Journal of Clinical Nutrition and Metabolism

Research Article

A SCITECHNOL JOURNAL

Docosahexaenoic Acid and Eicosapentaenoic Acid Supplementation Improves Plasma Lipid Profile in Late Pregnancy

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Abstract

Background: Pregnant women are exposed to atherogenic state during pregnancy which further characterized by hypertriglyceridemia a n-3 long chain of polyunsaturated fatty acids (LCPUFA) are proposed to have a lipid-lowering effect during pregnancy.

Objective: To explore the possible influence of maternal Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) supplementation during the last trimester of pregnancy on serum lipid concentrations.

Methods: The present controlled intervention trial was conducted at the Obstetrics and Gynecological Department of the Jordan University Hospital from November 2014 to May 2015. 84 pregnant women at 20 weeks of gestation were enrolled and divided into two groups: (1) women (n = 42) who received 600mg DHA and EPA supplementation daily and (2) women (n = 42) who didn't receive supplementation. Lipid profile and fatty acid levels were measured at the beginning and at the end of treatment.

Results: Women who received supplementation during pregnancy had significantly lower plasma concentration of triacylglycerol (TAG) and higher HDL-C than the control group. Maternal plasma concentration of TAG was independently and inversely associated with n-3 index, and positively associated with the ratio of n-6:n-3 fatty acids after adjusting of confounders.

Conclusion: DHA and EPA supplementation improves the blood lipid profile in pregnant women through hypotriglyceridemic effects and increase HDL-C concentrations which are associated with reduced incidence of atherogenic disease.

Keywords

Omega-3 fatty acids; Pregnancy; Plasma lipid profile; Triglycerides; LDL-cholesterol; HDL- cholesterol

Abbreviations

ALA: α-Linolenic Acid; ARA: Arachidonic Acid; COX: Cyclooxygenase; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; FA: Fatty Acid; HDL-C: High Density Lipoprotein Cholesterol; LCPUFAs: Long Chain Poly Unsaturated Fatty Acid; LDL-C: Low density lipoprotein cholesterol; LNA: Linoleic acid; LOX: Lipoxygenase; TAG: Triacylglycerol; VLDL-C: Very Low Density Lipoprotein Cholesterol

Introduction

The omega-3 fatty acid (n-3), α-linolenic acid (ALA, 18:3 n-3), is an essential FA [1]. The biologically active forms of n-3 FA are docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5 n-3) [2] that are synthesized from the parent n-3 essential FA ALA [3]. The primary sources of ALA are plant sources of flax seed, walnuts, and some vegetable oils, such as soybean oil and canola oil [4]. However, these plant sources are not sufficient to meet physiologic demands during pregnancy for both mother and fetus because only a small proportion (<1%) of ALA is converted to DHA. The richest sources of EPA and DHA are fatty fish like salmon, trout, tuna, sardines and fish oils. These sources provide EPA and DHA that is ready for immediate use by the body [3]. N-3 FAs reduces the risk factors which were responsible for the pathogenesis of thrombotic and atherosclerotic disease [5]. The benefit of n-3 FAs is attributed to their capacity to modulate cellular metabolic function by bringing about an alteration in cellular membrane structure and function [6], modulating various signal pathways and alteration in inflammatory process in which eicosanoids participate [7]. The long chain n-3 Fas, EPA and DHA as well as the n-6 FA, arachidonic acid (ARA, 20:4 n-6), which is derived from the linoleic acid (LNA; 18:2 n-6), are the main precursors of the regulatory eicosanoids.

Many studies reported the protective effects of n-3 FAs from marine sources on atherosclerotic disease [8-10]. The People whose diet contains large amount of n-3 FAs have reduced concentrations of plasma cholesterol and TAG when compared to those who ingest a Western- type diet [11-19]. Many studies observed positive effects of n-3 fatty acids intake on lowering of TAG and very low density lipoprotein cholesterol (VLDL-C) [10]. Some authors [11] found that a high dose of (3.4g/d) of EPA+DHA significantly lowered TAG in patients with moderate hyperlipidemia. Other authors [12] reported significant reduction in the levels of TAG and VLDL-C and marked elevation in the level of high density lipoprotein cholesterol (HDL-C) when comparing pre and post supplementary n-3 FAs in hyperlipidemic patients.

Recent research shows that pregnant women are exposed to hypertriglyceridemia that is characterized by its association with hypercholesterolemia [13]. This could be due to hormonal imbalance in late pregnancy [14]. Changes in lipid metabolism in early and mid-pregnancy occurs in response to increased estrogen, progesterone, and insulin concentrations which promote lipid deposition and inhibit lipolysis. However, in late pregnancy, at the time of maximal fetal growth, maternal fat metabolism shifts from an anabolic state to a catabolic state to meet maternal and fetal metabolic needs, promoting the use of lipids as a maternal energy source while

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Received: February 16, 2018 **Accepted:** April 03, 2018 **Published:** April 12, 2018

preserving glucose for the fetus [13,14]. TAG, FAs, cholesterol, and lipoprotein concentrations increase in blood plasma due to increase in lipolytic activity in adipose tissue in response to relative insulin resistance and under hormonal control of progesterone, cortisone, prolactinand leptin [15]. This increase of lipid and lipoprotein metabolism may reach the level of cardiovascular risk and related to preeclampsia during the second trimester [16, 17]. It has also been reported that dyslipidemia predisposing to atherosclerosis occurs in preeclampsia [18].

Maternal consumption of fish, or long chain n-3 (LCPUFA) supplementation, namely EPA and DHA, are associated with the hypotriglyceridemic effects and increased serum HDL-C concentration [19]. These FAs have antiatherogenic effects through one or multiple pathophysiological pathways. The present study was designed to explore the possible influence of maternal DHA and EPA supplementation during the last trimester of pregnancy on serum lipid concentrations.

Methods

Study group and design

This study is a controlled intervention study that was conducted at the Obstetrics and Gynecological Department of the Jordan University Hospital from November 2014 to May 2015. Eligible women were those aged 18-45 years at 20 weeks of gestational age and who were planning to deliver at Jordan University Hospital. We excluded women from the study having: high risk pregnancies, history of preterm delivery during an earlier pregnancy, intrauterine growth retardation, and history of stillbirth or fetal death. Along with this we also excluded smokers, women who were previously treated with anticoagulant medications, anti-platelet drugs and TAG lowering medications, and women who were taking the following herbs: clove, garlic, ginger, ginkgo, ginseng, red clover and turmeric [20]. A convenient sample of 120 pregnant women was recruited at their 20th weeks of pregnancy after providing informed written consent. This control intervention study was performed through utilizing computer balanced generated random numbers. The computer numbers of one to three digits organized in 120 blocks were generated from an excel computer program. Each number was inserted separately into an envelope by the investigator to obtain 120 sealed envelopes which were used for group' assignment. Using a randomization scheme, the participants were assigned to treatment groups at the baseline visit by a study coordinator. During the enrollment we excluded five subjects who were taking aspirin, 3 subjects who were diagnosed with hyperthyroidism, and 6 women who delivered in another hospital. In addition, 22 subjects did not continue the study: 6 subjects withdrew, 6 subjects felt discomfort from the supplement, 7 subjects disliked the taste of the supplement, and 3 subjects reported gastric pain. The remaining women (n = 84), were divided into two groups (42 women each): Group 1 received 600mg n-3 LCPUFA daily (containing 550mg DHA and 50mg EPA), while group 2 received no supplementation. The first group women were instructed to consume two soft gel capsules daily. Using structured questionnaires, interviewers collected information on maternal socio-demographic data that included: maternal age, height, pre-pregnancy weight, gestational weight, educational level, annual household income, medical histories and smoking status. Body mass index (BMI) for each woman was calculated as weight in kilograms divided by height in meters squared [21]. Weight was measured to the nearest 0.1kg using a digital floor scale while standing height measurement was made to the nearest 0.1cm using a wall-mounted stadiometer [22]. The study was conducted according to the guidelines in the Declaration of Helsinki and all procedures involving human subjects were approved by the committee for ethics in medical research at Jordan University Hospital.

Plasma lipid analysis

Blood samples were collected from all the pregnant women at 20 gestational weeks and at delivery time. Maternal fasting blood samples were collected in vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) and centrifuged at 5,000rpm for 10min to separate plasma from erythrocytes. TAG and cholesterol were enzymatically analyzed using commercial standard enzymatic kits. Total cholesterol was measured with the cholesterol oxidase peroxidase methods (CHOD- POD method, Shenzhen, Germany). TAG was measured with the Glycerolkinase Peroxidase-Peroxidase methods (GPO-POD method, Shenzhen, Germany). HDL cholesterol (HDL-C) was separated from VLDL, chylomicron and LDL-C by a chemical precipitation technique using phosphotungstic acid and magnesium chloride. After centrifugation the supernatant fluid that contains HDL fraction was assayed for HDL cholestrol with cholesterol liquicolor test kit (Human Gmbh 65205 Wiesbaden, Germany). The Friedewald equation was used to calculate LDL-C as follows: LDL-C = total cholesterol-[HDL+TG/5] [23]. All plasma lipid profile values were expressed as mmol/L.

Determination of plasma fatty acids

Plasma n-3 FAs are considered good biomarkers of n-3 FAs status, which rapidly respond to supplementation [24]. Plasma FA composition was measured as described by Cao et al. [25]. Briefly, membrane FAs was transmethyated by heating them at 100°C for 15 min in 1 ml boron trifluoride 14%. Then FA methyl esters were extracted with 500µl isooctane. Extracted methyl esters were analyzed using gas-liquid chromatography (GC 2010, Shimadzu Corporation, Japan) equipped with a 60m length Thermo Tr-Fame capillary column. The FA composition of plasma phospholipids was expressed as a relative percentage of the total area of all FA (mmol/% of total FAs) from 14:0 to 24:1 [25,26]. Based on FA analysis in 10 runs of replicate samples, the inter-assay coefficient of variation (CV) was (<2). The parameters of n-3 and n-6 FA were calculated based on n-3 index = ((DHA + EPA)/ total plasma FA)*100, Total n-3 = (ALA+DHA+ EPA+DPA)/ total plasma FA)*100, Total n-6 = (LA+ARA)/ total plasma fa)*100 and the ratio of n-6: n-3 = total n-6/ total n-3*100 [27-32].

Dietary Assessment

Three-day food records were used to obtain dietary intake information during recruitment. All participants were provided with verbal and written instructions on how to measure and record all foods and beverages consumed using food measures (cups or tablespoons) and a dietary food log book (pictures). The participants were instructed to record all foods and beverages consumed during three days that included two weekdays and one weekend in a seven days period at the time of eating to avoid reliance on memory. Participants were instructed to give details regarding amount consumed, methods of preparation, recipe, ingredients, portion size, and food brand name for packaged foods when possible. The participants were also instructed to estimate the weight of foods they

consumed away from home to the best of their ability. The researcher reviewed food records with the participants to clarify entries. To estimate the average daily dietary intake of n-3, we used the Food Processor SQL software (ESHA research, Version 10.90) [27]. To cover the program limitation of some composite dishes and food sources we used Middle East Food Composition Tables [28].

Statistical analysis

Statistical evaluation was performed using SPSS Version 22 (SPSS statistics for Windows V22, IBM Corp., Armonk, NY, USA). Differences in mean lipid concentrations between the two sample groups before and after supplementation were compared using repeated measure analysis (ANOVA). The associations of plasma FA levels with the serum lipid concentrations were determined using multivariable linear regression. To assess confounding factors, we have included the multiple linear regression model the covariates (additional independent variables) of those participant characteristics that independently predicted the outcomes, namely maternal age, pre-pregnancy body mass index, and body mass index at delivery sequentially. Then the unadjusted and adjusted regression models were compared. P value ≤0.05 were considered significant.

Results

Demographic and baseline characteristics of all pregnant women are listed in Table 1. The mean age for the supplementation group was (31.2 ± 5.7) years, whereas it was (29.8 ± 5.5) years for the control group with no significant differences between them. The educational level was more than twelve years for the majority of women in both the groups. There were no significant differences between the two study groups regarding gravidity, parity, total energy intake, or the percent of total energy from fat. Also, the initial prepregnancy BMI was not significantly different between groups.

Differences of FAs profile between the supplementation group and the control group at the beginning and at the end of the study are shown in Table 2. The results showed elevation in the plasma level of DHA and n-3 index and a reduction in ARA and n-6: n-3 ratio after the supplementation.

Changes and average percentage change of the maternal plasma lipid profile after supplementation are shown in Table 3. The supplementation group had significantly lower plasma TAG concentration (-35% mmol/L) as compared with the control group (-1.1% mmol/L). On the other hand, HDL-cholesterol significantly increases (+14.2% mmol/L) among supplementation group and a decrease in HDL cholesterol in the control group (-7.1% mmol/L). Regarding the LDL-C there was no statistical difference in LDL-C among supplementation group and control group (P>0.05) before and after supplementation.

Table 3 also shows the changes of maternal plasma TAG, low density lipoprotein (LDL) - cholesterol, high density lipoprotein (HDL)-cholesterol, and total cholesterol: HDL ratio in between two study groups. The table shows that there were no significant differences between the two experimental groups before and after of the supplementation. After supplementation women who received supplementation during the third trimester significantly have lower plasma TAG than those of the control group (-0.7mmol/L) and higher HDL-cholesterol (+0.4mmol/L), and lower total cholesterol-HDL ratio (-0.7mmol/L). There were no changes in LDL-C between the two groups (p>0.05).

After adjusting the confounding variables (maternal age, pre pregnancy body mass index, body mass index at delivery, and smoking) Table 4 shows that maternal mean plasma concentration of TAG was inversely and significantly associated with DHA (β = -2.1 mmol/L), and n-3 index (β = - 2.1mmol/L, p = 0.004). However mean plasma concentration of TAG was positively and significantly associated with ARA (β = 1.6 mmol/L, p = 0.01) and the ratio of n-6: n-3 FA (β = 1.6 mmol/L). Mean plasma concentration of HDL-C was positively and significantly associated with n-3 index (β = 1.4mmol/L) and negatively and significantly associated with ratio of n-6: n-3 FA (β = -1.7mmol/L). The ratio of (total cholesterol: HDL-C) was independently and significantly associated with ARA (β = 4.8 mmol/L). On the other hand there was no significant association between the ratios of total cholesterol: HDL-C with the other maternal plasma FAs (P>0.05). Also there was no significant association between plasma concentration of LDL-C and plasma FAs (ARA, DHA, EPA, n-3 index, and ratio of n-6: n-3).

Discussion

In this intervention study, we used a supplement of 600mg/day of combined (DHA and EPA). Intake of 500 - 1,000mg/day of EPA+DHA, which is within the current American Heart Association guidelines, is suggested to achieve cardioprotection through hypotriglyceridemia [29]. The cardiovascular benefit of n-3 supplementation is shown in large randomized control trials as indicated by a reduction of cardiovascular risk from 19%-45% in those with or without risk of coronary artery disease [30]. The American College of Obstetricians and Gynecologists, the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) recommend pregnant women to consume 2 servings of low mercury fish and seafood each week (approximately 340g or 12oz of seafood per week) [2]. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends 650mg of combined EPA and DHA per day, of which at least 300mg is DHA, during pregnancy [31]. Whereas the Dietitians of Canada and the Academy of Nutrition and Dietetics recommend

Table 1: Selected socio-demographic data of the study population.

Variables	Supplementation group (n=42)	Control group (n=42)
Age	31.2 ± 0.8	29.9 ± 0.9
Educational level		
<12 (y)	10 (23.8%)	12 (28.6%)
>12 (y)	31 (73.8%)	30 (71.4%)
Gravidity	3.2 ± 0.3	4.1 ± 0.4
Parity	2.4 ± 0.3	3 ± 0.4
Pre pregnancy BMI (Kg/m²)	24.27 ± 3.72	26.16 ± 5.31
≤18.5	2 (4.8%)	0 (0%)
>18.5- 24.999	25 (59%)	20 (48.8%)
≥25- 29.999	10 (23.8%)	15 (34.9%)
BMI at delivery	30.2 ± 3.8	31.6 ± 5.4
≤18.5	0 (0%)	0 (0%)
>18.5- 24.999	2 (4.9%)	3 (7%)
≥25- 29.999	22 (53.7%)	15 (35.7%)
≥30	17 (41.5%)	24 (57.1%)
Total energy, Kcal/day	2048.8 ± 116.5	1817 ± 85
% Daily energy from fats	37.4 ± 1.4	40.2 ± 0.6

Note: BMI: Body Mass Index. Values were expressed as mean ± SEM; within a row, values in brackets were expressed as percentage.

Table 2: Comparison of fatty acids profile between the supplementation group and the control group at the beginning and at the end of the study.

Maternal plasma fatty acids (mol/%)	Before supplementation		After supplementation	
	Supplementation group	Control group	Supplementation group	Control group
Palmatic acid 16:0	7.1 ± 0.3	6.4 ± 0.4	8.1 ± 0.4	8.2 ± 0.4
Palmitoleic acid 16:1	0.6 ± 0.1	1.4 ± 0.5	0.5 ± 0.1	0.7 ± 0.1*
Stearic acid 18:0	1.9 ± 0.1	1.3 ± 0.1*	1.9 ± 0.2	1.6 ± 0.1
Oleic acid 18:1 n-9	8.2 ± 2.0	5.2 ± 0.3	6.4 ± 0.4	5.7 ± 0.3
LNA 18:2 n-6	5.8 ± 0.4	5.0 ± 0.3	5.7 ± 0.3	5.7 ± 0.3
ALA 18:3 n-3	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
ARA 20:4 n-6	4.5 ± 0.2	2.7 ± 0.5*	1.5 ± 0.3	4.0 ± 0*
EPA 20:5n-3	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
DPA 20:5n-3	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.04	0.3 ± 0.1
DHA 22:6 n-3	0.3 ± 0.0	0.3 ± 0.1	1.0 ± 0.1	0.3 ± 0.0*
n-3 index	0.8 ± 0.2	0.8 ± 0.2	1.7 ± 0.2	0.7 ± 0.1*
n-6:n-3	10.1 ± 2	6.1 ± 1	2.7 ± 0.3	7.3 ± 1.5

Note: LNA: Linoleic Acid; ALA: α-Linoleic Acid; ARA: Arachidonic Acids; EPA: Eicosapentaenoic acid, DPA: Docosapentaenoic Acid; DHA: Docosahexaenoic Acid. The initial reading was taken before the start of the study and the final reading was taken at the end of the study.*significant ($p<0.05$) within a row.

Table 3: Distributions of maternal plasma concentration of TAG, LDL-C, high HDL-C, total cholesterol and total cholesterol: HDL ratio in the two study groups before and after supplementation.

Maternal plasma lipid and lipoprotein (mmol/L)	Supplementation group		Control group		Differences between groups	
	Before	After	Before	After	Before	After
	Mean ± SEM (mmol/L)		Mean ± SEM (mmol/L)		Mean ± SEM (mmol/L)	
Triacylglycerol	3.1 ± 0.1	2.0 ± 0.1	2.7 ± 0.2	2.7 ± 0.1*	0.3 ± 0.2	- 0.7 ± 0.1*
Mean changes in the same groups	-1.1 ± 0.0		-0.03 ± 0.2			
Percent changes	(-35.4%)		(-1.1%)			
HDL- C	1.4 ± 0.0	1.6 ± 0.0	1.4 ± 0.04	1.3 ± 0.0*	0.04 ± 0.7	0.4 ± 0.1*
Mean changes	0.2 ± 0.1		-0.1 ± 0.1			
Percent changes	(14.2%)		(- 7.1%)			
LDL-C	3.3 ± 0.1	3.2 ± 0.2	3.0 ± 0.1	3.8 ± 0.2*	0.3 ± 0.2	- 0.6 ± 0.2
Mean changes	-0.05 ± 0.2		0.9 ± 0.2			
Percent changes	(-1.5%)		(31%)			
Total cholesterol	6.1 ± 0.2		5.1 ± 0.2			
Mean changes	-0.6 ± 1.4	5.5 ± 0.2*	-0.03 ± 0.2	6.4 ± 0.2*	1.0 ± 0.2*	- 0.9 ± 0.3*
Percent changes	(9.8%)		(-0.6%)			
Total cholesterol/ HDL-C ratio	3.9 ± 0.2	3.7± 0.1	3.7 ± 0.1	4.6 ± 0.2*	0.02 ± 0.2	- 0.7 ± 0.3*
Mean changes	0.2± 0.9		0.9 ± 1.4			
Percent changes	(-5.4%)		(24.3%)			

Note: LDL-C: Low-Density Lipoprotein Cholesterol; HDL-: High-Density Lipoprotein Cholesterol; TAG: Triacylglycerol, Values were expressed as mean ± SEM; Within a row, values with sign* are significantly different at ($P<0.05$); Values were expressed as percentage; within a column

500mg/day of DHA and EPA for healthy individuals [32] and the European Food Safety Authority [33] recommends that all pregnant women should consume 700-1400mg DHA/week. These doses are in agreement with the US Food and Drug Administration (FDA) that has approved a dose of 4.0g/day of n-3 FAs for the treatment of hypertriglyceridemia. It is estimated that two meals of oily fish per week can provide 400 to 500mg/day of DHA and EPA [30].

During the second half of pregnancy, pregnant women are exposed to increase in plasma lipids. In many pregnant women, cholesterol, TAG, high density cholesterol lipoprotein (HDL-C), and low density lipoprotein cholesterol (LDL-C) were increased during pregnancy as a direct consequence of physiological gestational hyperlipidemia [34]. The TAG shows the largest increase and HDL-C shows the smallest. All these lipids are increased during the 40 weeks of pregnancy, except HDL-C which is stabilized during the second trimester. The changes in lipid profile during pregnancy occur as a result of hormonal control. Increased concentrations of prolactin inhibit adipose tissue lipoprotein lipase activities which in turn increase the concentration of plasma TAG [35]. Human placental lactogen (HPL) has lipolytic activity which may increase substrate supply of free fatty acids for the increased maternal hepatic TAG rich very low density lipoproteins (VLDL) production [13,15]. Estrogen can stimulate hepatic production of the VLDL, and thus increase TAG levels, by inhibition of hepatic and adipose tissue lipoprotein lipases [15]. Estrogens increase the concentration of HDL cholesterol by stimulating the production of apolipoproteins AI and AII, and by reducing the catabolism of HDL_2 to HDL_3 by hepatic lipase and stimulation of the production of LDL and apolipoprotein B. On the other hand, progesterone was found to increase the concentrations of LDL cholesterol and decrease the concentrations of HDL cholesterol [36]. There is an association between concentrations of total cholesterol, TAG and LDL-C and increased risk of coronary heart disease [24].

In the present study, our results showed that supplementation with 600mg/day of combined (DHA and EPA) significantly reduced

Table 4: Association of maternal plasma TAG, LDL-C, HDL-C, and total cholesterol concentrations with plasma n-3 and n-6 Fatty Acid concentrations at delivery.

Maternal plasma fatty acids (mol/% total)	Triglycerol mmol/L	HDL-C mmol/L	LDL-C mmol/L	Cholesterol: HDL-C
Un Adjusted	**β ± SEM**	**β ± SEM**	**β ± SEM**	**β ± SEM**
EPA 20:5 n-3	-2.5 ± 0.1*	1.3 ± 0.1*	-3.4 ± 0.2	-4.6 ± 0.2*
DHA 22:6 n-3	-2.5 ± 0.1*	1.4 ± 0.1*	-3.6 ± 0.2	-4.7 ± 0.2*
n-3 index Fatty Acid	-2.6 ± 0.1*	1.3 ± 0.1*	-3.9 ± 0.2*	-4.8 ± 0.2*
ARA 20:4 n-6	2.1 ± 0.1*	1.5 ± 0.1	3.4 ±0.2	4.2 ± 0.2
Ratio of n-6: n-3 Fatty Acids	1.9 ± 0.1*	-1.6± 0.1*	3.2 ± 0.2	4 ± 0.3
Adjusted†	**Adj β ± SEM**	**Adj β ± SEM**	**Adj β ± SEM**	**Adj β ± SEM**
EPA 20:5 n-3	-2.6 ± 0.7	0.9 ± 0.3	-3.6 ± 1.2	-3.6 ± 1.2
DHA 22:6 n-3	-2.1 ± 0.6*	1.4 ± 0.3	-3.7 ± 1	-4 ± 1.2
n-3 index Fatty Acid	-2.1 ± 0.7*	1.4 ± 0.3*	-3.9 ± 0.9	-3.9 ± 0.9
ARA 20:4 n-6	1.6 ± 0.6*	-1.6 ± 0.3	2.8 ± 0.2	4.8 ± 0.2*
Ratio of n-6: n-3 Fatty Acids	1.6 ± 0.6*	-1.7 ± 0.3*	3.2 ± 1.2	3.2 ± 1.2

Note: ARA: Arachidonic acids, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, LDL-C: low-density lipoprotein cholesterol, HDL- high-density lipoprotein cholesterol. Within a row, values with sign* significantly different (P<0.05). †Model adjusted for maternal age, pre pregnancy body mass index, body mass index at delivery, and smoking.

TAG levels and increased HDL-C levels in the subjects of the supplementation group. Also we noted that there were statistically significant inverse association between plasma TAG concentrations and n-3 index and DHA content in maternal plasma. Plasma n-6 fatty acids, particularly ARA, were positively associated with plasma TAG concentrations in the two study groups. Furthermore, there was a positive significant association between the ratio of n-6: n-3 and TAG and a negative significant association between the ratio of n-6: n-3 and HDL-C.

In parallel, the present study found a positive association between DHA and HDL-C and a negative significant association with TAG. It has been reported that n-3 LCPUFAs might lower plasma TAG probably due to their capacity to decrease hepatic TAG secretion and enhancing the TAG clearance from circulation [37]. Also it was suggested that EPA and DHA may have hypotriglyceridemic effects by their ability to influence hydroxyl methyl glutaryl CoA reductase enzyme (HMG-CoA) [38]. Further, n-3 LCPUFA may serve as ligands for sterol regulating element binding protein (SREBP) and peroxisome proliferators-activated receptor (PPAR). These two transcription factors are known to be involved in fatty acid oxidation and inhibition of TAG synthesis [39]. The activities of Δ6 and Δ5 desaturases are regulated by these two transcription factors. However, research on the association between maternal n-3 LCPUFA's consumption and plasma lipid and lipoprotein concentrations in late pregnancy is rare [19,40].

N-3 PUFA supplementation has been suggested by many researchers to improve lipid profile in hyperlipidemic patients; a minimum of 1g (EPA+DHA)/day significantly lowered TAG concentration and the DHA was negatively associated with HDL-C and the ratio of total cholesterol: HDL-C [5]. In another study [41], it was found that fish oil supplementation has a favorable effect on cardiovascular disease risk among postmenopausal women since fish oil supplementation was markedly associated with an increase in DHA and EPA in plasma phospholipids and with 26% reduction of serum TAG concentrations and lowered ratio of serum TAG to HDL cholesterol by 28%. Similarly, it was reported that n-3 LCPUFA lowered serum and liver TAG and increased serum HDL-cholesterol concentrations [42]. Likewise, an inverse relationship between increased fish consumption in early pregnancy and plasma TAG concentrations were observed in a cross sectional study [43]. Intakes of oily fish containing over 250mg DHA and EPA/day seemed to slow down the plaque progression [44,45]. However, in contrast to our results other authors found no association between maternal intake of n-3 PUFA and maternal lipid concentrations [46]. The differences in the results may be attributed to difference in timing of assessment and different fatty acid analytic approach.

Conclusion

In conclusion, we show that supplementation of DHA and EPA improved the lipid profiles of pregnant women. This suggests that intake of n-3 during pregnancy, whether from fish oil supplement or from dietary sources, is an effective way to increase plasma levels of DHA and EPA and should be considered as an effective way to improve lipid profile concentrations among high risk pregnant women. This may improve modifiable cardiovascular risks lipid factors and reduce the incidence of preeclampsia and gestational diabetes. Due to the relatively small sample size further research is suggested before the generalization of the results.

Acknowledgements

This work was supported the Deanship of Academic Research at The University of Jordan, the Scientific Research Support Fund, and the pharmaceutical product from Grupo Ferrer Internacional.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of medical research at Jordan University Hospital and with the 1964 world medical association declaration of Helsinki.

Informed Consent

Informed consent was obtained from all participants included in the study.

Human and Animal Rights

This article does not perform any studies with animals.

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