Three-Months Effect of EPA and DHA Supplementation on Red Blood Cell Fatty Acid Compositions and on Omega-3 Index in Patients with History of Cardiovascular Diseases: An Open Label Study

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ABSTRACT

Objective: This study was conducted to measure the fatty acid content in RBCs of CVD patients with intake of EPA and DHA supplementation for three months.

Design: A qualitative, single arm clinical trial open label study.

Setting: Mohammad bin Khalifa Al Khalifa Cardiac Centre and University of Bahrain, Bahrain.

Method: A triplicate dose (three capsules) of omega-3 supplements containing 180mg EPA and 120mg DHA was given to the CVD patients for 3 months. Blood samples were collected from the patients and the fatty acid content was extracted, methylated, and analyzed using Gas Chromatography.

Result: The O3I percentage of composition at baseline (2.54%) was unchanged after supplement consumption (2.99). However, a significant increase in the total omega-3 levels from 4.31% to 5.23%, a significant decrease in omega-6/omega-3 ratio from 5.89% to 4.51%, and a significant decrease in the total omega-6 contents from 25.38% to 23.59% were found.

Conclusion: This study provided the first experimental data on the effect of omega-3 supplement consumption in CVD patients in Bahrain. This study suggests the importance of ensuring the adequate intake of EPA+DHA supplements based on individuals/population O3I to achieve the desired O3I>8%.

Keywords: EPA+DHA, fatty acids; fish oil; omega-3 index; supplements

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INTRODUCTION

Western dietary habits are characterized by an unbalanced omega-6/omega-3 ratio, about 20-30:1, and a high intake of dietary fats, which is close to 30-35% with an amount of saturated fats (SFA) >10%[1-3]. This increase in the proportion of omega-6 fatty acids has become a major risk factor for many diseases including cardiovascular diseases (CVDs), cancer, and inflammatory and autoimmune diseases[4]. Several studies have stated that omega-3 (n-3) fatty acids intake, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is beneficial in reducing CVDs as well as other chronic diseases such as nonalcoholic fatty liver disease (NAFLD) and atrial hypertension[5-7]. The n-3 also helps to improve vascular and endothelial function[8,9] and lower the risk of non-fatal ischemic stroke[10]. A meta-analysis study of clinical trials with at least one-year duration, and published in the past 20 years have shown that n-3-supplements reduce the risk of sudden death, myocardial infarction, and cardiac death in secondary prevention studies[11]. However, several studies have reported that EPA monotherapy was found to be more effective in reducing cardiovascular risk in comparison to EPA+DHA therapy[7,12,13]. The required recommended consumption of n-3 fatty acids set by the Dietary Guidelines for Americans (DGAs) by the United States Department of Agriculture (USDA), Health and Human Services (HHS) and the American Heart Association (AHA) is 1g/day of EPA+DHA by consuming at least two fish meals per week[14].

One of the most important indexes to measure n-3 content at the level of red blood cells (RBCs) membrane is the Omega-3 index (O3I), which is the content of EPA+DHA expressed as a percentage of total fatty acids. A high O3I (≥ 8%) is associated with 90% less risk of mortality from sudden cardiac death compared to a low O3I (< 4%)[15]. Harris et al. (2018, 2019) have also concluded that low O3I levels can be one of the factors contributing to a higher risk of CVDs[16,17]. Several studies suggest the role of omega-3 supplement (EPA+DHA) intake on cardiovascular health[17,18]. In a study, an O3I of ≥ 8% was achieved in 44% of participants who took three servings of fish per week plus n-3 supplementation more than 3 times a week; whereas low O3I was noted in 10% of participants that were consuming 2 fish meals per week and not taking omega-3 supplements, and 2% in those not consuming fish or supplementations. In addition, the O3I increased by 2.2% when taking EPA+DHA supplements[19]. The study of McDonnell et al. (2019) has also shown that only those participants who consumed two fish servings per week and an average of 1100 mg/day of EPA+DHA supplements had a median O3I ≥8% required for improved health[20]. Therefore, the Current American Heart Association (AHA) recommendation of consuming two fish meals per week is very unlikely to achieve the O3I goal of more than 8%.

Several meta-analysis studies showed that EPA and/or DHA supplements provide additional support to cardiac health and resulted in a significant reduction in cardiac death[21,22]. On the other hand, some studies suggested the ineffectiveness of omega-3 supplement consumption on CVD[23-25]. The study by Aung et al. (2018) in which 77917 participants consumed n-3 fatty acid supplements (EPA 226-1800 mg/day) had shown no significant association with fatal or non-fatal coronary heart disease (CHD) or major vascular events[23]. The aim of this study was to measure the effect of EPA and DHA supplementation on red blood cell fatty acid compositions in a duration of 3 months, in patients with cardiovascular disease in Bahrain.

METHOD

Sample

The sample size (50 patients) of this study was calculated based on the study of Roke and Mutch (2014)[26]. The required sample size was calculated to be at a confidence limit of 95% and a confidence interval of 5%. Patients that were included in the study were Bahraini citizens diagnosed with stable cardiovascular disease that visited the cardiac outpatient clinic. In addition, they were willing to follow up and take omega-3 supplement for 3 months as well as blood collection before and after three months of the supplement consumption. Patients that were excluded from the study were CVD patients who were already taking n-3 supplements before taking part in the intervention study. The cardiology consultant checked the medical records of the participants. All patients were on medication that resulted in normal clinical and biochemical parameters.

Setting and ethical approval

Cardiac patients from Mohammad bin Khalifa Al Khalifa Cardiac Centre were selected from the cardiac outpatient clinic. Recruitment of patients was done from January 2018 till March 2018.

The study permission was obtained from the Research Ethics Committee at Bahrain Defence Force Royal Medical Services in which Mohammad bin Khalifa Al Khalifa Cardiac Centre is located. Patients signed an informed consent prior to blood sample donations and data collection.

Dietary counselling

Subjects were trained to follow a regimen that maintained a prudent balance of macronutrients: 28% of energy from fat (cholesterol < 200 mg), 57% of energy from carbohydrates (10% from simple carbohydrates), with 20–25 g of bran, and 15% of energy from protein. A registered dietician performed initial dietary counselling. Subjects were trained to restrict their daily energy intake by a moderate amount, 3344 kJ/d less than daily requirements based on WHO criteria (World Health Organization, 1985)[27]. The criteria include a regimen that maintained a prudent balance of macronutrients: 25–30% of energy from fat (cholesterol < 200 mg), 55–60% of energy from carbohydrates (10% from simple carbohydrates), with 25 g of bran and 15–20% of energy from protein.

Intervention

The recruited patients answered a face-to-face questionnaire, signed a consent form, and agreed to consume n-3 supplement daily for 3 months. The supplement given to the patients was 1000 mg of SEACOD fish oil (Geltec Private Limited, India) containing 180 mg of EPA and 120 mg of DHA. The patients were advised to take three tablets daily with their main meal for 3 months. Blood samples were collected, and the fatty acid content of RBC membranes was analyzed.

Data Collection

The weight and height of the patients were measured, and the body mass index (BMI) was calculated according to the classification of the Centers for Disease Control and Prevention (CDC). Patients were classified as normal, overweight, or obese based on their BMI in accordance with CDC recommendations[28]. A face-to-face questionnaire was carried out and the answers of the participants were recorded.

The questionnaire was divided into four sections. The first section included the demographic data regarding age, occupation, height, weight, gender, and education level. The second section of the questionnaire included clinical data regarding smoking, physical exercise, and the presence of chronic diseases such as diabetes, hypertension, high cholesterol, etc. the third section addressed the awareness of patients regarding omega-3 and omega-6 fatty acids, the enriched dietary sources of omega-3 and omega-6 and their beneficial health effects. The last section was about the nutritional data of the patients, including intake of n-3 enriched dietary sources such as fish, chia seeds, soybeans, flaxseed, and canola oil.

Fatty acids analysis

The procedure of Rodrigues et al. (2015) was used for the separation of RBCs[29]. Blood samples were centrifuged at 2500 rpm for 10 minutes and the buffy plasma layers were removed and discarded. The RBCs were suspended twice in 0.9% saline solution and centrifuged for 10 minutes at 2500 rpm. Fatty acids were then extracted and methylated following the one-step procedure of Bicalho et al. (2008)[30]. To 150µl of the RBCs sample, 2 ml of boron trifluoride solution was added. The mixture was vigorously vortexed for 30 seconds and kept in a water bath at 90 °C for 60 minutes. The mixture was kept at room temperature to cool down and then mixed vigorously again for 30 seconds. The supernatant layer was separated and evaporated under nitrogen gas. Finally, 100µl of n-heptane was added to suspend the fatty acid methyl esters (FAMEs).

The capsules of omega-3 supplements utilized in the present study (SEACODE) were analyzed for their fatty acid content following the same procedure described above[30]. An internal standard of 50 µg of heptadecanoic acid (C17:0) was added to the supplement's samples. The analysis of the supplement capsules has revealed that they contained EPA (183.77±0.19 mg/capsule), and DHA (120.72±0.98 mg/capsule) expressed as mean ± SD.

Individual FAMEs were identified using Perkin Elmer, Clarus 500 GC-FID. A fused carbon-silica column (Stabilwax, Crossbond, Carbowax, polyethylene glycol) was used for the separation of samples with a temperature range of 40 °C to 260 °C. The column had a length of 30 m with an internal diameter (width) of 0.25 mm and particle size (df) = 0.25 m. The temperature of the injector was kept constant at 250 °C with a split ratio of 1:20. The sample injected volume was 5 µL.

Nitrogen gas was used as a carrier gas which had a total flow rate of 0.76 mL/min. The oven temperature was set at 200 °C. This constant temperature was maintained for 80 minutes (the total run for each sample). The temperature of the FID was set at 300 °C. The flow rate of 450 mL/min for air and 45 mL/min for hydrogen and a sampling rate of 12.5 Hz were used. FAMEs were identified by comparing the peak of samples against authentic standards (PUFA No. 1 and PUFA No. 2) supplied by SUPELCO, USA.

Statistical analysis

The statistical analysis was done using the Statistical Package for Social Sciences (SPSS 26.0 for Windows, SPSS Inc., Chicago, IL. along with Microsoft excel□ 2013). Descriptive statistics including means, standard deviations, medians, interquartile ranges, and Δ changes were carried out. After the verification of the normal distribution with Shapiro-Wilk and Kolmogorov Smirnov of the continuous variables, the data were analyzed and compared statistically within the group using the Wilcoxon t-test for non-parametric data. Statistically significant values were considered at the level of p-value < 0.05. Delta changes mean differences and Pearson's correlations were assessed in order to find the multiple effect associations.

RESULT

In the present study, the effect of EPA and DHA supplementation on RBCs fatty acid content during 3 months was determined. Out of 60 patients assessed for the study, 10 were excluded, and after allocation and follow up, only 20 were remained for the further analysis (Figure 1).

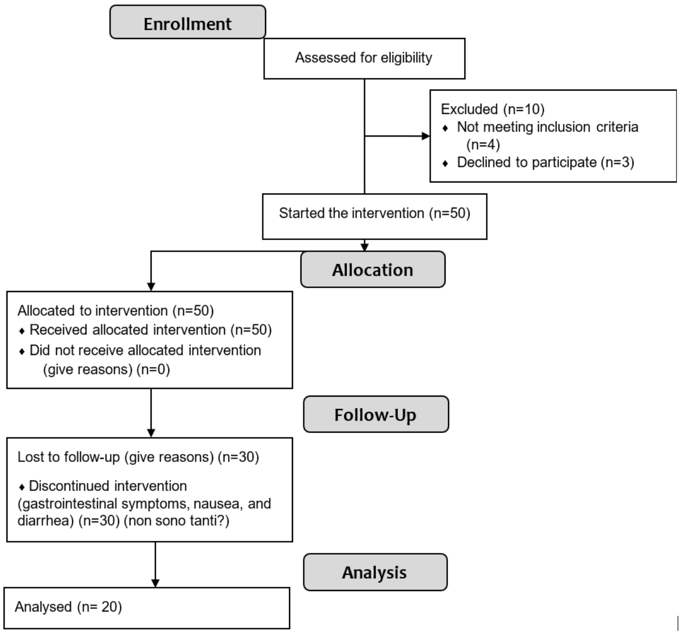

Figure 1. Analytical sample flowchart

The patients' demographic data are presented in Table 1. The age of the patients ranged between 44 and 73 years, 66.7% were in the age range between 61-73 years and 33.3% were between 44-55 years. Most of the patients were males (83.3%), and 16.7% were females. It was found that most of the participants (55.6%) were in the obese category (BMI: 30.0 to 39.9), whereas 27.8% were in the overweight category (25.0-29.9). The class III category (BMI > 40) was 5.6% and only 11.0% had the ideal weight (BMI: 18.5 to 24.9). Most of the patients (55.6%) had primary education, whereas 38.9% had intermediate or secondary education and only 5.6% had a bachelor's degree.

Most of the patients (94.4%) were either nonsmokers or quit smoking more than three years because of heart attack or other CVDs. Only 27.8% of the patients were exercising at least three times a week, mainly vigorous walking for at least 30 minutes.

Table 1. Demographical characteristics of the sample

Variable	Percentage %
Age (years)	
44-55	33.3
61 -73	66.7
Gender	
Male	83.3
Female	16.7
Education	
Primary	55.6
Intermediate/secondary	38.9
Bachelor's degree (B.Sc.)	5.6
BMI (kg/m²)	
Ideal weight (18.5-24.9)	11.0
Overweight (25-29.9)	27.8
Obese (30-39.9)	55.6
Over Obese (>40)	5.6

The medical record of the patients had shown that 88.9% had coronary heart disease, high cholesterol levels (83.3%), diabetes (66.7%), hypertension and heart attack (55.6%), stroke (11.1%), carotid artery disease and peripheral vascular disease (5.6%) (Table 2). Most of the patients (72.2%) had no prior knowledge regarding omega-3 fatty acids, and only 27.8% claimed to have some previous knowledge.

Table 2. Description of chronic diseases among patients.

Variable	Percentage %
Diabetes	66.7
Hypertension	55.6
Hypercholesterolemia	83.3
Coronary heart disease	88.9
Heart attack	55.6
Stroke	11.1
Carotid artery disease	5.6
Peripheral vascular disease	5.6

The participants' self-reported consumption habits regarding n-3 enriched dietary sources is shown in Table 3. The serving size of fish is approximately 3.5 oz., a handful of walnuts, one cup of cooked soybeans, one tablespoon of flaxseed and about 100 ml of canola oil per day.

Among all patients, 66.7% were consuming fish 1-3 times a week, and 27.8% were consuming fish more than 4 times a week accounting for a total of 94.4%. Regarding the consumption of a handful of walnut, one third of the participants have been consuming it for more than 4 times a week, while one third of them were consuming it 1-3 times a week, and the last third were never consuming walnut at all. None of the participants included soybeans, chia seed or flax seed in their diet because they did not know much about their benefits. A small number of patients (22.2%) were consuming canola oil for cooking (Table 3).

Table 3. Patient's consumption of omega-3-rich dietary sources

Variable	Percentage %
Fish (n=2)	
>4 times a week	27.8
1-3 times a week	66.7
Rarely or never	5.6
Walnuts (a handful)	
>4 times a week	33.3
1-3 times a week	33.3
Rarely or never	33.3
Soybeans (one cup)	
Rarely or never	100
Chia seeds (one tablespoon)	
Rarely or never	100
Flax seed (one tablespoon)	
Rarely or never	100
Canola oil (100 ml)	
Every day	22.2
Rarely or never	77.8

The percentage of fatty acid content in RBCs membranes before and after consuming n-3 supplements for 3 months was analyzed and compared. The values were expressed as % of total fatty acid methyl esters, median, interquartile ranges, and Δ Changes (Table 4). There was a significant effect ($p<0.01$) of n-3 supplements consumption on the total unsaturated fatty acids (UFA) which increased from 41.87% to 42.12%. The C16:1n7, which is a monounsaturated fatty acid (MUFA) decreased significantly ($p<0.01$) after treatment from 0.51% to 0.28%. However, a significant increase ($p<0.01$) in the total polyunsaturated fatty acid (PUFA) from 28.32% to 28.82% was recorded after treatment. The total omega-6 fatty acids decreased significantly ($p<0.01$) from 25.38% to 23.59%, while the total omega-3 fatty acids significantly increased from 4.31% to 5.23%. A significant increase ($p<0.01$) in the omega-3 fatty acid C22:5n3 from 1.54% to 2.11% was recorded. No significant changes in the omega-3 fatty acids EPA and DHA were found, whereas a significant decrease ($p<0.01$) in the omega-6/omega-3 ratio from 5.89% to 4.51% was recorded.

Table 4. Effects of a single daily dose treatment with EPA+DHA (180+120mg) consumption on fatty acids content in red blood cell membranes

Fatty acids	Baseline		After 3 months		Δ Changes	P- value
	Median	IQR	Median	IQR		
Total SFA	45.67	15.89	42.47	2.49	-3.20	0.616
C14:0 (%)	0.48	0.36	0.59	0.21	0.27	0.265
C16:0 (%)	25.60	10.02	24.58	1.11	-1.02	0.768
C18:0 (%)	19.59	3.33	17.30	2.37	-2.29	0.375
C16:1n7 (%)	0.51	0.46	0.28	0.11	-0.23	**<0.001**
C18:1n9 (%)	11.35	2.92	12.05	1.42	0.7	0.625
C18:1n7 (%)	1.10	0.62	0.97	0.21	-0.13	0.638
C18:2n6 (%)	12.45	0.66	11.5	3.62	-0.95	0.790
C18:3n6 (%)	0.09	0.33	0.02	0.13	-0.07	0.073
C20:4n6 (%)	11.80	2.58	10.35	1.92	-1.45	0.683
C22:5n6 (%)	1.04	0.30	1.72	1.18	0.68	0.780
C18:3n3 (%)	0.23	0.54	0.13	0.44	-0.10	0.234
C20:5n3 EPA (%)	0.95	1.19	1.04	0.30	0.09	0.381
C22:5n3 (%)	1.54	1.13	2.11	1.47	0.57	**<0.001**
C22:6n3 DHA (%)	1.59	2.04	1.95	1.73	0.36	0.879
Omega-6/ Omega-3	5.89	5.16	4.51	1.28	-1.38	**<0.001**
EPA+DHA (O3I) (%)	2.54	1.61	2.99	1.81	0.45	0.626
Total UFA (%)	41.87	7.45	42.12	2.01	2.50	**<0.001**
Total MUFA (%)	12.96	3.38	13.30	1.18	0.34	0.521
Total PUFA (%)	28.32	4.16	28.82	4.85	0.50	**<0.001**
Total Omega-6 (%)	25.38	4.00	23.59	2.83	-1.79	**<0.001**
Total Omega-3 (%)	4.31	2.46	5.23	3.76	0.92	**<0.001**

SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

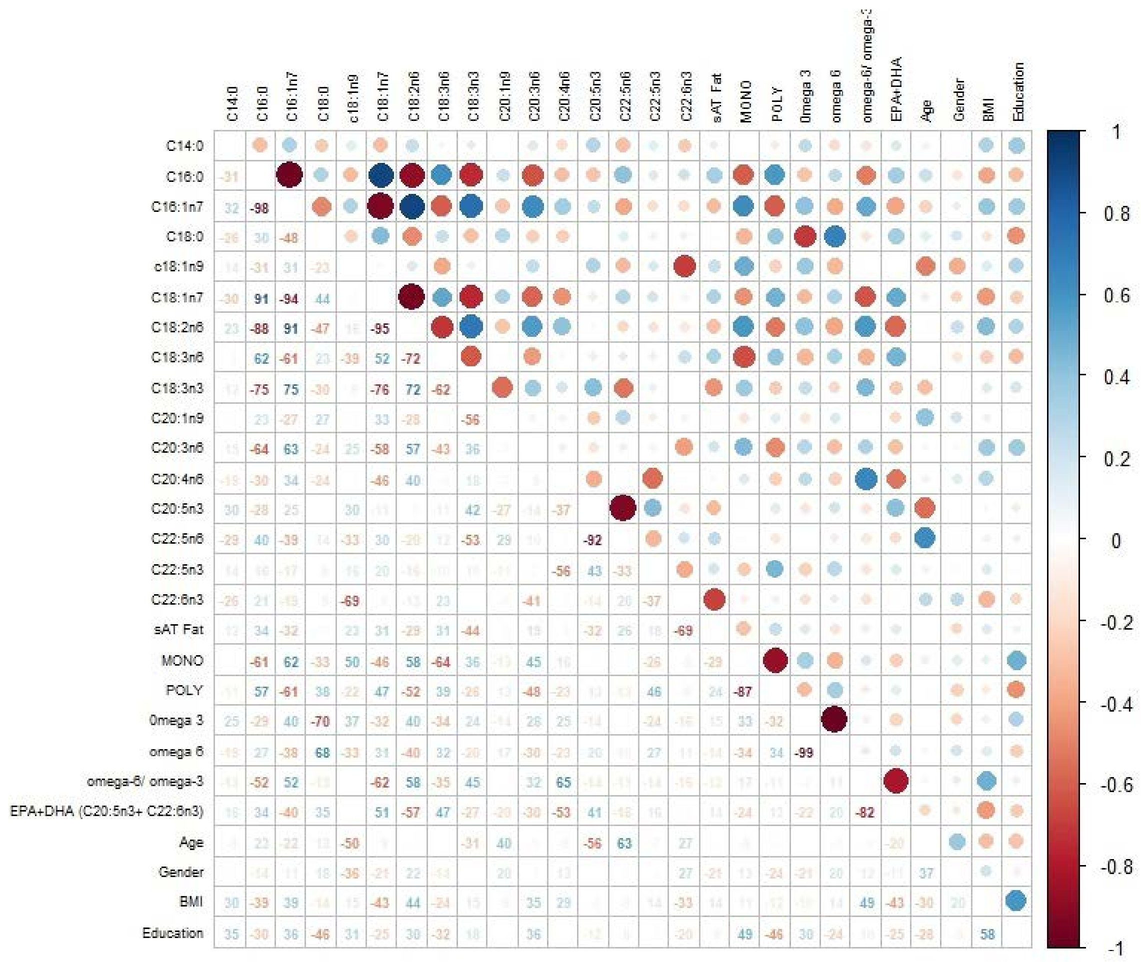

Figure 2. Correlation heatmap among the mean difference changes of the fatty acid content in RBCs of DVD patients before and after treatment with n-3 supplementation (pre-post effects)

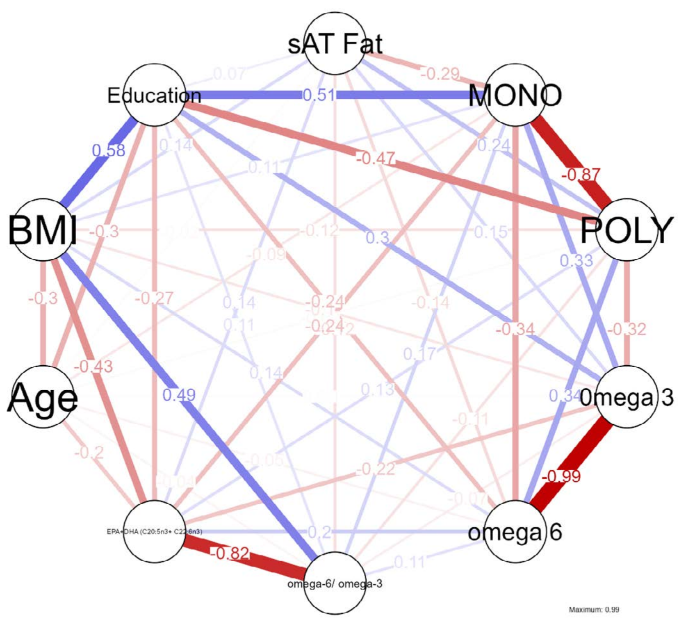

Figure 3. Correlation Network analysis among BMI, age, education and monounsaturated, polyunsaturated and saturated fatty acids mean difference changes of the fatty acid content in RBCs of DVD patients before and after treatment with n-3 supplementation (pre-post effects)

The correlation between BMI, age, education and monounsaturated, polyunsaturated and saturated fatty acids mean difference changes of before and after the intake of the supplementation are shown in figures 2 and 3. The total UFA decreased synergically with a counterbalance in terms of an increase in total Omega-3. Specifically, there was a strong relationship between the decrease in the saturated fatty acid C14 and the increase in the n-3 fatty acid C22:6n3 (Figure 2).

DISCUSSION

The effectiveness of n-3 supplement consumption on patients with cardiovascular diseases in terms of O3I has scarcely been investigated in the literature. This study primarily showed that 3-month EPA and DHA supplementation in CVD patients lead to a statistically significant decrease in total UFA (-2.50%), C16:1n7(-0.23%), total omega-6 (-1.79) and omega-6/omega-3 ratio (-1.38). Furthermore, this study showed a statistically significant increase over time of total PUFA (0.50%), total omega-3 (0.92%) and the total increase of EPA+DHA (O3I) (0.45%) as the primary outcome of the study.

The baseline O3I in the current study was also low (2.54%) and a very high n-6/n-3 ratio was recorded in this cohort of CVD patients. The low O3I recorded in the current study (<4%) is inconsistent with several other intervention studies in which the basal O3I was between 4-5%[31,32].

Several intervention studies have reported an increase in O3I after the Omega 3 supplement consumption[20,31-33]. The low O3I in the present study was slightly improved after taking an omega-3 supplement for 3 months. The main reason behind such results could be attributed to three limitation factors: the supplement dosage (1000 mg/day, containing 180mg EPA and 120mg DHA), the short period of consumption (3 months), and the quality of local fish in regard to their omega-3 content[34].

The intervention study of Drobnic et al. (2017) in which participants consumed two different daily dosages (760 mg or 1140 mg) of fish oil supplements for 4 months reported an increase in O3I from 4.9% to 7.0% in the group consuming 1140mg/ day, whereas an increase from 5.0% to 6.8% was reported in the group consuming 760mg/ day[32]. Similarly, Flock et al. (2013) intervention study used 5 different doses (0, 300, 600, 900, 1800 mg) of EPA+DHA supplements daily for approximately 5 months and showed the highest increment in O3I in the group receiving 1800 mg/day[31]. McDonnell et al. (2019) suggested an approximate intake of 2000 mg/day of EPA+DHA supplement to achieve the desired O3I>8% in 90% of the population[20]. The current study has adopted the recommended daily intake of 1000 mg of EPA+DHA supplement in addition to consuming two fatty fish meals per week for CHD patients based on the study of Kris-Etherton et al. (2002)[35], and Dietary Guidelines for Americans (DGAs) and the American Heart Association (AHA)[36].

Although most patients (94.5%) in the present study consumed fish meals 1-4 times per week in addition to 1000 mg/day of the EPA+DHA supplement, this was not sufficient to show a statistically significant change in the O3I levels. Based on the study of Freije (2009), this can be attributed to the low omega-3 levels in local fish due to the high degrees of salinity and temperature of the Arabian Gulf water[34]. Furthermore, participants were not consuming any other sources of omega-3 enriched food such as soybeans, chia seeds and flax seeds, except for a small number of patients (22%) using canola oil for daily cooking. This is mainly due to the patient's low level of awareness regarding the important nutritional value of omega-3 fatty acids. Indeed, 72% of them reported that they "never heard of omega-3".

Obesity is another factor that can affect the O3I levels. It has been reported that the O3I increases largely in patients having lower body mass index[37]. Diabetes associated with insulin resistance can lead to a defect in the enzymes Δ6 and Δ5 desaturases that are required for PUFAs metabolism. this results in a decrease in plasma and tissue concentrations of PUFAs such as γ-linolenic acid (18:3 n-6), EPA and DHA[38]. Most of the patients in the present study were diabetic (66%), overweight (27.8%), obese (55.6%), and class III obese (5.6%) adding an extra burden on their low O3I.

Nevertheless, such intervention has resulted in a significant increase in total omega-3 content, lower total omega-6 content, and reduced Omega-6/Omega-3. However, both baselines of n-6/n-3 ratio (5.89%) and post consumption ratio (4.51%) were not within the recommended Omega-6/Omega-3ratio of up to 4:1[39-41]. An elevated Omega-6/ Omega-3 is a major risk factor for several types of cancers, stroke, allergic hyperactivity, thrombotic diseases, and CVD[4]. Furthermore, the Japan Society for Lipid Nutrition recommends n-6/n-3 of less than 2:1 for the prevention of chronic disease in the elderly[42]. Such findings can be applied to the age group of the present study in which most of the participants (66.7%) were in the age group 61-73 years old.

Another limitation of the present study could be the duration of the supplementation, which was for 3 months, based on the studies of Harris and Von Schacky (2004), and Jackowski et al. (2013)[15,43]. This period seems to be not sufficient to show significant changes in the O3I. as suggested, the approximate lifespan of RBCs[44] is 4 months, and it is the time required for the membrane fatty acids to reach a new steady state[45]. The period of supplement consumption in most other intervention studies in which O3I increased significantly was longer. For example, it was 4 months in the study of Drobnic et al. (2017)[32], 5 months in the Folck et al. study (2013)[31], 6 months in McDonnell et al. study (2019)[20], 12 months in Browning et al. study (2012)[46], and 30 months in Alfaddagh et al. study (2019)[18].

Therefore, this study supports the findings of previous studies[19,20,32,47] in which the recommendations regarding 2 fish meals per week plus 1000 mg of EPA+DHA supplement did not increase the O3I to the optimal >8%. The intake should reflect the average amount needed based on the extreme variations in EPA+DHA contents in seafood[34,48]. In addition, it is recommended that the individual O3I or at least the population average O3I should be determined to estimate the required intake of supplements needed to achieve the desired O3I to prevent CVD.

CONCLUSION

This study characterized the fatty acid contents in CVD patients prior to and after the consumption of omega-3 supplements for 3 months. Although the increase of the O3I was not statistically significant, a significant increase in total omega-3 levels, and a significant decrease in omega-6/omega-3 ratio, mainly due to a significant decrease in total omega-6 contents was demonstrated. These findings highlight the importance of corroborating the evidence regarding the effect of omega-3 supplementation and to better investigate the role of the O3I in the context of CVDs.

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