; 14.2	7.8; 13.0
Range	4; 28	4; 36
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	13.3 (8.68)	11.8 (7.91)
Median	11.0	8.3
Q1; Q3	8.6; 15.6	7.5; 12.9
Range	5; 64	4; 50
Folic acid (ug)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	443.9 (197.89)	411.6 (187.20)
Median	402.2	384.2
Q1; Q3	324.8; 543.3	266.6; 510.5
Range	123; 1280	160; 887
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	415.4 (215.92)	409.3 (248.85)
Median	374.7	357.1
Q1; Q3	282.2; 478.5	261.4; 459.7
Range	110; 1434	98; 1560
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	431.3 (235.22)	407.4 (321.25)
Median	379.5	329.6
Q1; Q3	277.5; 526.0	222.2; 441.7
Range	129; 1339	69; 2069
C20:5 (EPA) (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65

Table 7-1: Descriptive statistics of nutrient intake; PP population		
	No supplementation (N=76)	Elevit supplementation (N=65)
Mean (SD)	0.3 (0.29)	0.2 (0.17)
Median	0.2	0.2
Q1; Q3	0.2; 0.4	0.1; 0.3
Range	0; 2	0; 1
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	0.3 (0.18)	0.2 (0.12)
Median	0.3	0.2
Q1; Q3	0.2; 0.4	0.1; 0.3
Range	0; 1	0; 1
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	0.3 (0.28)	0.2 (0.20)
Median	0.3	0.2
Q1; Q3	0.2; 0.4	0.1; 0.3
Range	0; 2	0; 1
C22:6 (DHA) (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	0.5 (0.44)	0.4 (0.29)
Median	0.4	0.3
Q1; Q3	0.2; 0.6	0.2; 0.5
Range	0; 2	0; 2
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	0.5 (0.32)	0.3 (0.20)
Median	0.5	0.3
Q1; Q3	0.3; 0.6	0.2; 0.4
Range	0; 2	0; 1
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	0.5 (0.47)	0.4 (0.24)
Median	0.4	0.3
Q1; Q3	0.3; 0.6	0.2; 0.4
Range	0; 3	0; 1
Animal proteins (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	50.4 (25.99)	47.3 (21.33)
Median	44.7	43.8
Q1; Q3	34.1; 60.5	32.9; 57.6
Range	15; 197	14; 142
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	49.2 (22.21)	44.3 (18.68)
Median	47.5	40.1
Q1; Q3	34.2; 59.2	32.0; 54.6
Range	14; 148	12; 114
Visit 4 (GA Week 34/36)		
n	76	65

Table 7-1: Descriptive statistics of nutrient intake; PP population		
	No supplementation (N=76)	Elevit supplementation (N=65)
Mean (SD)	50.7 (22.75)	47.0 (24.07)
Median	47.6	39.3
Q1; Q3	35.2; 59.5	33.2; 49.2
Range	16; 140	14; 147
Vegetable proteins (g)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	31.2 (17.60)	27.2 (10.66)
Median	27.7	25.3
Q1; Q3	21.5; 36.6	19.9; 32.8
Range	7; 126	9; 59
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	27.3 (11.15)	26.6 (10.39)
Median	26.4	24.8
Q1; Q3	19.9; 33.8	18.4; 35.3
Range	10; 69	6; 49
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	27.5 (12.27)	25.7 (14.06)
Median	24.4	22.4
Q1; Q3	19.3; 34.0	16.0; 31.8
Range	8; 74	2; 89
Drinking water (mL)		
Visit 2 - Baseline (GA Week 13/15)		
n	76	65
Mean (SD)	1873.8 (752.24)	1756.4 (710.10)
Median	1827.3	1765.8
Q1; Q3	1379.8; 2194.0	1454.4; 2027.9
Range	197; 4493	86; 4148
Visit 3 (GA Week 24/26)		
n	76	64
Mean (SD)	1910.5 (625.97)	1746.9 (631.45)
Median	1810.8	1765.9
Q1; Q3	1652.7; 2063.8	1501.9; 2001.8
Range	211; 4619	357; 3376
Visit 4 (GA Week 34/36)		
n	76	65
Mean (SD)	1863.0 (623.04)	1783.2 (784.87)
Median	1841.4	1748.7
Q1; Q3	1710.7; 2022.0	1313.8; 2089.2
Range	131; 3766	74; 3577

PP=Per Protocol, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, GA=Gestational Age.

Table 7-2: Descriptive statistics of consumption of dietary supplements, diet and physical activities; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Food supplements during pregnancy		
Visit 2 - Baseline (GA Week 13/15)		
No	14 (18.42)	14 (21.54)
Yes	62 (81.58)	51 (78.46)
Visit 3 (GA Week 24/26)		
No	21 (27.63)	17 (26.56)
Yes	55 (72.37)	47 (73.44)
Visit 4 (GA Week 34/36)		
No	18 (23.68)	22 (33.85)
Yes	58 (76.32)	43 (66.15)
Diet followed in the last 3 months		
Visit 2 - Baseline (GA Week 13/15)		
No	72 (94.74)	61 (93.85)
Yes	3 (3.95)	2 (3.08)
Missing	1 (1.32)	2 (3.08)
Visit 3 (GA Week 24/26)		
No	72 (94.74)	59 (92.19)
Yes	3 (3.95)	3 (4.69)
Missing	1 (1.32)	2 (3.13)
Visit 4 (GA Week 34/36)		
No	70 (92.11)	58 (89.23)
Yes	3 (3.95)	1 (1.54)
Missing	3 (3.95)	6 (9.23)
Reason for diet [a]		
Visit 2 - Baseline (GA Week 13/15)		
Weight control	2 (66.67)	1 (50.00)
Other	1 (33.33)	0
Missing	0	1 (50.00)
Visit 3 (GA Week 24/26)		
Weight control	1 (33.33)	1 (33.33)
Other	1 (33.33)	0
Missing	1 (33.33)	2 (66.67)
Visit 4 (GA Week 34/36)		
Because you have high sugar or diabetes	1 (33.33)	0
Weight control	1 (33.33)	0
Weight control, because you have high blood pressure or heart problems	1 (33.33)	0
Missing	0	1 (100.00)
Physical activity level		
Visit 2 - Baseline (GA Week 13/15)		
Sedentary (seated almost all the time, no physical activity, no sport, in care)	3 (3.95)	3 (4.62)
Fairly inactive (seated jobs or activities, housewives with electric appliances, little sport)	31 (40.79)	24 (36.92)
Somewhat active (manual jobs, housewives without electric appliances, light sport, etc.)	30 (39.47)	26 (40.00)
Fairly active (work or activities standing or walking, intense sport, etc.)	9 (11.84)	10 (15.38)
Very active (very strenuous work, intense daily sport)	1 (1.32)	2 (3.08)
Missing	2 (2.63)	0

Table 7-2: Descriptive statistics of consumption of dietary supplements, diet and physical activities; PP population

	No supplementation (N=76)	Elevit supplementation (N=65)
Visit 3 (GA Week 24/26)		
Sedentary (seated almost all the time, no physical activity, no sport, in care)	3 (3.95)	1 (1.56)
Fairly inactive (seated jobs or activities, housewives with electric appliances, little sport)	29 (38.16)	23 (35.94)
Somewhat active (manual jobs, housewives without electric appliances, light sport, etc.)	30 (39.47)	31 (48.44)
Fairly active (work or activities standing or walking, intense sport, etc.)	13 (17.11)	7 (10.94)
Very active (very strenuous work, intense daily sport)	0	1 (1.56)
I do not know/ no answer	0	1 (1.56)
Missing	1 (1.32)	0
Visit 4 (GA Week 34/36)		
Sedentary (seated almost all the time, no physical activity, no sport, in care)	2 (2.63)	4 (6.15)
Fairly inactive (seated jobs or activities, housewives with electric appliances, little sport)	34 (44.74)	24 (36.92)
Somewhat active (manual jobs, housewives without electric appliances, light sport, etc.)	29 (38.16)	30 (46.15)
Fairly active (work or activities standing or walking, intense sport, etc.)	10 (13.16)	6 (9.23)
Missing	1 (1.32)	1 (1.54)

PP=Per Protocol, GA=Gestational Age.

Percentages were computed on subjects performing the Food Frequency questionnaire at each visit.

[a] Percentages were computed on subjects who answered Yes at the question Diet followed in the last 3 months.

7.2 Delivery information

Delivery information by study group are shown in Table 7-3. Overall, one hundred thirty-one (131) subjects in the PP population performed the Delivery Visit: sixty-nine (69) in the control group and sixty-two (62) in the Elevit group. Twenty-seven (27, 20.61%) subjects had Caesarean delivery: fourteen (14, 20.29%) in the control group and thirteen (13, 20.97%) in the "Elevit supplementation" group. Delivery complications were reported for a total of twenty-four (24, 18.32%) subjects: sixteen (16, 23.19%) in the control group and eight (8, 12.90%) in the "Elevit supplementation" group. Labor was induced in twenty-two (22, 16.79%) women: thirteen (13, 18.84%) in the control group and nine (9, 14.52%) in the "Elevit supplementation" group. Overall, seventy-five (75, 57.25%) newborns were male and fifty-six (56, 42.75%) were female. Same results were reported for the ITT population.

Table 7-3: Delivery information; PP population

	No supplementation (N=76) n (%)	Elevit supplementation (N=65) n (%)	Total (N=141) n (%)
Subjects performing delivery visit	69	62	131
Type of delivery			
Vaginal	55 (79.71)	49 (79.03)	104 (79.39)
Caesarean	14 (20.29)	13 (20.97)	27 (20.61)
Delivery complications			
No	53 (76.81)	54 (87.10)	107 (81.68)
Yes	16 (23.19)	8 (12.90)	24 (18.32)
Induced labor			
No	56 (81.16)	53 (85.48)	109 (83.21)
Yes	13 (18.84)	9 (14.52)	22 (16.79)
Infant sex			
Male	39 (56.52)	36 (58.06)	75 (57.25)
Female	30 (43.48)	26 (41.94)	56 (42.75)

PP=Per Protocol.
Percentages were computed on subjects belonging to Per Protocol population that performed delivery visit.

8. DISCUSSION AND OVERALL CONCLUSIONS

Nutritional requirement increases during pregnancy in order to maintain maternal metabolism and to support fetal growth and development (Mousa et al., 2019) and therefore supplementation with multiple micronutrient supplements can be helpful in preventing potential micronutrient deficiencies (Parisi et al., 2014). This study shows that supplementation of pregnant women with a multiple micronutrients & DHA supplementation during the 2nd and 3rd trimester of pregnancy is beneficial for the maternal nutritional status. The primary maternal efficacy endpoint of this study was met.

Elevit soft gels capsules consist of 13 vitamins, 6 minerals and the long-chain polyunsaturated fatty acids (LCPUFAs) DHA (200 mg) and EPA (80 mg). In particular, DHA has been widely investigated for its critical importance on fetal growth and especially on central nervous system development (Cetin et al., 2009a).

In the present study, baseline characteristics were similar between the two study groups. Indeed, there were no relevant differences in mean age, weight, height, BMI, history of previous birth outcomes, smoking and alcohol habits, and vital signs between the control and the Elevit groups at screening. Background dietary intake assessed by means of a food frequency questionnaire was slightly higher in the control group at baseline and remained higher throughout the study.

Maternal baseline percentages of DHA within total fatty acids in red blood cells were similar and increased by visits in both groups. Change in RBC DHA (wt% TFA) at 34/36 weeks of gestation from gestational week 11/14 was significantly higher (p-value <0.0001) in the group daily supplemented with Elevit (1.48 [95% CI: 1.22 to 1.74]) compared with the non-supplemented one (0.52 [95% CI: 0.28 to 0.76]). Maternal plasma DHA content commonly declines during pregnancy and particularly during late gestation (Al et al., 1995) when fetal requirements for LCPUFAs to support brain growth and visual development are highest (Cetin et al., 2009b). However, a decrease in maternal DHA was not observed in the control group in the current study, possibly because the initial maternal stores were high and because of an appropriate background dietary intake. Under less optimal conditions, maternal stores could be considerably compromised (Dunstan et al., 2004). The significant increase in the DHA fraction of phospholipids in maternal red blood cells reported in this study upon supplementation with 200 mg DHA per day is in line with results from previous studies. Indeed, prior reports show that daily supplementation during pregnancy with fish oil preparations (Dunstan et al., 2004; Krauss-Etschmann et al., 2007), DHA (200mg)-containing supplements (Bergmann et al., 2008) and marine algae-oil (Carlson et al., 2013) can lead to a significant increase from baseline in the proportions of DHA in maternal erythrocytes phospholipids. In this study, a benefit on maternal DHA status could be observed despite the lower daily DHA supplementation dose compared with the dose in previous studies (Carlson et al., 2013; Dunstan et al., 2004; Krauss-Etschmann et al., 2007), where the daily DHA intake was 2240 mg, 500 mg and 600 mg, respectively.

Furthermore, maternal baseline percentages of EPA within total fatty acids in erythrocytes were slightly higher in the control group (0.54 ± 0.242) than in the Elevit one (0.49 ± 0.222). There was a slight decrease in EPA levels at GA 34/36 in the first group (0.49 ± 0.323), whereas levels remained unvaried in the second one (0.49 ± 0.194). However, the difference between the two groups was not significant (p-value 0.4291). This result is in contrast with data from a previous study where daily supplementation with fish oil from gestational week 20 significantly increased the proportion of EPA in RBCs of pregnant women at week 30, 37 and delivery (Dunstan et al., 2004). This different outcome might be explained by the greater amount of EPA present in the fish oil preparation, where the estimated amount of EPA given to subjects was 1100 mg per day (Dunstan et al., 2004) compared with the 80 mg per day administered in the present study. Furthermore, an additional

report described a significant increase in the levels of EPA in RBCs at gestational week 30 and at delivery in women daily supplemented with a fish oil preparation containing 150 mg of EPA from pregnancy week 20 (Krauss-Etschmann et al., 2007). However, in the mentioned study, the daily dose of EPA was higher and baseline levels of EPA were markedly lower than baseline levels in our study. This might partially explain why a significant increase in EPA levels could be observed upon supplementation.

Similar trends as for DHA (wt% TFA) were reported for the DHA/TFA ratio and the Omega 3 index (calculated as the proportion of EPA and DHA in RBCs membranes) that increased in both study arms at gestational week 34/36 and to a greater extent in the "Elevit supplementation" group compared with the "No supplementation" one. The difference between study arms was significant for both variables (p-value <0.0001).

Levels of 25-hydroxyvitamin D, the major circulating form of vitamin D and the most common measure of vitamin D status, were monitored throughout the study. Vitamin D is known for its role in maintaining calcium homeostasis and bone integrity (Mousa et al., 2019) and the fetus relies completely on maternal vitamin D stores for its development (De-Regil et al., 2016). In adults, levels of 25-hydroxyvitamin D below 20 µg/L are known as hypovitaminosis (Podd, 2015). In this study, levels of 25-hydroxyvitamin D decreased from baseline to gestational week 34/36 in the "No supplementation" group: absolute change from baseline was -3.48 µg/L [95% CI: -5.62 to -1.33], with mean 25-hydroxyvitamin D levels of 17.75 ± 9.717 µg/L at GA week 34/36. It has been reported that vitamin D deficiency is common during pregnancy worldwide (Palacios et al., 2016), including the Mediterranean region (Karras et al., 2016). On the other hand, supplementation with Elevit was beneficial for the maternal 25-hydroxyvitamin D status. Indeed, levels of 25-hydroxyvitamin D remained stable during later pregnancy in the "Elevit supplementation" group: absolute change from baseline at week 34/36 was 0.48 µg/L [95% CI: -1.81 to 2.77], with mean 25-hydroxyvitamin D levels of 21.38 ± 9.073 µg/L. This demonstrates a beneficial effect of Elevit on maintaining maternal vitamin D stores during pregnancy.

In this study the GSH/GSSG ratio, levels of ROMS and 8-isoprostane were analyzed as oxidative stress markers. Oxidative stress is thought to be a key mechanism underlying the pathophysiology of several pregnancy complications including preeclampsia, preterm birth, intrauterine growth restriction and premature rupture of membranes (Mousa et al., 2019). For example, systemic oxidative stress and inflammatory status are greatly enhanced in preeclampsia (Berti et al., 2011). Therefore, maintaining a balanced oxidative and inflammatory status is crucial during pregnancy. GSH/GSSG ratio and levels of 8-isoprostane slightly increased during later pregnancy compared with baseline, whereas levels of ROMs remained unvaried. These changes were observed in both groups.

Maternal nutritional status during pregnancy is a major determinant for birth outcomes (Chia et al., 2019) and there is growing interest in the relationship between LCPUFA status and pregnancy and infant outcomes (Dunstan et al., 2004). In this study, an effect of supplementation with Elevit could not be observed on any of the variables of the infant analyzed.The term and gestational age of most of the babies at delivery were similar between the two groups (39.92 ± 1.203 weeks in the control arm and 39.86 ± 1.121 weeks in the Elevit one). This lack of difference was in line with some previous studies (Dunstan et al., 2004; Krauss-Etschmann et al., 2007) but in contrast with other reports demonstrating an increase in gestational length in subjects supplemented with DHA compared with non-supplemented ones (Carlson et al., 2013; Harris et al., 2015). The lack of difference observed in our study could be attributed to the background nutrient intake and health of the population analyzed, which could explain why we observed no difference between the two supplementation groupsA recent Cochrane systematic review shows that preterm birth < 37 weeks and early preterm

birth < 34 weeks are reduced in women receiving omega-3 LCPUFAs compared with no omega-3 (Middleton et al., 2019).

Furthermore, in the current study, no difference between the two study arms could be observed concerning head circumference, weight, length, ponderal index, skinfold thickness, APGAR score and bone density. These results are in line with those from other studies (Dunstan et al., 2004; Harris et al., 2015; Krauss-Etschmann et al., 2007), where no effect of supplementation with DHA could be observed on newborns' weight, length and head circumference. A recent systematic review shows that the quality of the evidence demonstrating an effect of supplementation with LCPUFAs on infants parameters, other than gestational length, remains low (Middleton et al., 2019).

Due to the small number of subjects included in the subgroup of women undergoing elective Caesarean section, no comparison between study groups concerning the exploratory variables analyzed could be performed. However, a trend could be observed describing higher medians of most cord blood and placental RBC fatty acids parameters in the supplementation group compared with the control group. Previous studies also demonstrated a positive effect of LCPUFA supplementation on DHA levels in cord blood (Carlson et al., 2013; Krauss-Etschmann et al., 2007) and in placental tissue (Keelan et al., 2015), and on both DHA and EPA levels in cord blood (Dunstan et al., 2004). In general, enhanced maternal dietary intake of DHA increases fetal supply and leads to higher DHA concentrations in cord blood (Koletzko et al., 2007). In particular, when DHA stores are low in less well-nourished women, low DHA supplementation may primarily affect the fetal DHA status, because DHA is preferentially transported to the fetus (Krauss-Etschmann et al., 2007). Our results suggest a positive effect of "Elevit supplementation" on fetal PUFAs status but need to be confirmed in a larger population.

On the other hand, no clear trend could be described for the oxidative and inflammatory parameters in the placenta and in placental cytotrophoblast cells. Omega-3 LCPUFAs are well known anti-inflammatory and antioxidative nutrients (Wu et al., 2015). Reactive oxygen species could harm placental development, but they are also reported to regulate gene transcription and downstream activities such as trophoblast proliferation, invasion, and angiogenesis. Therefore, an appropriate balance between the generation of reactive oxygen species and their clearance by antioxidant mechanisms is critical for pregnancy outcomes (Wu et al., 2015). The same is true for the inflammatory cytokines, as some pregnancy outcomes such as preterm birth are thought to be related with an inflammatory status (Middleton et al., 2019). Analyses of the presented exploratory variables in a larger group of subjects might be required in order to draw conclusions about a possible impact of supplementation with Elevit on the explored variables.

No safety concerns were identified during this study and safety parameters did not differ among the two study groups. This study did not identify any serious safety concern related to Elevit supplementation for mothers or newborns. The only reported adverse event thought to be related to Elevit intake was vomiting, of mild intensity. This event was transient and lasted from one day to maximum one week. Based on the above, there are no safety concerns linked to the vitamins, minerals and trace elements contained in Elevit soft gel capsules when the product is taken as recommended (one capsule daily) in the target population in the claimed benefit area.

A question remains open regarding the long-term effects of supplementation with Elevit. Further follow-up trials are needed to assess longer-term outcomes for mother and child, to improve understanding of metabolic, growth and neurodevelopment pathways in children and to establish if, and how, outcomes may vary depending on different baseline and background characteristics of women.

Overall conclusions

In conclusion, supplementation with Elevit soft gel capsules during pregnancy positively impacts maternal nutritional status in blood. No concerns related to maternal and infants' health are to be reported. Therefore, the benefits of supplementation with Elevit outweigh the risks related to product intake.

9. PUBLICATIONS BASED ON THE STUDY

Manuscript Accepted by Nutrients

Multiple micronutrients and docosahexaenoic acid supplementation during pregnancy: a randomized controlled study

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Abstract: Maternal dietary intake during pregnancy needs to meet increased nutritional demands to maintain metabolism and support fetal development. Docosahexaenoic acid (DHA) is essential for fetal neuro-/visual development and in immunomodulation, accumulating rapidly within the developing brain and central nervous system. Levels available to the fetus are governed by the maternal diet. In this multicenter, parallel, randomized controlled trial, we evaluated once-daily supplementation with multiple micronutrients and DHA (MMS) on maternal biomarkers and infant anthropometric parameters during the second and third trimesters of pregnancy compared with no supplementation. Primary efficacy endpoint: change in maternal red blood cell (RBC) DHA (wt% total fatty acids) during the study. Secondary variables: other biomarkers of fatty acid and oxidative status, vitamin D, and infant anthropometric parameters at delivery. Supplementation significantly increased RBC DHA levels, the omega-3 index, and vitamin D levels. Subscapular skinfold thickness was significantly greater with MMS in infants. Safety outcomes were comparable between groups. This first randomized controlled trial of supplementation with multiple micronutrients and DHA in pregnant women indicated that MMS significantly improved maternal DHA and vitamin D status in an industrialized setting – an important finding considering the essential roles of DHA and vitamin D.

Keywords: docosahexaenoic acid; long-chain polyunsaturated fatty acids; maternal biomarkers; micronutrients; neurodevelopment; pregnant women; supplementation; vitamin D

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1. Introduction

During pregnancy, an adequate maternal dietary intake is essential to meet the increased nutritional demands required to maintain metabolism and support fetal development [1]. Micronutrients, such as folic acid, B vitamins, vitamin D, vitamin C, calcium, copper, magnesium, iodine, selenium, zinc, iron all have vital roles throughout all stages of pregnancy [2-4]. Poor dietary intakes or deficiencies in both micro- and macronutrients can have adverse effects on pregnancy outcomes and neonatal health [5], including an increased risk of neural tube defects, preeclampsia, miscarriage, and low birth weight [6,7]. Many women are at risk of insufficient nutrient intake, in industrialized as well as developing countries [8-10]. Therefore, micronutrient supplementation is frequently recommended during pregnancy to help improve pregnancy outcomes in the mother and child [11,12]. International guidelines (i.e., from the World Health Organization)

currently recommend supplementation of iron and folic acid (0.4 mg/day) during the whole pregnancy for the purpose of improving pregnancy outcomes and reducing maternal anemia in pregnancy [13]. Recently, there have been extensive scientific and medical discussions around the need to include vitamin D as a standard nutrient to be supplemented during pregnancy, due to low intake. Vitamin D regulates calcium and phosphate body stores and is therefore critical for bone health [14]. Furthermore, low concentrations of blood vitamin D in pregnant women have been associated with pregnancy complications [15,16].

In addition to micronutrients, a balanced macronutrient intake is recommended. In particular, the long-chain polyunsaturated fatty acids (LCPUFAs) found at high concentrations within the brain and central nervous system are essential for the development of the fetal brain [17]. Docosahexaenoic acid (DHA) – representing the largest proportion of LCPUFAs in the brain and retina – plays a key role during the pre- and early postnatal period [17-20]. After the first trimester, when the neural tube has closed and grey matter begins to form [21], DHA begins to rapidly accumulate in the brain [18,22]; accumulation continues up to 2 years [23,24].

However, the human body is not efficient at producing essential LCPUFAs [22], and maternal concentrations decrease over the course of gestation [25]. Of note, the levels of DHA available to the fetus during pregnancy are governed by the diet of the mother [17,26-28]. Studies suggest that consumption of a diet rich in omega-3 LCPUFAs including DHA may have a reduced risk for common pregnancy complications such as intrauterine growth restriction, preeclampsia, and preterm deliveries [29-31]. Supplementation with DHA can also increase the expression of fatty acid transport proteins, thus increasing transport through the placenta and improving the fatty acid status of both the mother and child [32,33].

Meta-analyses have demonstrated that there are clinical benefits associated with prenatal multiple micronutrient [34] and LCPUFA supplementation [35] during pregnancy. However, there is limited data on the effects of prenatal supplementation in industrialized countries, particularly when used in combination. Clinical guidelines for pregnant women tend to focus on single nutrients for supplementation [36,37]. Given the interest in the potential beneficial effects of supplementation with micronutrients and DHA during pregnancy, we carried out a randomized trial to evaluate the effects of multiple micronutrient plus DHA supplementation during the second and third trimesters of pregnancy on maternal biomarkers compared with no supplementation in the control group in an industrialized country. The primary variable, i.e., the concentration of DHA (weight percent of total fatty acids (wt% TFA)) in maternal red blood cells (RBC), was considered indicative for LCPUFA status. Secondary explorative variables were other biomarkers of fatty acid and oxidative status, vitamin D, and anthropometric parameters of infants at delivery. We included vitamin D status as a secondary endpoint to investigate whether vitamin D supplementation is needed to maintain adequate status , and whether the levels of vitamin D in the supplement would be sufficient to maintain adequate status. We hypothesized that supplementation might help to improve maternal DHA and vitamin D status in a healthy population of pregnant women, whereas dietary intake would be insufficient to meet the increased needs during pregnancy.

2. Materials and Methods

2.1. Trial Design

This was a multicenter, parallel, randomized controlled trial conducted at two centers in Italy to compare the effects of once daily supplementation with multiple micronutrients plus DHA (hereafter referred to as MMS) versus no supplementation during pregnancy on maternal biomarkers and infant anthropometric parameters. Supplementation began at gestational week 13-15 until delivery. Six visits were conducted during the trial, from screening to final follow-up, as outlined in Figure 1 and Supplementary Table 1. At baseline (Visit 2; gestational week 13-15), women who fulfilled the eligibility criteria were randomized to the supplementation or control group in a 1:1 ratio. The sequential randomization list (generated through a validated SAS program by an independent statistician) was generated according to permutated block codes. A randomization number was assigned to each woman at each site by means of randomization cards. The study was not blinded. All blood parameters were measured at Visits 1, 3, and 4 in all women, while dietary intake was recorded at Visits 2, 3, and 4.

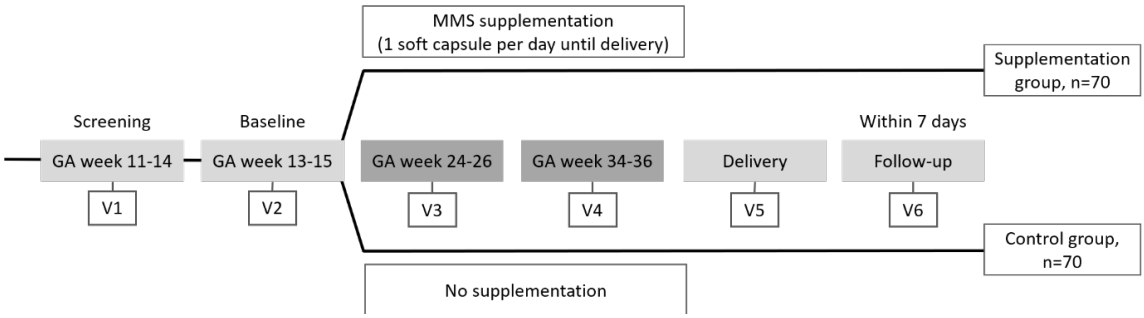


Figure 1. Study design. Visit 1 (screening): pregnant women were screened for study eligibility and blood collection was performed. Visit 2 (baseline): eligible women meeting the inclusion and exclusion criteria were randomized equally to one of the two study groups; nutritional status was assessed using a semi-quantitative food frequency questionnaire (FFQ). Visits 3 and 4 (MMS supplementation or no supplementation): FFQ was administered and blood sampling took place-the red blood cell DHA level measured at Visit 4 was compared to the value measured at Visit 1 to assess the primary endpoint. Visit 5 (delivery): obstetric evaluations were performed in all women and infant anthropometric parameters were measured. Concomitant medications and adverse events were assessed at all Visits.

The study was approved by an independent ethics committee (Comitato Etico Milano, Milan, Italy). The Institutional Review board Project no. of the study: 2016/ST/024. The study was approved on 30-Mar-2016. The study was conducted in accordance with the Declaration of Helsinki and in compliance with all current Good Clinical Practice guidelines, local laws, regulations, and organizations. The trial was registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT04438928). The trial protocol can be obtained from the corresponding author, upon reasonable request.

2.2. Study Population

Healthy, pregnant Caucasian women aged 18-42 years were screened during their first trimester prenatal visit (gestational age (GA) week 11-14) at Hospital Sacco and Hospital Buzzi in Milan, Italy. The study was proposed to all pregnant women with a singleton pregnancy within the gestational age indicated. Women were included in the study if they were having a singleton pregnancy, hemoglobin level >105 g/L, normal ultrasound examination and inconspicuous fetal anomaly screening, taking at least 400 μg folate per day, and provided written, signed informed consent for participation in the study. Women were excluded if they had experienced previous adverse pregnancy outcomes, followed a specific diet, or were already taking DHA/multivitamin supplements (except folate or iron). Full inclusion and exclusion criteria are listed in Supplementary Table 2.

2.3. Study Product

The study product was an oral MMS soft gel capsule (Elevit, Bayer) that contained 12 vitamins, six minerals and DHA (200 mg) to meet the requirements of women during pregnancy, especially during the second and third trimester [38,39] (Supplementary Table 3). One capsule was taken per day with a sufficient amount of liquid, from GA week 13-15 (Visit 2, baseline) until delivery (Visit 5; approximately 27 weeks of supplementation). The control group did not receive a placebo during this time.

2.4. Parameters Assessed

Analyses were performed at the "Luigi Sacco" Department of Biomedical and Clinical Sciences (Università degli Studi di Milano) and ASST Fatebenefratelli Sacco, Milan, Italy. In total, approximately 56 mL of blood was taken in the fasted state from each subject for the efficacy and safety assessments during the whole study

The efficacy parameters assessed are outlined in Supplementary Table 4. The change in RBC DHA (wt% TFA) from Visit 1 to Visit 4 was the primary maternal variable, to assess the beneficial effects of supplementation with micronutrients and DHA during the second and third trimesters of pregnancy. Secondary maternal variables included other RBC fatty acid parameters (TFA, eicosapentaenoic acid (EPA)

wt% TFA, DHA/TFA ratio, and omega-3 index), calcidiol (25-hydroxyvitamin D), and oxidative stress markers in blood including reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio, plasma reactive oxygen metabolites (ROMs, which are hydroperoxides), and plasma 8-isoprostane. The erythrocyte membrane fatty acid composition was determined by gas chromatography of fatty acid methyl esters. [41-43], The amount of each considered fatty acid was calculated as μg/mL of RBCs and expressed as a percentage of the total fatty acid concentration. The omega 3 index was calculated by summing the percentage of EPA and DHA (WS Harris, C von Schacky, The Omega-3 Index: a new risk factor for death from coronary heart disease?,Preventive Medicine, 2004 39 (1): 212-220,). Calcidiol levels were measured using radioimmunoassay [44], the GSH/GSSG ratio using fluorimetric assay [1], ROMs using photometric assay [45,46], and 8-isoprostane using competitive enzyme immunoassay [47].. Dietary intake was evaluated using a semi-quantitative Food Frequency Questionnaire of five food categories to assess the usual daily intake of foods and nutrients (adapted from Vioque et al. [48], which was validated in pregnant women) at Visits 2, 3 and 4. Dietary intake data and results of a small subgroup analysis in women who underwent a cesarean section (cord blood and placenta samples) will be presented elsewhere.

Safety and tolerability were assessed by evaluating the incidence and severity of adverse events (AEs) and their relationship to trial treatment. Laboratory parameters, physical examination and vital signs were also recorded.

2.5. Statistical Analysis

Assuming a treatment difference of 1.6 (standard deviation (SD) 3.4), as observed by Bergmann et al 2008 [49], 70 subjects per arm were required to achieve 80% power with 0.05 of alpha to detect the treatment difference between the supplementation and control groups. To account for a drop-out rate of 15%, approximately 164 subjects (82 per treatment group) were to be randomized to get 140 evaluable subjects.

The primary efficacy analysis was performed on the per protocol (PP) population (all subjects with efficacy data for the primary efficacy endpoint at Visit 4 who did not have protocol violations). Results were corroborated using data from the intent-to-treat (ITT) population (i.e., all subjects in the safety population who had at least one post-baseline measurement of efficacy data). The safety population comprised all subjects who were randomized into the study, and took at least one dose of the supplement for those randomized to the treatment group.

The primary efficacy endpoint was defined as the change in maternal RBC DHA (wt% total fatty acids) from Visit 1 to Visit 4, analyzed using the analysis of covariance (ANCOVA) with treatment as fixed effect and the Visit 1 value as covariate. Secondary maternal efficacy endpoints were changes from Visit 1 to Visit 4 in blood fatty acid parameters (RBC EPA (wt% total fatty acids), DHA/EPA ratio, RBC omega-3 index), 25-hydroxyvitamin D, and antioxidant status (GSH/GSSG ratio, plasma ROMs, 8-isoprostane). All secondary endpoints were analyzed similarly to the primary endpoint. Secondary infant efficacy endpoints (gestational age, head circumference, weight and length measurements, ponderal index, infant skinfold thickness, Apgar score, bone density) were collected at delivery (Visit 5) or within 10 days after delivery for bone density and analyzed using ANCOVA with treatment as fixed effect.

Safety and tolerability variables were assessed by evaluating incidence and severity of AEs, their relationship to trial treatment, and the incidence of abnormal findings in measurement of objective tolerability through vital signs, physical examination, and clinical laboratory findings. Only treatment-emergent AEs (TEAEs) were analyzed, i.e., AEs that began or worsened after randomization.

Two-sided p-values <0.05 were considered statistically significant. Results are presented as mean ± standard deviation (range), n (%), or LSMEANS (least squares means) of change from Visit 1 (95% confidence interval, CI), as appropriate. All statistical tables, listings, and analyses were produced using SAS® release 9.4 or later (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Subject Characteristics

The study took place between September 2016 to December 2019. After screening, 176 subjects were randomized to the MMS (n=87) or control (n=89) groups (Figure 2). All subjects were included in the safety

population. Forty-six subjects discontinued the study, mainly because of adverse events (32 (69.6%) subjects). The PP population comprised 141 subjects (MMS, n=65; control, n=76). The mean study duration was 24.5±6.49 (1.0-30.9) weeks, and was comparable in both groups. Overall compliance was ≥80% in 63 (72.4%) of MMS subjects, ≤80% in four (4.6%), and unknown in 20 (23.%).

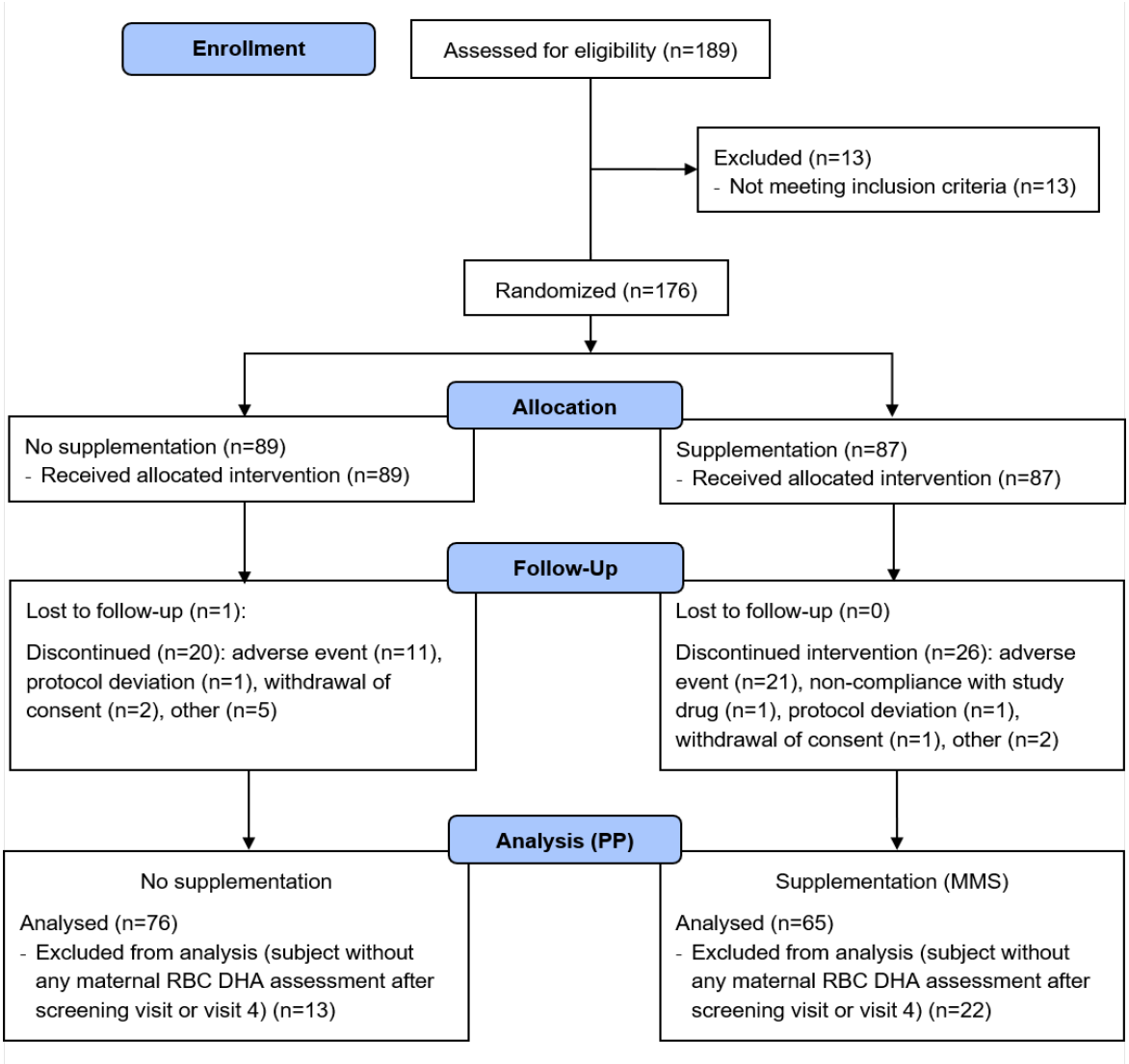


Figure 2. Flow diagram for study participants. MMS, multiple micronutrient supplementation; PP, per protocol.

Subject baseline demographics, clinical characteristics, and delivery information are shown in Table 1. The mean age was 31.9±4.64 (18-41) years and all subjects were Caucasian. All demographics were similar between groups, with no significant differences. No abnormalities in physical or gynecological examinations were reported at Visit 1 or Visit 2. Although not statistically significant, a higher proportion of subjects in the control group compared with the MMS group experienced delivery complications (16 (23.2%) vs. eight (12.9%) subjects, respectively) or had an induced labor (13 (18.8%) vs. nine (14.5%) subjects). The groups were well balanced regarding infant sex (male 58.1% in the MMS group, 56.5% in the control group).

Table 1. Subject characteristics at baseline (values expressed as n, mean ± standard deviation, and median (range), unless otherwise stated) and delivery information (values expressed as n (%), unless otherwise stated) (per protocol population).

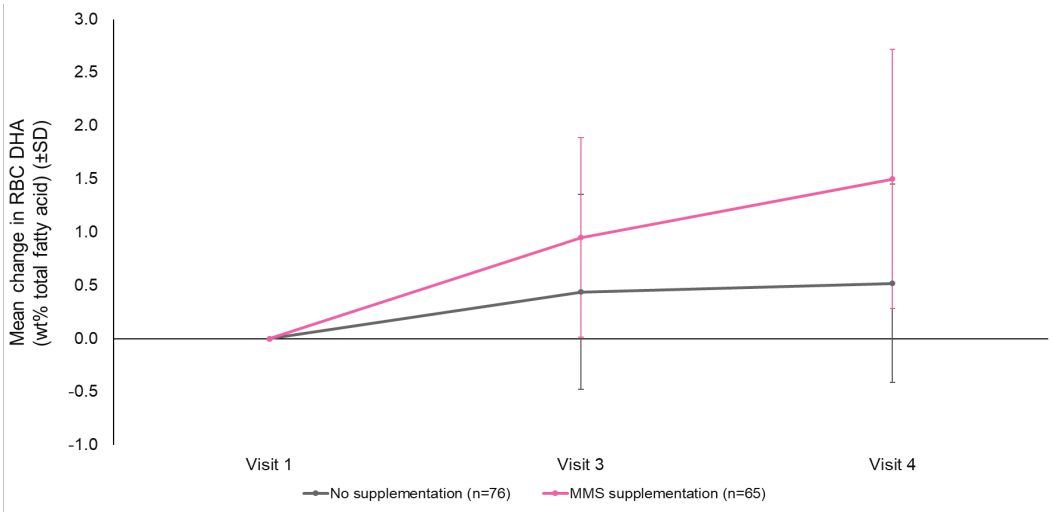
Characteristics	No supplementation (N=76)	MMS (N=65)
Age (years)	76	65
	32.3±4.72	31.4±4.52
	33.0 (18-41)	32.0 (20-40)
Weight (kg)	76	65
	61.5±9.96	63.2±9.48
	59.0 (45-87)	47.0 (47-95)
Height (cm)	76	65
	164.1±7.08	165.9±5.60
	165.0 (147-184)	165.0 (150-178)
Body mass index (kg/m^2)	76	65
	22.8±3.24	22.9±3.10
	21.7 (18.0-29.7)	22.0 (18.1-29.9)
Previous pregnancy, n (%)		
No	30 (39.5)	30 (46.2)
Yes	46 (60.5)	35 (53.9)
Smoking status, n (%)		
Never	49 (64.5)	49 (75.4)
Former [a]	27 (35.5)	16 (24.6)
Delivery information		
Subjects performing delivery visit	69	62
Type of delivery		
Vaginal	55 (79.7)	49 (79.0)
Caesarean	14 (20.3)	13 (21.0)
Delivery complications		
No	53 (76.8)	54 (87.1)
Yes	16 (23.2)	8 (12.9)

Characteristics	No supplementation (N=76)	MMS (N=65)
Induced labor		
No	56 (81.2)	53 (85.5)
Yes	13 (18.8)	9 (14.5)
Infant sex		
Male	39 (56.5)	36 (58.1)
Female	30 (43.5)	26 (41.9)

[a] Stopped smoking prior to pregnancy/when becoming aware of pregnancy consent signature plus one day. MMS, multiple micronutrient supplementation.

3.2. Efficacy Endpoints

Primary. Maternal RBC DHA (wt% TFA) increased every visit in both groups (Figure 3 and Table 2), but the mean change from Visit 1 to Visit 4 was significantly greater in the MMS group compared with the control group, with an estimated treatment difference of 0.96 (95% CI 0.61, 1.31) (p<0.0001) (Table 2). Furthermore, RBC DHA levels in women at the lower ranges increased by a greater extent in the MMS group (1.1% at Visit 3 and 1.6% at Visit 4 vs. Visit 1) compared to those in the control group (increase of 0.2% at Visit 3 and 0.5% at Visit 4 vs. Visit 1), and reached threshold levels (5% [50]) by Visit 4 (Table 2).



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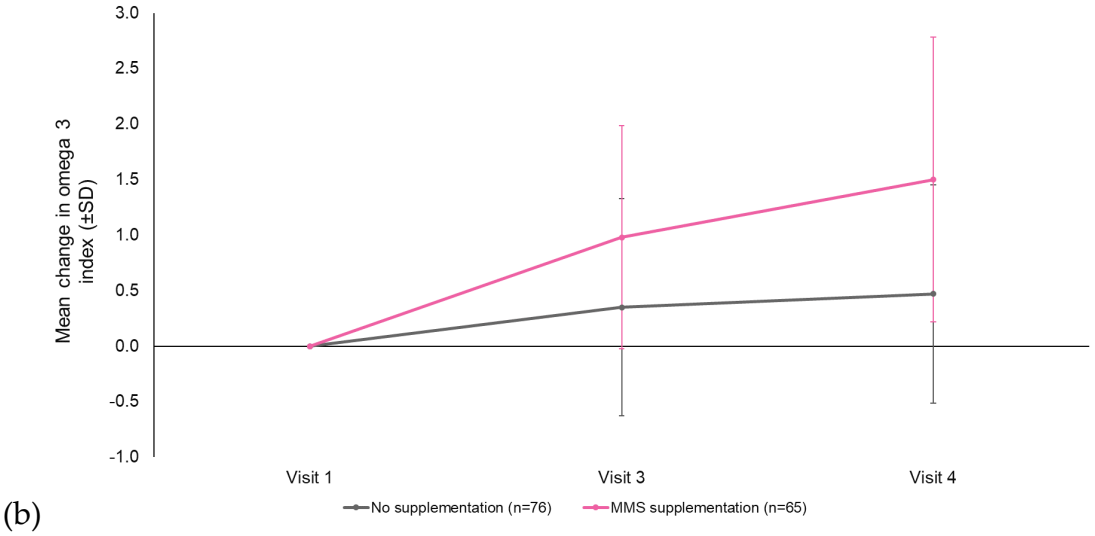

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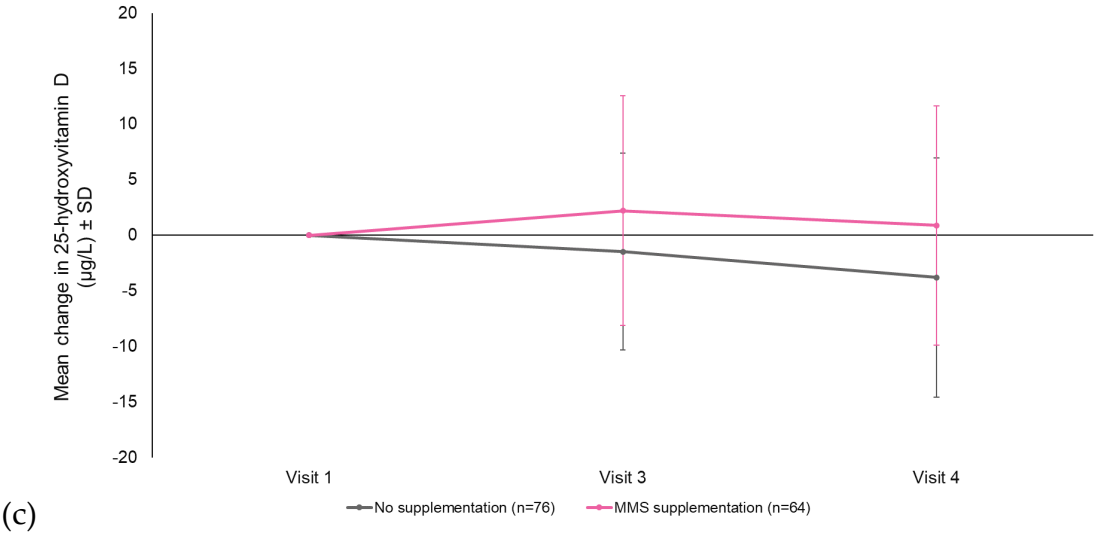

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Figure 3. Mean change (± standard deviation) from Visit 1 to Visit 4 in maternal (**a**) RBC DHA (wt% TFA) (p<0.0001 in favor of MMS), (**b**) omega 3 index (p<0.0001 in favor of MMS), and (**c**) calcidiol (25-hydroxyvitamin D) (p=0.0122 in favor of MMS) (per protocol population; LOCF approach). Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36. DHA, docosahexaenoic acid; GA, gestational age; LOCF, last observation carried forward; RBC, red blood cells; SD, standard deviation; TFA, total fatty acids; wt, weight.

Table 2. Primary and secondary outcomes at each visit (LOCF approach; values presented as mean ± standard deviation (range)) and differences between groups from Visit 1 to Visit 4 (presented as LSMEANS (95% confidence interval), ANCOVA model) (per protocol population).

	No supplementation (n=76)			MMS (n=65)		
	Visit 1	**Visit 3**	**Visit 4**	**Visit 1**	**Visit 3**	**Visit 4**
RBC DHA (wt% TFA)	6.1±1.23 (3.8-9.3)	6.6±1.30 (4.0-10.4)	6.7±1.34 (4.3-9.6)	6.1±1.26 (3.4-10.2)	7.0±1.30 (4.5-10.5)	7.5±1.48 (5.0-13.0)
LSMEANS	—			—		0.96 (0.61, 1.31)/
difference/*p* value						<0.0001 *
RBC DHA/TFA ratio	0.06±0.01 (0.04-0.09)	0.07±0.01 (0.04-0.10)	0.07±0.01 (0.04-0.10)	0.06±0.01 (0.03-0.10)	0.07±0.01 (0.04-0.11)	0.08±0.01 (0.05-0.13)
LSMEANS	—			—		0.010 (0.006, 0.013)/
difference/*p* value						<0.0001*
Omega 3 index (%)	6.7±1.38 (4.2-10.1)	7.0±1.43 (4.2-10.7)	7.1±1.45 (4.5-10.0)	6.5±1.40 (3.7-10.9)	7.5±1.43 (4.7-11.1)	8.0±1.59 (5.3-13.6)
LSMEANS	—			—		1.00 (0.64, 1.37)/
difference/*p* value						<0.0001 *
Calcidiol (ug/L)	21.6±8.94 (5.5-48.8)	19.9±9.87 (4.6-64.1)	17.8±9.72 (4.0-45.0)	20.5±7.54 (4.4-36.5)	22.8±8.94 (4.0-48.6)	21.4±9.07 (5.5-42.7)
LSMEANS	—			—		3.96 (0.88, 7.04)/
difference/*p* value						0.0122 *

* Two-sided *p* value <0.05 considered statistically significant. Visit 1: Screening (GA Week 11/14); Visit 3: GA Week 24/26; Visit 4: GA Week 34/36. DHA, docosahexaenoic acid; GA, gestational age; LOCF, last observation carried forward; LSMEANS, least squares means (difference = supplementation – no supplementation); MMS, multiple micronutrient supplementation; RBC, red blood cells; TFA, total fatty acids; wt, weight.

Secondary maternal endpoints. Significant differences were observed in favor of MMS for maternal RBC DHA/TFA ratio (estimated difference 0.01 (95% CI 0.006, 0.013); $p<0.0001$), omega-3 index (estimated difference 1.00 (95% CI 0.64, 1.37); $p<0.0001$), and calcidiol (estimated difference 3.96 (95% CI 0.88, 7.04) μg/L; $p=0.0122$) (Figure 3 and Table 2).

The remaining secondary efficacy endpoints (maternal RBC TFA, RBC EPA (wt% TFA, GSH/GSSG ratio, ROMs, 8-isoprostane) were comparable between groups, albeit slightly higher in the MMS group, with no significant differences (Supplementary Table 5).

Secondary infant endpoints. As outlined in Supplementary Table 6, infant variables were comparable between groups, with no statistically significant differences apart from subscapular skinfold thickness (thicker in the MMS group, $p=0.0292$) and bone density in m^2 (borderline significantly greater in the control group, $p=0.0486$).

Dietary intake. Assessment of dietary intake showed that consumption of the macro- and micronutrients measured was comparable between groups at each visit (Supplementary Table 7).

3.3. Safety Analysis

As outlined in Table 3, 125 (71.0%) subjects reported at least one TEAE pertinent to the mother (232 TEAEs overall) and 23 (13.1%) subjects reported them as serious, with a comparable number in each group. In the MMS group, 19 (21.8%) had one TEAE that led to permanent treatment discontinuation. Only three (3.5%) subjects in the MMS group had at least one suspected related TEAE (vomiting, with mild severity). At least one TEAE pertinent to the fetus/child were reported in ten (5.7%) subjects (13 TEAEs overall), and five (2.8%) reported them as serious. A higher proportion of subjects reported a TEAE in the MMS group, but none were considered treatment related. One (1.6%) subject had one TEAE pertinent to the fetus/child that led to permanent discontinuation. There was one fatality in the MMS group, unrelated to study treatment. No relevant changes in clinical laboratory parameters (i.e., hematology, kidney function, liver function, blood coagulation, CRP) were observed, although there was a decrease in mean ferritin levels in both groups over the course of the study. Physical and gynecological examinations were normal throughout.

Table 3. Summary of participants with treatment-emergent adverse event (safety population; values expressed as n (%) subjects).

Parameters	No supplementation (N=89)	MMS (N=87)	Total (N=176)
Number of TEAEs pertinent to the mother	114	118	232
Any TEAEs pertinent to the mother	64 (71.9)	61 (70.1)	125 (71.0)
At least one suspected related [a]	NA	3 (3.5)	3 (1.7)
At least one serious TEAE	11 (12.4)	12 (13.8)	23 (13.1)
At least one leading to temporary treatment interruption [b]	NA	1 (1.2)	1 (0.6)
At least one leading to permanent treatment discontinuation [c]	NA	19 (21.8)	19 (10.8)
Fatal outcome			
Number of TEAEs pertinent to fetus/child	0	0	0

Any TEAEs pertinent to fetus/child	4	9	13
At least one suspected related [a]	3 (3.4)	7 (8.1)	10 (5.7)
At least one serious TEAE	NA	0	0
At least one leading to temporary treatment interruption [b]	2 (2.3)	3 (3.5)	5 (2.8)
At least one leading to permanent treatment discontinuation [c]	NA	1 (1.2)	1 (0.6)
Fatal outcome	NA	1 (1.2)	1 (0.6)
	0	1 (1.2)	1 (0.6)

[a] Suspected related adverse events were those events with causal relationship equal to related; [b] adverse events leading to temporary treatment interruption were those events with action taken equal to drug interrupted; [c] adverse events leading to permanent treatment discontinuation were those events with action taken equal to drug withdrawn. MMS, multiple micronutrient supplementation; NA, not applicable; TEAEs, treatment-emergent adverse events.

4. Discussion

Supplementation with MMS plus DHA throughout the second and third trimester of pregnancy led to a significant increase in RBC levels of DHA, as well as the proportion of DHA compared with EPA and TFA. There was also a significant increase in the omega-3 index, while vitamin D levels increased during the course of the study compared to a decrease in women who did not receive supplementation. In the infant, a significantly greater subscapular skinfold thickness was observed in the MMS group. Safety outcomes were comparable between groups and MMS was well tolerated.

Our findings demonstrate that RBC DHA levels were significantly higher in the MMS than the control group. In pregnant women, the target RBC DHA level is 5% [50] (with <4.3% considered very low [51]). In our study, although average RBC DHA levels were above 6% at each visit (with higher levels in the MMS group), the lower ranges indicated that some women in both groups fell below this value. Nevertheless, RBC DHA levels in women at the lower ranges increased by a greater extent in the MMS group compared to those in the control group over the course of the study, and reached the threshold by the third trimester (Table 2).

The omega-3 index was also significantly higher after supplementation. As RBC EPA values were comparable between groups, the increase in omega-3 index must be the result of an increase in DHA. In cardiovascular disease, the target range for the omega-3 index is 8-11%; it has been suggested that this range might also be suitable during pregnancy and lactation [52]. Reference values of 7.5-10.0% have also been recommended in pregnant women [53]. In our study, while the omega-3 index increased from 6.7% to 7.1% in the control group, the increase was greater (6.5% to 8.0%) in the MMS group. Therefore, supplementation with DHA helped women to reach target levels during pregnancy.

Current nutritional recommendations indicate that pregnant and lactating women should aim to achieve an average dietary intake of at least 200 mg DHA/day [54]. However, consumption of omega-3 fatty acids remains low particularly in pregnant and lactating women [55]. This is of relevance considering the vital roles of DHA in neurodevelopment, visual development, and neuroinflammation [56]. Moreover, pregnancy syndromes such as gestational diabetes and preeclampsia have also been associated with altered maternal omega-3 status and placental omega-3 metabolism [57-59].

The finding that there was a significant increase in calcidiol levels in supplemented women, but not in the non-supplemented control group, is also of interest. Vitamin D is essential for the health of both the developing fetus and the mother [60], and insufficient levels may have an adverse effect on skeletal homeostasis in the infant [61] and increase the maternal risk of preeclampsia [5].

In our study, no significant differences were observed between supplemented and control women regarding markers of oxidative status. Oxidative stress has been implicated in many pathological processes during pregnancy [5]. However, this particular population of pregnant women was selectively chosen as a low-risk population, likely not at risk for decreased antioxidant status. Moreover, the sample size of the study was calculated based on the primary outcome; therefore, these results must be considered exploratory.

To our knowledge, this is the first randomized controlled trial evaluating the combination of MMS plus DHA in pregnant women. Our results indicate that in a high-income country setting supplementation with micronutrients in combination with DHA can optimize maternal DHA status [62-64] – despite the women in our supplemented group having a slightly lower intake of DHA from food. The timing of supplementation is important, and should occur in line with the development and growth of the embryonic brain, particularly during the later stages of pregnancy [17,21] when DHA rapidly begins to accumulate [18,22]. Furthermore, supplementation with MMS during pregnancy, as in our study, can improve maternal and infant outcomes, leading to reductions in the incidence of pre-eclampsia [65], neural-tube defects [65,66], low birthweight and small-for-gestational age babies [3], limb reduction defects, and congenital urinary tract abnormalities [65]. There may also be long-term benefits in children [4] (e.g., cognitive development [67,68]). Although many of these results have been reported from low- to middle-income countries, micronutrient levels in pregnant women are often insufficient even in industrialized countries, where dietary resources are more readily available [12]. However, the routine use of multivitamins during pregnancy has not yet been recommended in high-income countries, despite the benefits on clinical outcomes [69]. Currently, only folic acid and iron are recommended as standard interventions in pregnancy in industrialized countries [37].

Further research is necessary to better understand whether the improvements in maternal DHA status, as well as other improvements in omega-3 index and calcidiol levels, have a positive impact on maternal and infant clinical outcomes. Large, long-term randomized controlled trials on MMS supplementation including DHA are essential.

Our study has some limitations, including the lack of a placebo control group and the consequent unblinded nature of the study (which could have led to expectation bias[70]), the small sample size, and the fact that only Caucasian women were included (which limits the generalizability of the results). Adequately-powered studies with a varied study population are necessary to better establish the impact of different baseline characteristics in pregnant women and evaluate clinical outcomes.

5. Conclusions

Supplementation with MMS plus DHA in pregnant women can complement dietary intake and significantly improve maternal DHA and vitamin D status. This finding is important in light of the essential roles of DHA in the developing brain of the fetus, in visual development, and in immunomodulation.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Assessment schedule, Table S2: Full list of inclusion and exclusion criteria, Table S3: Composition of the multimicronutrient supplement (MMS) compared to the recommended dietary allowance (RDA) and upper tolerable limits (UL) for pregnant women, Table S4: Blood and plasma sampling for efficacy parameters in all pregnant women, Table S5: Change in primary and secondary maternal efficacy endpoints from Visit 1 to Visit 4 (gestational age week 34/36), Table S6: Infant assessments, Table S7: Daily macronutrient intakes during the study (per protocol population) compared with recommended allowances for pregnant women.

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Conflicts of Interest: SM and ES are employed by Bayer consumer Care AG. All other authors (MM, CN, CM, SDF, MDP, RC, MP, VS, IC) state that they have no conflicts of interests.

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