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Fatty acids profile in pregnancies affected by neural tube defects

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Abstract

This study aimed to determine if maternal fatty acids (FA) levels during pregnancy are associated with the occurrence of neural tube defects (NTDs) and to explore the correlation between FA and maternal vitamin D, homocysteine, vitamin B_{12}, and folate in cases. Plasma FA composition was assessed using capillary gas chromatography. Comparisons between cases and controls were performed by independent samples *t*-test for continuous variables. Cases had significantly higher levels of heptadecanoic acid, linolelaidic acid, and arachidonic acid (ARA):(eicosapentaenoic acid+docosahexaenoic acid) ratio than controls ($p < 0.05$). Nervonic acid, ARA, adrenic acid, eicosapentaenoic acid, docosahexaenoic acid, and omega-3 polyunsaturated fatty acids (n-3 PUFA) levels were significantly lower in cases ($p < 0.05$). Maternal 25-hydroxyvitamin D (25(OH)D) levels were positively correlated with maternal polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids. RBC folate levels were negatively correlated with n-3 PUFA.

Further research is required to clarify the association of FA metabolism with NTDs.

Introduction

Appropriate maternal nutrition supply has a major impact on the growth and development of the placental-fetal unit. Micronutrient deficits can modify placental development and the fetus's capacity to absorb nutrients by changing the expression of critical signaling molecules involved in the growth and regulation of the placental-fetal unit.[1]

Folic acid, vitamin D, iron, calcium, and omega-3 fatty acids (n-3 PUFA) are among these micronutrients that affect placental growth and functionality.[2]

Fatty acids (FA), primarily polyunsaturated fatty acids (PUFA), are essential for a normal growth and development in infants, in addition to the regulation of oxidative stress, angiogenesis, and inflammation in the placenta.

PUFA are crucial for early brain development, being involved in both the structure and function of the nervous system. In addition, PUFA are precursors of highly bioactive compounds such as prostaglandins, leukotrienes, lipoxins, resolvins, and protectins. These compounds are involved in the launching and resolution of inflammation that can influence the outcome of acute and chronic diseases. There is evidence that not only is the amount of various PUFA important but also the balance between n-3 and n-6 PUFA is essential for good health.

This is why a sufficient amount of PUFA, particularly n-3 polyunsaturated fatty acids (n-3 PUFA) and n-6 polyunsaturated fatty acids (n-6 PUFA), must be transferred from the mother to the child through the placenta for appropriate growth and development.

Numerous beneficial impacts on health have been demonstrated for n-3 PUFA. An adequate consumption of n-3 PUFA during pregnancy and the early childhood safeguards the membrane phospholipids and the biochemical development of the brain and retina during infancy, guaranteeing visual maturation and cognitive function, as well as preventing the infant from developing allergies.[3]

Besides, higher intakes of n-3 PUFA as well as a higher maternal n-3:n-6 ratio during pregnancy have been associated with a higher duration of pregnancy and a reduction in the risk of premature birth but also, with the fetal growth velocity from mid-pregnancy onward, better child neurodevelopment and an improvement in the overall health of the neonate.

A-linoleic acid (ALA), eicosapentanoic acid (EPA), docosapentanoic acid, arachidonic acid (ARA), and docosahexanoic acid (DHA) are the main n-3 PUFA that have been demonstrated essential for neuronal and visual development, playing a beneficial role in health status and illness prevention.

DHA and EPA are involved in brain functions, including neuronal membrane dynamics and neurotransmitter functions. ARA, besides other functions, protects the brain from oxidative stress and is involved in early neurological development.[4]

Additionally, ARA is crucial for the hormonal control of healthy bone development and the body's overall mineral metabolism during infancy and childhood growth. ARA facilitates vitamin D3-regulated chondrocyte maturation and proliferation for the mineralization of skeletal growth plates during long bone growth when bone tissue is formed.[5]

Lipid metabolism is known to change significantly during pregnancy, mostly as a result of changed lipoprotein profiles. Higher levels of lipid production and metabolism are caused by increased estrogen and lipolysis from the placenta's lactogen.[6]

Lu *et al.* (2021) have reported that changes in lipid concentration are risk factors for the onset preeclampsia, which was associated with elevated levels of triglycerides, cholesterol, and low-density lipoprotein cholesterol in early pregnancy.[7]

The study of Rendeli *et al.* (2008) has shown higher levels of total cholesterol and very-low-density lipoprotein cholesterol in the myelomeningocele female group with respect to the control group.[8]

In our previous study, we studied the association of 5,10-methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) and methionine synthase reductase (MTRR A66G) gene polymorphisms with maternal FA levels in the occurrence of neural tube defects (NTDs) and whether these associations were modified by environmental factors.[9]

Then in another research, we studied the effect of maternal status in (plasma and red blood cell) folate, vitamin B_{12}, homocysteine, and vitamin D, as well as their interaction with MTHFR (C677T and A1298C) and MTRR A66G polymorphisms, on maternal plasma DHA, EPA, and ARA levels and the risk of NTDs.[10]

In this current study, we were interested to evaluate the association between individual maternal FA levels and the risk of NTDs. Therefore, this study aimed to investigate whether maternal blood FA levels during pregnancy are associated with the occurrence of NTDs in their offspring.

On the other hand, this study was conducted to explore the correlation between FA and maternal vitamin D, homocysteine, vitamin B_{12}, plasma-RBC folate, and alpha-fetoprotein (AFP) in pregnancies affected by NTDs and to determine whether there is any association between FA levels and some maternal/fetal characteristics including maternal age, gravidity, parity, consanguinity, folic acid supplementation, antecedents, maternal blood rhesus (rh±), fetal sex, fetal term, and compatibility of the subtype with life.

Materials and methods

Study population

This prospective study was performed in the embryo-fetopathology unit of Wassila Bourguiba Maternity and Neonatology Center in Tunis, Tunisia. In addition to those in the center, pregnant women carrying a fetus with severe NTDs came from all regional hospitals and private clinics in Tunisia. The study group was recruited between January 1, 2012 and December 30, 2013.

For each woman registered in the NTDs group, a matched control woman with normal ultrasound and normal obstetrics history (no fetal death, previous spontaneous abortion, stillbirth, or fetal intrauterine growth restriction) was recruited on a similar date or same month of conception and the presence or absence of folate supplementation.

Women with heart disease, high blood pressure, or atherosclerosis were excluded from the study. Ethics approval for the study was obtained from the ethics committee of Wassila Bourguiba Maternity and Neonatology Center in Tunis. All participants gave a written informed consent.

Fatty acids analysis

Blood samples were taken from the participants in tubes containing ethylenediaminetetraacetic acid. Plasma (300 ml) was supplemented with 20 ml of butylhydroxytoluene (25 mg in 100 ml of ethanol) as an antioxidant and stored at 80°C until analysis. Plasma FA were extracted and transformed into FA methyl esters according to the method of Moser and Moser.[11]

FA analysis was carried out using a gas chromatograph model 6890N (Agilent Technologies, Santa Clara, CA), equipped with a capillary column (DB-23, 60m_0.25 mm; 0.25 lm film thickness), a split/splitless Intel capillary system and flame ionization detector. Nitrogen flow was set as a carrier gas at 1.5 ml/min with an injector and detector temperatures of 230°C and 280°C, respectively. The oven temperature was held at 160°C for 1 min, then programmed at 2.5°C/min at 240°C, and finally programmed at 250°C at a rate of 4°C/min. The FA were identified by comparing the results to known manufactured standard mixtures. Findings were expressed as a percentage of the total weight of FA (% in moles). The fatty acids contents were calculated as follows:

Sum of the saturated fatty acids (SFA) = C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0.

Sum of the monounsaturated fatty acids (MUFA) = C16:1n7 cis + C16:1n7 trans + C18:1n9 cis + C18:1n7 cis + C20:1n9 + C24:1n9.

Sum of the omega-6 fatty acids (n-6 PUFA) = C18:2n6 + C18:2n6 9 cis-11 trans + C18:2n6 10 trans-12 cis + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:4n6.

Sum of the omega-3 fatty acids (n-3 PUFA) = C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3.

Sum of the polyunsaturated fatty acids (PUFA) = Sum of the omega-6 fatty acids + sum of the n-3 PUFA.

Sum of the unsaturated fatty acids (UFA) = Sum of the MUFA and PUFA.

Statistical analysis

Statistical analysis was carried out using SPSS 18 for Windows Software (SPSS Inc., Chicago, IL). The chi-squared test was used to assess differences between case and control groups among the following variables: fetal term, gravidity, parity, and consanguinity. The differences in means of maternal age were examined by student *t* test between cases and controls.

Continuous variables were examined for normality using the Kolmogorov–Smirnov test, and data were expressed as mean ±standard deviation (SD).

Comparisons between cases and controls were performed by independent samples *t*-test for continuous variables.

To compare FA levels by subtypes of NTDs, we used the ANOVA test.

The correlation between FA and vitamin D, homocysteine, vitamin B_{12}, plasma-RBC folate, and AFP levels was tested by the rank Pearson's correlation (two-tailed) in cases. Concentration values of vitamin D, homocysteine, vitamin B_{12}, plasma-RBC

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Table 1. Maternal characteristics of case and control group

Characteristics of mothers	Cases ($n = 42$)	Controls ($n = 30$)	*P*-value
Age (years)	40.66 ± 5.844	29.38 ± 3.899	0.134
Fetal term (weeks of gestation)	22.73 ± 6.481	22.87 ± 6.699	0.823
Gravidity	2.05 ± 1.129	2.19 ± 0.622	**0.008**
Parity	0.76 ± 0.895	1.19 ± 0.622	**0.006**
Consanguinity (N)			
(+)	6	4	0.594
(-)	29	18	

Data are given as mean ± SD.
N, number of cases.
Significant values were marked in bold.

folate, and AFP used in this study were obtained from our previous studies.[12,13]

Then, to better understand the association between maternal/fetal characteristics and FA levels, we evaluated differences in the distribution of SFA, MUFA, PUFA, UFA, n-3 PUFA, n-6 PUFA, n-6:n-3 ratio, and ARA:(EPA + DHA) ratio by maternal/fetal characteristics including maternal age, gravidity, parity, consanguinity, folic acid supplementation, antecedents, maternal blood rhesus (rh±), fetal sex, fetal term, and compatibility of the subtype with life (i.e., the malformation will lead to the death of the fetus or not). This distribution was studied with a stratified analysis in cases and controls.

Results were considered statistically significant when the *P*-value was ≤ 0.05.

Because several mothers' information were missing, the sample numbers varied depending on the parameter.

Results

Table 1 subjects, including 42 cases and 30 controls, were part of our study group in previous studies.[12,13] No significant differences were observed between cases and controls in maternal age, fetal term, and consanguinity. However, the differences were significant in parity and gravidity (Table 1).

Plasma FA composition in cases and controls were shown in Table 2.

Cases had significantly higher levels of heptadecanoic acid, linolelaidic acid and ARA:(EPA + DHA) ratio than controls ($p < 0.05$). In contrast, the level of nervonic acid, ARA, adrenic acid, eicosapentaenoic acid, docosahexaenoic acid, and n-3 PUFA were significantly lower in cases ($p < 0.05$).

Comparisons of the distribution of FA levels by NTDs subtypes have shown no significant differences between different subtypes (Table 3).

Correlations between SFA, MUFA, PUFA, UFA, n-3 PUFA, n-6 PUFA, n-6:n-3 ratio, and ARA:(EPA + DHA) ratio and vitamin D, homocysteine, vitamin B_{12}, plasma-RBC folate, and AFP levels in cases were shown in Table 4.

Maternal 25(OH)D levels were positively correlated with maternal PUFA and n-6 PUFA. Maternal RBC folate levels were negatively correlated with maternal n-3 PUFA. ARA:(EPA + DHA) ratio was positively correlated with maternal plasma folate, RBC folate, and vitamin B_{12}.

When analyzing the distribution of FA levels by maternal/fetal characteristics in cases as presented in Table 5, we noted that cases older than 30 years had significantly higher levels of SFA and ARA:(EPA + DHA) ratio but significantly lower levels of UFA. The levels of n-3 PUFA were significantly higher in the group of multiparous cases and in the group of multigravida cases. Furthermore, cases supplemented with folic acid had higher levels of n-3 PUFA but lower levels of n-6 PUFA and n-6:n-3 ratio. Cases with an antecedent malformed child, in addition to cases with rhesus-positive blood type, had significantly higher levels of SFA and significantly lower levels of MUFA and UFA. While cases with a rhesus-positive blood type did have a lower n-6:n-3 ratio, this result did not reach statistical significance. This group did however demonstrate a lower ARA:(EPA + DHA) ratio, and this result was statistically significant. In addition, cases of a male NTD baby had higher levels of n-6:n-3 ratio. Significantly higher levels of n-6 PUFA were found in cases of fetuses aged less than 20 weeks of gestation. The results have also shown that cases of fetuses affected with NTD subtypes compatible with life had higher levels of PUFA and n-6 PUFA.

No significant differences were found in the distribution of FA levels by maternal/fetal characteristics in controls.

Discussion

To the best of our knowledge, this study is the first demonstrating an association of maternal FA levels with the occurrence of NTDs in fetuses in Tunisia.

It is well-documented that an optimal FA profile during pregnancy, particularly concerning DHA and EPA, is crucial for maternal and fetal health.[14] Despite the abundance of literature on fatty acid levels and congenital birth defects, including neurological abnormalities, this study uniquely dissects individual fatty acids, revealing significant associations not previously identified.

This study showed that cases had higher but not significant levels of total SFA. The only significant difference among studied SFA was found for heptadecanoic acid.

Currently, it is recognized that consuming large amounts of SFA alters metabolism in the central nervous system as well as in the peripheral organs. Even while the exact mechanisms and signaling pathways in the brain that are impacted by exposure to various SFA concentrations are unknown, there is evidence that involves altered energy metabolism, decreased neuronal viability, and neuroinflammation. As a result, high SFA intake is thought to pose a serious risk for Alzheimer's disease, pathological brain aging, and cognitive decline.[15]

In contrast, the amount of nervonic acid, ARA, adrenic acid, eicosapentaenoic acid, docosahexaenoic acid, and n-3 PUFA were

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Table 2. Plasma fatty acids composition in NTD mothers and controls (mol%)

	Cases ($n = 42$)	Controls ($n = 30$)	*P*-value
Saturated fatty acids (%)	28.02 ± 14.5	25.95 ± 13.6	0.401
C14:0	1.02 ± 0.64	0.97 ± 0.62	0.96
C16:0	10.21 ± 12.62	10.61 ± 12.47	0.811
C17:0 heptadecanoid acid	8.28 ± 4.38	7.67 ± 1.81	**0.048**
C18:0	7.30 ± 2.95	6.52 ± 1.69	0.502
C20:0	0.30 ± 0.11	0.30 ± 0.10	0.922
C22:0	0.61 ± 0.26	0.73 ± 0.36	0.094
C24:0	0.25 ± 0.12	0.28 ± 0.10	0.518
Monounsaturated fatty acids	36.05 ± 15.72	35.03 ± 14.74	0.685
C16:1n7 cis + C16:1n7 trans	15.60 ± 14.22	13.59 ± 13.46	0.349
C16:1n7 trans	14.41 ± 14.03	12.34 ± 13.51	0.479
C16:1n7 cis	1.19 ± 0.76	1.24 ± 1.02	0.400
C18:1n9 cis	16.93 ± 6.46	18.69 ± 5.98	0.720
C18:1n7 cis	2.93 ± 2.49	2.07 ± 1.39	0.178
C20:1n9	0.21 ± 0.19	0.21 ± 0.11	0.473
C24:1n9 nervonic acid	0.36 ± 0.24	0.44 ± 0.34	**0.049**
Polyunsaturated fatty acids	35.92 ± 8.59	37.85 ± 8.93	0.885
n-6 PUFA	32.16 ± 8.56	33.46 ± 9.67	0.637
C18:2 n6	24.40 ± 9.30	25.13 ± 10.79	0.426
C18:2n6 9 cis-11 trans linolelaidic acid	0.17 ± 0.21	0.12 ± 0.07	**0.009**
C18:2n6 10 trans-12 cis	0.19 ± 0.11	0.21 ± 0.15	0.098
C18:3n6	0.27 ± 0.11	0.23 ± 0.09	0.285
C20:2n6	0.46 ± 0.27	0.50 ± 0.18	0.233
C20:3n6	1.42 ± 0.71	1.62 ± 0.62	0.955
C20:4n6 arachidonic acid	4.84 ± 2.58	5.16 ± 1.68	**0.031**
C22:4n6 adrenic acid	0.38 ± 0.20	0.45 ± 0.34	**0.04**
n-3 PUFA	3.75 ± 1.37	4.38 ± 1.40	**0.042**
C18:3n3	0.57 ± 0.31	0.63 ± 0.27	0.612
C20:5n3 eicosapentaenoic acid	0.36 ± 0.27	0.49 ± 0.53	**0.006**
C22:5n3	0.49 ± 0.18	0.48 ± 0.18	0.505
C22:6n3 docosahexaenoic acid	2.31 ± 1.04	2.76 ± 1.05	**0.048**
Unsaturated fatty acids	71.97 ± 14.50	72.88 ± 12.84	0.191
ARA:(EPA + DHA) ratio	1.82 ± 0.77	1.64 ± 0.47	**0.026**
n-6:n-3 ratio	9.26 ± 3.11	8.42 ± 3.10	0.782

Data are expressed as mean ± SD; *P*-values derived from independent samples *t*-tests.
Significant values were marked in bold.
ARA:(EPA + DHA)ratio, arachidonic acid/(eicosapentaenoic acid+docosahexaenoic acid)ratio; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-6:n-3 ratio, omega-6 polyunsaturated fatty acids:omega-3 polyunsaturated fatty acids ratio; NTD, neural tube defect.

significantly lower in cases ($p < 0.05$). Over the last decade, studies reported that ARA and DHA predominate in the composition of the brain. Both ARA and DHA are preferentially transported across the placenta during pregnancy and are stored in the developing brain from the early stages of its development.[16]

ARA accumulates rapidly in the brain during development, which occurs from the beginning of the third trimester of pregnancy until about 2 years of age.[17]

Because NTDs are common major congenital anomalies that result from very early disruption in the development of the brain and spinal cord,[18] this may explain the reduced levels of ARA, DHA, and EPA in cases.

Various functions have been shown to be related to ARA. It is required for brain development, where it is involved in cell division and communication. It regulates neuronal firing, signaling, and long-term potentiation.[19] It also contributes to the maintenance of

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Table 3. Distribution of fatty acids levels by neural tube defect subtypes

Fatty acids (mol%) NTD subtypes (N)	SFA	MUFA	PUFA	UFA	n-3 PUFA	n-6 PUFA	n-6:n-3 ratio	ARA:(EPA + DHA) ratio
Rachischisis (6)	23.42 ± 12.79	39.49 ± 12.57	37.08 ± 3.46	76.57 ± 12.79	3.23 ± 0.52	33.84 ± 3.13	10.59 ± 1.23	1.76 ± 0.68
Myelomeningocele (6)	34.01 ± 16.20	29.23 ± 16.92	36.74 ± 6.33	65.98 ± 16.20	4.90 ± 2.45	34.28 ± 5.72	12.74 ± 2.79	2.27 ± 0.29
Meningocele (2)	15.75 ± 2.05	47.73 ± 4.72	37.08 ± 6.78	84.82 ± 2.05	2.80 ± 1.06	34.28 ± 5.72	12.74 ± 2.79	2.27 ± 0.29
Craniorachischisis (1)	43.36	28.40	28.23	56.63	4.18	24.05	5.74	0.51
Anencephaly (22)	28.95 ± 15.22	36.01 ± 16.48	35.03 ± 10.02	71.04 ± 15.22	3.36 ± 1.12	31.39 ± 10.10	9.40 ± 3.53	1.92 ± 0.82
Encephalocele (5)	24.31 ± 14.50	37.12 ± 19.31	38.56 ± 11.02	75.68 ± 12.56	3.84 ± 1.26	34.72 ± 10.07	9.23 ± 2.10	1.75 ± 0.89
P	0.468	0.757	0.902	0.468	0.282	0.881	0.261	0.538

Results are expressed as mean ± SD.
N, number of cases; ARA:(EPA + DHA)ratio, arachidonic acid/(eicosapentaenoic acid+docosahexaenoic acid)ratio; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-6:n-3 ratio, omega-6 polyunsaturated fatty acids:omega-3 polyunsaturated fatty acids ratio; NTD, neural tube defect; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

Table 4. Correlations between fatty acids and vitamin D, homocysteine, vitamin B_{12}, plasma-RBC folate, and AFP levels in cases

	SFA		MUFA		PUFA		UFA		n-3 PUFA		n-6 PUFA		n-6:n-3 ratio		ARA:(EPA + DHA) ratio	
	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value	(r)	*P*-value
Vit D	− 0.153	0.334	− 0.034	0.830	0.321	**0.038**	0.153	0.334	0.081	0.611	0.309	**0.047**	0.073	0.646	0.094	0.552
Hcy	0.127	0.422	− 0.192	0.222	0.137	0.386	− 0.127	0.422	− 0.048	0.763	0.145	0.395	0.061	0.702	0.131	0.408
Plasma folate	0.045	0.779	− 0.138	0.385	0.176	0.264	− 0.045	0.779	− 0.044	0.784	0.184	0.243	0.179	0.256	0.521	**<0.001**
RBC folate	0.047	0.773	− 0.012	0.939	− 0.055	0.735	− 0.047	0.773	− 0.272	**0.045**	− 0.012	0.940	0.211	0.191	0.271	**0.041**
Vit B_{12}	− 0.131	0.407	0.130	0.412	− 0.016	0.920	0.131	0.407	− 0.245	0.119	0.023	0.884	0.209	0.183	0.367	**0.017**
AFP	− 0.126	0.428	0.018	0.912	0.180	0.245	0.126	0.428	0.027	0.867	0.176	0.264	0.033	0.834	− 0.04	0.803

Pearson's correlation (r).
Significant values were marked in bold.
AFP, α-fetoprotein; Hcy, homocysteine; RBC folate, red blood cell folate; vit B_{12}, vitamin B_{12}; vit D, vitamin D; ARA:(EPA + DHA)ratio, arachidonic acid/(eicosapentaenoic acid+docosahexaenoic acid)ratio; n-3 PUFA, omega-3 polyunsaturated fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; n-6:n-3 ratio, omega-6 polyunsaturated fatty acids:omega-3 polyunsaturated fatty acids ratio; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

Table 5. Distribution of fatty acids levels by maternal/fetal characteristics in cases

Fatty acids maternal/fetal characteristics (N)	SFA	MUFA	PUFA	UFA	n-3 PUFA	n-6 PUFA	n-6:n-3 ratio	ARA:(EPA + DHA) ratio
Maternal age								
≤ 30 (N = 18)	24.95 ± 11.19	39.39 ± 15.76	35.64 ± 8.29	75.04 ± 11.19	3.44 ± 1.07	32.2 ± 8.26	9.94 ± 2.74	1.62 ± 0.53
>30 (N = 13)	33.97 ± 15.18	30.27 ± 16.56	35.74 ± 9.90	66.02 ± 15.18	4.16 ± 1.87	31.57 ± 10.09	8.69 ± 3.63	1.65 ± 0.80
P	**0.048**	0.130	0.977	**0.042**	0.185	0.851	0.289	**0.04**
Gravidity								
G1 (N = 15)	29.66 ± 14.51	36.54 ± 17.03	33.78 ± 17.03	70.33 ± 14.57	3.33 ± 0.80	30.44 ± 10.38	9.64 ± 3.56	1.73 ± 0.93
G>1 (N = 22)	28.98 ± 14.86	34.91 ± 15.89	36.18 ± 7.58	71.10 ± 14.86	3.98 ± 1.69	32.19 ± 7.76	9.14 ± 3.15	1.84 ± 0.73
P	0.877	0.768	0.423	0.877	**0.047**	0.561	0.660	0.692
Parity								
P = 0 (N = 19)	29.89 ± 14.77	35.08 ± 16.77	35.01 ± 9.66	70.10 ± 14.77	3.46 ± 0.89	31.55 ± 9.64	9.67 ± 3.41	1.80 ± 0.90
P ≥ 1 (N = 18)	28.84 ± 14.64	36.09 ± 15.93	35.41 ± 8.03	71.51 ± 14.64	3.99 ± 1.81	31.42 ± 8.14	9.00 ± 3.20	1.80 ± 0.72
P	0.773	0.852	0.893	0.773	**0.043**	0.964	0.541	0.981
Consanguinity								
(+)*(N = 6)	30.56 ± 16.57	32.75 ± 17.62	36.68 ± 8.29	69.43 ± 16.57	3.84 ± 1.19	32.84 ± 7.8	8.94 ± 2.23	1.78 ± 0.60
(-)**(N = 29)	29.25 ± 14.36	36.26 ± 16.05	34.47 ± 9.05	70.74 ± 14.48	3.70 ± 1.53	30.77 ± 9.16	9.34 ± 3.53	1.77 ± 0.86
P	0.845	0.634	0.587	0.845	0.838	0.611	0.796	0.979
Folic acid supplementation								
Yes (N = 20)	28.23 ± 15.05	36.95 ± 18.22	34.21 ± 9.55	71.16 ± 15.05	3.97 ± 1.78	30.23 ± 9.76	8.77 ± 3.58	1.72 ± 0.63
No (N = 22)	27.28 ± 14.31	35.22 ± 13.44	37.48 ± 7.5	72.71 ± 14.33	3.55 ± 0.85	33.92 ± 7.08	9.91 ± 2.59	1.91 ± 0.89
P	0.735	0.727	0.222	0.735	**0.032**	**0.046**	**0.045**	0.440
Antecedents								
Yes (N = 3)	43.56 ± 9.48	18.41 ± 6.78	38.02 ± 5.88	56.43 ± 9.48	4.08 ± 1.42	33.93 ± 6.52	9.07 ± 3.55	1.71 ± 0.97
No (N = 31)	27.16 ± 14.8	38.76 ± 15.63	34.07 ± 8.9	72.83 ± 14.80	3.67 ± 1.50	30.39 ± 8.93	9.23 ± 3.39	1.82 ± 0.83
P	**0.048**	**0.034**	0.461	**0.042**	0.656	0.511	0.936	0.833
Maternal blood type								
Rhesus+ (N = 21)	27.91 ± 14.87	37.25 ± 18.09	34.82 ± 8.6	72.08 ± 14.87	3.77 ± 1.54	31.04 ± 8.62	9.02 ± 3.04	1.75 ± 0.68
Rhesus- (N = 6)	23.57 ± 10.29	41.92 ± 10.64	34.496.02	76.42 ± 10.29	3.77 ± 1.62	30.71 ± 9.05	9.70 ± 4.45	1.82 ± 1.16
P	**0.01**	**0.045**	0.934	**0.016**	0.999	0.935	0.663	**0.048**
Fetal sex								
Female (N = 18)	29.13 ± 14.51	35.88 ± 16.25	34.98 ± 7.24	70.86 ± 14.51	4.02 ± 1.83	30.96 ± 7.73	8.01 ± 3.64	1.52 ± 0.67
Male (N = 14)	26.00 ± 15.20	39.29 ± 17.27	34.69 ± 9.39	73.99 ± 15.20	3.50 ± 1.00	31.18 ± 8.99	9.92 ± 2.56	1.79 ± 0.57
P	0.558	0.570	0.922	0.558	0.356	0.940	**0.04**	0.241

Table 5. (*Continued*)

Fetal term								
≤ 20 (N = 18)	25.37 ± 15.15	38.13 ± 16.38	36.13 ± 10.66	74.26 ± 15.15	3.72 ± 0.8	39.41 ± 0.57	8.98 ± 3.09	1.91 ± 0.89
>20 (N = 23)	28.54 ± 13.09	35.52 ± 14.48	35.93 ± 7.01	71.45 ± 13.1	3.52 ± 1.2	32.4 ± 6.78	9.98 ± 2.82	1.74 ± 0.70
P	0.529	0.597	0.944	0.529	0.548	**0.047**	0.287	0.498
Compatibility of the subtype with life***								
Subtypes compatible with life (N = 13)	26.78 ± 14.64	36.27 ± 14.85	36.93 ± 4.85	73.21 ± 14.64	3.88 ± 1.83	33.04 ± 5.1	9.62 ± 2.83	1.79 ± 0.64
Subtypes incompatible with life (N = 29)	28.64 ± 14.67	35.93 ± 16.40	35.42 ± 1.00	71.35 ± 14.67	3.69 ± 1.11	31.72 ± 9.91	9.24 ± 3.29	1.84 ± 0.84
P	0.701	0.949	**0.048**	0.701	0.668	**0.046**	0.711	0.835

Results are expressed as mean ± SD.
Significant values were marked in bold.
N, number of cases; ALA, alpha-linolenic acid; AFP, alpha-fetoprotein; ARA, arachidonic acid; ARA:(EPA + DHA)ratio, arachidonic acid/(eicosapentaenoic acid+docosahexaenoic acid)ratio; DHA, docosahexaenoic acid; EPA,eicosapentaenoic acid; FA, fatty acids; Hcy, homocysteine; LA, linoleic acid; MUFA, monounsaturated fatty acids; n-3 PUFA, omega-3 polyunsaturated Fatty acids; n-6 PUFA, omega-6 polyunsaturated fatty acids; SFA, saturated fatty acids; n-6:n-3 ratio, omega-6 polyunsaturated fatty acids: omega-3 polyunsaturated fatty acids ratio; NTDs, neural tube defects; OA, oleic acid; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; RBC folate, red blood cell folate; vit B_{12}, vitamin B_{12}.
(+)* Consanguineous parents.
(-)**Non-consanguineous parents.
****Anencephaly, rachischisis, and craniorachischisis were classified as subtypes incompatible with life. Myelomeningocele, meningocele, and encephalocele were considered as subtypes compatible with life.

membrane organization and hippocampal plasticity, protects the brain from oxidative stress in the hippocampus by activating the peroxisome proliferator-activated receptor gamma, and aids in the production of new protein in the tissue.[20]

ARA is also involved in the hormonal regulation of appropriate bone formation and whole-body mineral metabolism during baby and childhood development. Eicosanoids convey cellular, organ, and systemic signals during skeletal development to balance calcium and phosphate requirements for bone formation and other metabolic activities. When bone tissue is formed during long bone growth, ARA mediates vitamin D3-regulated chondrocyte maturation and proliferation for the mineralization of skeletal growth plates.[5]

On the other hand, EPA and DHA can function as competing inhibitors of ARA release from cell membranes and binding to the active site of COX-2. EPA also works as an alternate substrate for this enzyme, resulting in less production of pro-inflammatory and pro-carcinogenic ARA derivatives.[21] This could explain the deduced suggestion of higher NTD risk with an increased ARA:(EPA + DHA) ratio.

In animal models, roughly half of the quantities of ARA and DHA accumulate in the rat brain before myelination and at 15 d after birth, when myelination has just begun. Myelination is principally responsible for the highest rate of brain growth, and the synthesis of myelin lipids, which are required early in brain development, has been demonstrated affected by low-PUFA diets.[22]

Cases of this study had significantly lower levels of DHA than controls.

DHA has been shown to have a neuroprotective role during neonatal asphyxia in rats and in prematurely born pigs. In rats, a maternal diet enriched in DHA during pregnancy prevents neonatal brain injury.[4]

In other studies, lower levels of maternal DHA status during pregnancy were suggested to be involved in compromised maternal selective attention, which is a key component of cognition.[23] A declining maternal DHA status has also been associated with a higher incidence of postpartum depression.[24]

In our study, linolelaidic acid (a cis-trans isomer of linoleic acid (LA)) was significantly higher in cases than in controls.

According to brain kinetics in fetal baboons, in addition to maternal-produced ARA, LA may be transported across the blood-brain barrier despite its very low level in brain lipids. The brain contains an active desaturation/elongation machinery that converts LA to ARA.[25]

This may explain high linolelaidic acid levels in cases which may be due to its inability to be converted to ARA or to decreased levels of ARA found in our cases.

In the present study, n-3 PUFA levels were significantly lower in cases than in controls.

In the study of Koletzko *et al.* (2008), a low n-3 PUFA intake during pregnancy resulted in a little shorter gestation, a slightly lower birth weight, and an increased chance of premature delivery.[26]

Contrariwise, the ingestion of n-3 PUFA has been shown to have a wide range of health benefits. The brain effect of n-3 PUFA is permanent, as neuropsychiatric illnesses such as Parkinson's disease[27] and mild cognitive impairment[28] have been reversibly related to n-3 PUFA deficit.

In our study, nervonic acid, a MUFA, was found with significantly lower levels in cases.

It is commonly proved that MUFA have anti-inflammatory benefits and may improve endothelial function.[29]

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Neuroinflammation, a complex process involving the activation of various immune cells within the central nervous system, was reported as an important pathological feature of NTDs.[30]

Adrenic acid levels were significantly lower in cases than in controls in our study group.

Adrenic acid is the third most abundant PUFA in the brain, and it is rich in myelin lipids, especially phosphatidylethanolamine. Like ARA, it accumulates rapidly in babies during the early postnatal period of the brain growth spurt. As an early precursor to adrenic acid, ARA plays a potentially important role in its metabolism in vivo. Adrenic acid conversion may be a key mechanism for ARA use in babies in order to fulfill the rapid increase in adrenic acid required for neural tissue development.[31]

This could explain decreased adrenic acid levels in cases of our study, which may be due to the decreased levels of its precursor, ARA, found in our case group or a possible non-conversion of ARA to adrenic acid.

In this current research, we also studied the correlation between FA and vitamin D levels in cases. To our knowledge, this study for the first time reports the association of 25(OH)D levels with fatty acid levels in pregnancies affected by NTDs.

Maternal 25(OH)D levels were positively correlated with maternal PUFA and n-6 PUFA.

The same finding was observed in other studies, which demonstrated a positive association of vitamin D with total PUFA and n-6 PUFA levels in women with preeclampsia. However, in contrast with our results, they also showed that vitamin D was negatively associated with SFA and MUFA levels.[32]

The anti-inflammatory and immune-regulating effects of both vitamin D and PUFA are well established.[33,34] Neuroinflammation was identified as the significant pathological feature in both the central nervous system and amniotic fluid from fetuses with NTDs.[30]

In the study of Nandy *et al.* (2019), lower levels of ALA and EPA were observed in vitamin D deficiency as compared to controls. However, ARA and n-3 PUFA levels were similar in both groups.[26]

PUFA, such as ARA, have been demonstrated to be significant in the homeostatic regulation of vitamin D and parathyroid hormone during bone formation.[35]

This could explain why bones are affected in some NTD subtypes in mothers with decreased levels of ARA.

Furthermore, an animal study demonstrates that maternal deficiency of vitamin D influences FA metabolism and alters FA levels. Nandi *et al.* (2019) observed that maternal vitamin D deficiency increases the ratio of vasoconstrictor and vasodilator lipid metabolites of PUFA by increasing Hcy and oxidative stress levels.[36]

It is important to remind that significant low levels of vitamin D and increased Hcy levels were associated with the occurrence of NTDs in in this study group in previous studies.[12,13]

Our results have shown that maternal RBC folate levels were negatively correlated with maternal n-3 PUFA.

According to Kulkarni A *et al.* (2011), there was no correlation between folic acid or plasma vitamin B_{12} and erythrocyte n-3 PUFA in either the normotensive or pre-eclamptic groups.[37]

These findings suggest an intrinsic link between biochemical parameters and FA profiles in the occurrence of NTDs.

We have also studied the distribution of FA levels by maternal/fetal characteristics in cases. Results have shown decreased SFA levels and increased UFA levels in cases aged less than 30 years.

The study of Carver JD *et al.* (2001) indicated that the relative levels of PUFA decreased with age with the exception of DHA.[38] In our previous study, DHA level was positively correlated with maternal age.[10]

In our study group, maternal n-3 PUFA levels were significantly higher in the group of multiparous cases and in the group of multigravida cases. This finding is in contrast with the study of Bokor *et al.* (2007), who reported a maternal PUFA depletion with increasing parity.[39]

This result may be due to that multiparous women are more likely exposed to health promotion messages regarding folate during previous pregnancies and thus have a greater awareness of the need for periconceptional folic acid. This suggestion is confirmed by our finding where cases supplemented with folic acid had higher levels of n-3 PUFA than cases not supplemented with folate (Table 5).

Further studies are needed to establish the association between FA levels and folic acid supplementation.

In addition, we have found that cases with previous history of NTDs had higher levels of SFA but lower levels of MUFA and UFA (Table 5).

This finding confirms our hypothesis of an association between increased SFA levels (heptadecanoic acid), related to altering metabolism in the central nervous system, decreasing neuronal viability, and inducing neuroinflammation, as well as decreased MUFA levels (nevronic acid), characterized by its anti-inflammatory benefits, as shown previously, in the occurrence of NTDs. Cases with fetal term ≤ 20 weeks of gestation had higher levels of n-6 PUFA. However, the absolute amount of n-6 PUFA increases in all organs with increasing gestational age, especially the absolute amount of ARA, which increases because of the brain growth.[40]

However, due to the absence of a major part of the brain, skull, and scalp (anencephaly)[41] and to the very early disruption in the development of the brain and spinal cord in NTDs subtypes, we have observed decreased n-6 PUFA with increasing fetal term.

Finally, our study is limited by the lack of some mothers' information resulting in varied sample numbers depending on the parameter and by the small sample size of case and control groups, which may be due to the fact that Tunisia is a small country. More research with a larger sample is required to support our findings.

Conclusions

To summarize, our findings suggest that lower levels of ARA, DHA, EPA, and n-3 PUFA in mothers are associated with the occurrence of NTDs in Tunisian fetuses. We also report a positive association of maternal vitamin D with PUFA and n-6 PUFA in cases.

Further research is required to clarify the association of FA metabolism with vitamin D, folic acid, and vitamin B_{12} status during pregnancy.

This will further help to define optimal strategies for FA and vitamins supplementations according to the vitamins and FA status of each woman in order to prevent the occurrence of NTDs during subsequent pregnancies.

Data availability statement. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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