Fatty Acid Profile of the Erythrocyte Membranes of Healthy Bahraini Citizens in Comparison with Coronary Heart Disease Patients
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Abstract: We compared the fatty acid compositions including the n-3 and n-6 polyunsaturated fatty acids families in the red blood cell membranes of 26 healthy normal subjects to those with coronary heart disease. The main finding was a significant decrease in the level of docosahexanoic acid (DHA, 22:6 n-3) in coronary heart disease patients. In addition, an increase in n-6/n-3 ratio, and a decrease in the ratio of 22:6/18:3 (n-3) and in omega-3 index was also observed in coronary heart disease patients. The reduction in 22:6/18:3 (n-3) ratio suggests a defect in the elongation and desaturation steps in the n-3 series. The findings in this study also suggest that the low dietary value of fish from Bahraini water may have resulted in a modest dietary intake of DHA and eicosapentanoic acid (EPA, 20:5 n-3), which might have been the reason behind the high incidence of coronary heart disease in Bahrain.

Key words: CHD, DHA, EPA, fatty acids, omega 3, red blood cells

1 INTRODUCTION
Diets have changed rapidly in the past 100-150 years mainly in the type and quantity of essential fatty acids (EFAs), giving rise to a number of chronic diseases such as hypertension, obesity, diabetes, cardiac diseases and many cancers. Several studies have suggested that coronary heart disease (CHD) is associated with the blood and/or tissue fatty acids (FAs) proportions. Those FAs of interest include the n-6 FAs (linoleic acid [LA], dihomo-gamma linolenic acid [DGLA] and arachidonic acid [AA]), and the n-3 FAs (α-linolenic acid [ALA], eicosapentaenoic acid [EPA] and docosahexanoic acid [DHA]).

Humans originally consumed diets that contained a 1:1 ratio of omega-6 to omega-3 polyunsaturated fatty acids (PUFAs) based on wild and free-ranging animals with higher omega-3 FAs than the present-day commercial livestock. A ratio of omega-6 to omega-3 PUFAs of 15.0-16.7:1, instead of 1:1 has been estimated in the present Western diet resulting in a diet deficient in omega-3 FAs. The increased proportion of omega-6 FAs is considered a major risk factor for many diseases such as cardiovascular disease, cancer, and inflammatory and autoimmune diseases.

EPA and DHA are the major n-3 fatty acids found in fish and shellfish and are thought to be responsible for their cardioprotective effect, whereas, n-6 fatty acids are mostly found in plants, especially seeds. The fish that are rich in EPA and DHA are mainly oily fish. These fatty acids are produced by unicellular algae (microalgae) or phytoplankton which are consumed and then accumulate in the fish flesh.

The importance of omega-3 PUFAs in the prevention of CHD is well documented. Blood or tissue omega-3 (n-3) PUFAs levels are considered a biomarker of fish intake which is also associated with reduced risk of fatal coronary heart disease, nonfatal coronary heart disease and sudden cardiac death (SCD). Many other beneficial effects are also associated with n-3 PUFAs including antiarrhythmic effect, inhibition of platelet aggregation, reduction of blood viscosity, suppression of leukotriene formation, reduction of insulin resistance, lowering of triglycerides, fibrinogen and blood pressure levels.

Omega-3 index is a new risk factor, which can be measured in red blood cells and expressed as a percentage of EPA+DHA of total fatty acids. An omega-3 index of > 8% is considered to be associated with 80% less risk of SCD, in comparison to an index of <4%. The n-3 index might be recommended in the future as a goal for treatment with EPA and DHA.

A daily dose of 850mg of n-3 FAs supplement, mainly as EPA and DHA was proven to reduce the risk of CHD death.
by 25% and SCD by 45%\(^\text{16}\). A recent recommendation by the American Heart Association/American College of Cardiology, the European Society for Cardiology and national cardiatic societies that an intake of 1 g/day of EPA and DHA n-3 marine FAs can be taken for secondary prevention, cardiovascular prevention, post myocardial infarction treatment and prevention of SCD\(^\text{17-19}\). However, consumption of at least two preferably oily fish meals per week, which provides about 500 mg of EPA + DHA per day, is recommended for those with no known disease (primary prevention)\(^\text{15}\).

No studies are available today about the fatty acid composition of red blood cells (RBCs) and omega-3 index in adult Bahraini citizens. Therefore, the present study is a first attempt to provide information about the fatty acid composition of RBCs in normal people as well as CHD patients. Our investigation also aimed to determine whether any relationship is present between the fatty acid composition of red blood cell membranes and fish consumption in Bahrain. The results are also discussed in relation to fatty acid metabolism. In addition, the levels of EPA+DHA (omega-3 index), expressed as percent of total FAs, were investigated.

2 MATERIALS AND METHODS

2.1 Study subjects

Students and employees at the University of Bahrain, College of Science, at Sakhir campus were invited to participate in this study. Blood samples were drawn after ≥ 10 h of fasting. Venous blood samples from 26 healthy adults were obtained by a trained phlebotomist using venipuncture methods. The group included 19 females and 7 males with an age range of 18-45 and 18-52 years old respectively. Approximately, half of the group (14 subjects) were between the ages 18-24 years, whereas the other half (12 subjects) were between the ages 45-52 years.

Eleven patients (6 females and 5 males), aged 49-57 years, with CHD, admitted at SH. Mohamed Bin Khalifa Al Khalifa Cardiac Center, Bahrain Defense Force Hospital (BDF Hospital), participated in the study. A sample of 5 mL blood collected in heparinized bottles was used directly for fatty acids measurement whereas blood samples (5 mL) collected in plain bottles, were centrifuged to obtain serum which was stored at -70°C until lipid profile analysis. Lipid profile analysis was carried out at the BDF Hospital, Pathology Laboratory.

2.2 Ethics

The ethical committee at the BDF Hospital approved the study, and the patients gave their verbal consent.

2.3 Sample preparation

2.3.1 Lipid extraction

Blood samples were extracted with a mixture of isopropanol: chloroform (11 v/v) by the procedure of Rose and Oklander (1965)\(^\text{20}\). Aliquots of blood samples (1 mL) were mixed with 1 mL deionized water, 11 mL isopropanol, and 7 mL chloroform, with vigorous mixing for 5 min after each addition. The mixtures were then centrifuged for 30 min at 3000 rpm. After extraction, the lipid extracts in the chloroform layer were then stored at -20°C.

2.3.2 Fatty acids methylation

Fatty acid methylation was performed on the extracted stored samples according to the method of Morrison and Smith (1964)\(^\text{21}\). Fatty acid methyl esters (FAMEs) were prepared by drying 5 mL of the chloroform phase under nitrogen in leak-proof reaction tubes, followed by the addition of 4 mL boron trifluoride-methanol (14%) to the residue. The tubes were then flushed with nitrogen before sealing, heated in a water bath for 90 min at 100°C. After cooling, methanol (4 mL) and benzene (3.45 mL) were added, tubes were incubated at 100°C water bath under nitrogen atmosphere for 30 min. The mixture was then transferred into 50 mL Pyrex reaction tubes, extracted by adding 22.9 mL pentane and 11.45 mL water. The upper pentane phase containing FAMEs was then separated, dried under nitrogen, and the residues resuspended in methylene dichloride.

2.3.3 Fatty acids analysis

FAMEs analysis was carried out with a Perkin Elmer Autosystem XL Gas Chromatography (GC) Flame Ionization Detector (FID) equipped with metal column 6 ft in length, 1/8 in diameter, CSP 2310 3% and 2300% on 100/120 chrom WAW support (Supelco, Switzerland). Column temperature was 250°C, injector and detector temperature was 300°C, and injection volume was 2 μL. The carrier gas was nitrogen at a flow rate of 36.5 mL/min. Identification of FAMEs was based on the comparison of their retention times and peak areas against authentic standards (PUFA No. 2, Cat. No. 47015-U, and FAMEs Cat. No. 241136) supplied by SUPELCO USA.

2.4 Statistical analysis

The statistical analysis was performed using the statistical package from Excel 97 (Microsoft Corporation). Results are presented as means of 3 replicates ± standard deviation (SD). The mean values of each measured parameter were also statistically compared using ANOVA single factor and student’s t-test. Differences with p value <0.05 were considered statistically significant.

3 RESULTS

3.1 Physical characteristics of the subjects

Clinical characteristics of the subjects participated in
the study are described in Table 1. Values of serum cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol did not differ between the two groups (controls and patients). Parameters from all the subjects were within the normal range.

3.2 Fatty acids composition of RBCs

The fatty acid content of red blood cell membranes in CHD patients and controls is reported in Table 2 and Fig. 1. Since no significant difference was observed between the two different age groups (age range 18-24 and 45-52 years) of the normal subjects, as well as the two different groups of the CHD patients (diabetic and non diabetic), the results of the FA compositions were pooled together and represented as normal subjects versus CHD patients.

There were no significant differences in saturated, unsaturated, polyunsaturated, and monounsaturated fatty acid values (%) between CHD patients and control subjects. However, CHD patients were characterized by lower DHA value (0.41 ± 0.7%) in red blood cell membranes when compared with controls (2.81 ± 1.48%) (p = 0.00021). As a consequence, both total n-3 fatty acids value (0.82 ± 1.19%, p = 0.008) and omega-3 index (0.64 ± 0.96%, p = 0.002) were reduced, whereas n-6/n-3 ratio was significantly higher (40.76 ± 7.7%, p = 0.004) than the mean value obtained in our control population (3.2 ± 1.78, 3.14 ± 1.68, and 11.12 ± 3.41%) respectively. Only 1 of the 26 control subjects (3.8%) and none of the 11 patients (0%) were smokers. Therefore, the low proportion of normal subjects who smoked did not allow statistical analysis.

In order to evaluate whether the specific reduction of DHA was due to alterations in the metabolic pathway, the following parameters suggested by Reddy et al. (2004)²² as biochemical indices of product-substrate relationship of elongation and desaturation in the PUFA pathways were used: (1) 18:0/16:0 ratio-saturated fatty acids; (2) 22:6/20:5, and 20:5/18:3 ratios -the n-3 family; (3) 22:4/20:4, 20:4/18:3, and 18:3 n-6/18:2 n-6 ratios -the n-6 family; and 18:1/18 (n-9) family (Table 3). The ratios of 18:0/16:0 and 22:6/20:5 were significantly lower in patients (0.66 ± 0.12 and 0.72 ± 1.13 respectively) than in normal control subjects (0.83 ± 0.10 and 9.21 ± 2.34, p = 0.0037 and < 0.0001 respectively). No significant differences were observed in the ratios of 20:4/18:2 (n-6), 22:4/20:4 (n-6), 20:5/18:3 (n-3), and18:1/18 (n-9) between normal controls and patients.

4 DISCUSSION

It is well established that dietary FAs affect several physiological processes and hence their impact on health and disease²³,²⁴. Since, a balanced diet of essential fatty acids is vital for good health and normal development, the evaluation of FAs status is very important especially that n-3 FAs supplementation is now adopted in the management and treatment of many diseases²⁵,²⁶. Plasma FAs composition is considered a reflection of short term fat intake, whereas erythrocytes FAs are a better long-term marker of fat intake because of their slower turnover than that of plasma or platelet lipids²⁷. We com-

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### Table 1 Description of Patients and Normal Controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal controls (n = 26)</th>
<th>Patients (n = 11)</th>
<th>Normal*** range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females %</td>
<td>73.07</td>
<td>54.54</td>
<td>—</td>
</tr>
<tr>
<td>Males %</td>
<td>26.92</td>
<td>45.45</td>
<td>—</td>
</tr>
<tr>
<td>Fish consumption*</td>
<td>0-3</td>
<td>0-3</td>
<td>—</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus %</td>
<td>0</td>
<td>45.45</td>
<td>—</td>
</tr>
<tr>
<td>Age (18-24) %</td>
<td>53.85</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age (45-57) %</td>
<td>46.15</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol (μmol/L)**</td>
<td>4.26 ± 1.06</td>
<td>3.83 ± 0.84</td>
<td>3.8-5.2</td>
</tr>
<tr>
<td>Triglycerides (μmol/L)**</td>
<td>0.87 ± 0.49</td>
<td>1.41 ± 1.05</td>
<td>0.1-2.3</td>
</tr>
<tr>
<td>HDL-cholesterol (μmol/L)**</td>
<td>1.35 ± 0.36</td>
<td>1.01 ± 0.30</td>
<td>0-1.7</td>
</tr>
<tr>
<td>LDL-cholesterol (μmol/L)**</td>
<td>0.0025 ± 0.0008</td>
<td>0.0023 ± 0.0008</td>
<td>0-3.4</td>
</tr>
</tbody>
</table>

* Fish intake per week
** Values are expressed as mean ± standard deviation (SD)
*** Supplied by BDF hospital
Table 2  Main Fatty Acid Contents of Red Blood Cell Membranes.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Normal controls (n = 26)</th>
<th>Patients (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>19.24 ± 4.03</td>
<td>20.27 ± 2.95</td>
<td>NS</td>
</tr>
<tr>
<td>18:0</td>
<td>15.59 ± 1.67</td>
<td>13.66 ± 4.49</td>
<td>NS</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>16.48 ± 3.22</td>
<td>15.13 ± 4.13</td>
<td>NS</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>11.20 ± 0.98</td>
<td>9.68 ± 2.49</td>
<td>NS</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.06 ± 0.10</td>
<td>0.18 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>10.14 ± 2.61</td>
<td>10.89 ± 6.59</td>
<td>NS</td>
</tr>
<tr>
<td>20:4n-6 (AA)</td>
<td>14.25 ± 1.44</td>
<td>12.85 ± 5.13</td>
<td>NS</td>
</tr>
<tr>
<td>20:5n-3 (EPA)</td>
<td>0.33 ± 0.20</td>
<td>0.23 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>22:4</td>
<td>2.29 ± 1.49</td>
<td>3.01 ± 1.38</td>
<td>NS</td>
</tr>
<tr>
<td>22:6n-3 (DHA)</td>
<td>2.81 ± 1.48</td>
<td>0.41 ± 0.70</td>
<td>0.00021</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ SFA</td>
<td>34.83 ± 5.70</td>
<td>33.93 ± 7.44</td>
<td>NS</td>
</tr>
<tr>
<td>Σ UFA</td>
<td>57.56 ± 11.52</td>
<td>52.38 ± 20.91</td>
<td>NS</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>41.08 ± 8.30</td>
<td>37.25 ± 16.78</td>
<td>NS</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>16.48 ± 3.22</td>
<td>15.13 ± 4.13</td>
<td>NS</td>
</tr>
<tr>
<td>Σ n-6</td>
<td>35.59 ± 5.03</td>
<td>33.42 ± 14.21</td>
<td>NS</td>
</tr>
<tr>
<td>Σ n-3</td>
<td>3.20 ± 1.78</td>
<td>0.82 ± 1.19</td>
<td>0.008</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>11.12 ± 3.41</td>
<td>40.76 ± 7.70</td>
<td>0.004</td>
</tr>
<tr>
<td>EPA + DHA</td>
<td>3.14 ± 1.68</td>
<td>0.64 ± 0.96</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The values are expressed as % of total fatty acids; mean ± SD
SFA: saturated fatty acids, UFA: unsaturated fatty acids, PUFA: polyunsaturated fatty acids
MUFA: monounsaturated fatty acids. NS: not significant

![Graph](image)

Fig. 1  Relative Composition (% of total FAME) of Selected Fatty Acids in Red Blood Cell Membranes (mean ± SD).
pared the FA composition of the RBCs of CHD patients with those of normal control subjects. The current study showed that there is indeed significant reduction in a key RBC membrane FA (DHA) in CHD patients which resulted in a decrease in the n-3 PUFA’s, an increase in the n-6/n-3 ratio, and a decreased omega-3 index. Concerning other FAs, no significant differences among CHD and normal control groups were observed. It is possible that differences in DHA level between patients and healthy subjects in this study can be accounted for by environmental factors, such as diet, and other risk factors including CHD, and diabetes, which are known to affect RBC membrane PUFA levels).

The result of the present study is consistent with several studies on PUFAs which have shown that the levels of DHA are reduced in patients with CHD, hypertension, and type 2 diabetes mellitus.

Although fatty acid compositions of serum and erythrocytes reflect the individual dietary intake of lipids, they also reflect endogenous fatty acid metabolism including fatty acid synthesis (de novo lipogenesis) and fatty acid desaturation and elongation. The conversion of EPA to DHA involves several elongation, desaturation and β-oxidation steps rather than a Δ4 desaturation step. The current study demonstrated a significant reduction in the ratio of 22:6/20:5 (p<0.0001) in CHD patients which suggests a defect in the elongation and desaturation steps of the n-3 metabolic pathway. However, the normal 18:3 n-6/18:2 n-6 ratio suggests a normal Δ6 desaturase activity. Since the Δ6 desaturase enzyme is involved in one step of AA synthesis and three steps of the complex conversion to DHA, its availability may be limited to the earlier step (i.e. AA synthesis) in the pathway in diabetic patients. An alternative explanation may be an enhanced peroxisomal β-oxidation of DHA which may also contribute to the decreased DHA levels in diabetic patients in this study.

The results from several studies have demonstrated that low activity of Δ5 desaturase is related to several metabolic and cardiovascular disorders. It is also known that Δ5 and Δ6 desaturases activities are enhanced by insulin. Bahrainis are known to have high incidence of CHD, hypertension, and type 2 diabetes mellitus, which are known to be associated with insulin resistance and hyperinsulinemia.

The result of the present study is consistent with several studies on PUFAs which have shown that the levels of DHA are reduced in patients with CHD, hypertension, and type 2 diabetes mellitus.

Table 3  Ratios of Some Selected Fatty Acids.

<table>
<thead>
<tr>
<th>Fatty acid ratio</th>
<th>Normal controls</th>
<th>Patients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 26)</td>
<td>(n = 11)</td>
<td></td>
</tr>
<tr>
<td>18:0/16:0 (SFAs)</td>
<td>0.83 ± 0.10</td>
<td>0.66 ± 0.12</td>
<td>0.0037</td>
</tr>
<tr>
<td>20:5/18:3 (n-3)</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.03</td>
<td>0.99</td>
</tr>
<tr>
<td>22:6/20:5 (n-3)</td>
<td>9.21 ± 2.34</td>
<td>0.72 ± 1.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>22:4/20:4 (n-6)</td>
<td>0.20 ± 0.09</td>
<td>0.23 ± 0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>20:4/18:2 (n-6)</td>
<td>1.27 ± 0.04</td>
<td>1.29 ± 0.29</td>
<td>0.85</td>
</tr>
<tr>
<td>18:3/18:2 (n-6)</td>
<td>1.05 ± 0.16</td>
<td>1.17 ± 0.68</td>
<td>0.24</td>
</tr>
<tr>
<td>18:1/18:0 (n-9)</td>
<td>1.05 ± 0.10</td>
<td>1.11 ± 4.31</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

SFA: saturated fatty acids

a Reflects the activity of elongase
b Reflects the activities of Δ 6 and Δ 5 desaturases
c Reflects the activities of Δ 6 desaturase
d Reflects the activity of Δ 9 desaturase

Similar results have been reported by other workers in India in which the population suffer a high incidence of CHD, type 2 diabetes mellitus, and hypertension. Das (1995) has clearly demonstrated that EPA and DHA are low in CHD, type 2 diabetes mellitus, and hypertension patients. He also suggested that changes in the FAs composition may be responsible for the insulin abnormalities described in CHD, hypertension, and type 2 diabetes mellitus and might influence the action of insulin by acting as precursors for eicosanoids such as prostaglandin E1 (PGE1), prostacyclin (PGI2), and PGI3. Those eicosanoids are produced from DGLA, AA, and EPA and function as potent antiaggregators and vasodilators and can prevent thrombosis and atherosclerosis associated with type 2 diabetes.
The n-6/n-3 ratio in the present study is considered very high in normal subjects (11.12 ± 3.41) and extremely high in CHD patients (40.76 ± 7.7). The World Health Organization (FAO/WHO, 1993) and the British Nutrition Foundation (1992) are recommending a ratio of 3-4/1 for omega-6/omega-3 for healthy adults. Furthermore, the Japan Society for Lipid Nutrition recommended a ratio of omega-6/omega-3 of less than 2/1 for the prevention of chronic diseases of the elderly.

There is growing evidence about a beneficial relation between n-3 PUFA and lower risk of death from coronary heart disease. Therefore, several intervention studies of varying quality have been reported. However, the largest and the most well controlled was the GISSI Prevenzione study, in which a relatively small intake of EPA+DHA (one capsule of omega-3 FA ethyl esters, 850mg per day) was proven to reduce the cause of any death by 20%, and the risk of sudden death by 45%.

Moreover, based on the study of von Schacky and Harris (2007), an omega-3 index of >8% is associated with 90% less risk of sudden cardiac death, in comparison to an index of 4%, and a low ratio of n-6/n-3 fatty acids is needed for the prevention and management of many diseases including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune disease. The omega-3 index in both groups investigated in the present study, 3.14 ± 1.68%, and 0.64 ± 0.96% in normal subjects and CHD patients respectively, was less than 4%.

Marine organisms are known to contain a wide variety of PUFAs. However, the n3-PUFA (EPA and DHA) are found to play an important role against cardiovascular diseases, the improvement of learning ability and the enhancement of the immune system. Several studies have confirmed the importance of balanced omega-6/omega-3 ratio fatty acids from marine sources in the prevention of sudden death, and the prevention and management of cardiovascular disease and cancer mortality. DHA is the major n-3 fatty acid incorporated into myocardial membranes following feeding on fish oils. Although, fish is considered one of the most important food sources in Bahrain, cardiovascular diseases have been reported to be the leading cause of death among Bahrainis (Table 4).

All fish contain EPA and DHA; however, their concentrations vary between species as well as within the same species due to environmental conditions such as diet, and whether they are wild or farm-raised fish. In Table 5, EPA and DHA content of five fish types of important commercial values in Bahrain as well as the amount required to provide 1 g of EPA+DHA is presented. We compared the level of EPA+DHA in local fish from Bahrain with data from the USDA Nutrient Data Laboratory (Table 6).

It was shown that level of EPA+DHA in local fish from Bahrain is much lower (0.030-0.239 g/3oz) than that reported by the USDA Nutrient data Laboratory (0.13-1.81 g/3oz), most probably due to the high degrees of salinity and temperature of the Arabian Gulf water which decreases the extent of unsaturation of fatty acids of microalgae and thus reduce the dietary value of fish from Bahrain water.

The amount required to provide ~1 g of EPA+DHA presented in Table 5 seems to be difficult to attain and sustain through fish consumption over a long period mainly because of high cost, availability, and ability to eat fish. Based on fish consumption in Bahrain estimated by the Directorate of Fisheries (2005) (0.045 kg/day), equivalent to 0.02-0.13 g of EPA+DHA per day, it is apparent that intake does not meet recommendation for fish or EPA+DHA. Consequently, fish oil supplement may be considered as an alternative. Typical recommendations of 0.3-0.5 g/day of EPA+DHA and 0.8-1.1 g/day of α-linolenic acid have been adopted by many countries (Canada, Sweden, United Kingdom, Australia, Japan) as well as the World Health Organization and North Atlantic Treaty Organization; whereas, CHD patients are encouraged to increase their EPA and DHA to ~1 g/day, and 2-4 g/day for patients who need to lower their triglyceride levels.

The findings of the present study highly recommend an immediate intervention in CHD patients, i.e. to eat 1 g of EPA+DHA per day, preferably from oily fish (oom and kan nad), they could also take EPA+DHA supplements in consultation with their cardiologist in order to reduce the risk of fatal myocardial infarction. Whereas individuals without documented CHD are advised by the American Heart Association to eat at least two fish meals per week mainly

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Top Leading Causes of Death in Bahrain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causes of death</td>
<td>Rates per 10000 population</td>
</tr>
<tr>
<td>Circulatory system</td>
<td>60.5</td>
</tr>
<tr>
<td>Endocrine, nutritional and metabolic disorders</td>
<td>46.3</td>
</tr>
<tr>
<td>Accidents and injuries</td>
<td>36.6</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>34.1</td>
</tr>
<tr>
<td>Respiratory disorders</td>
<td>17.1</td>
</tr>
</tbody>
</table>
Table 5  Amounts of EPA + DHA in Five Fish Types of Important Commercial Values in Bahrain and the Amount of Fish Consumption Required to Provide ~1 g of EPA + DHA per Day.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Local name</th>
<th>EPA (20:5)* mg/100 g</th>
<th>DHA* (22:6) mg/100 g</th>
<th>EPA+DHA content mg/100 g</th>
<th>EPA+DHA content g/100 g</th>
<th>EPA+DHA content g/3-oz</th>
<th>Amount required to provide ~1 g of EPA+DHA per day, oz (fish)</th>
<th>Amount required to provide ~1 g of EPA+DHA per day, kg (fish)</th>
<th>EPA+DHA** intake per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish Mackerel</td>
<td>Kannad</td>
<td>55 ± 5.40 161 ± 19.80</td>
<td>216</td>
<td>0.216</td>
<td>0.183</td>
<td>16.40</td>
<td>0.46</td>
<td>0.098</td>
<td></td>
</tr>
<tr>
<td>Rabbitfish</td>
<td>Saffi</td>
<td>37.50 ± 3.90 76.40 ± 6.70</td>
<td>113.90</td>
<td>0.114</td>
<td>0.097</td>
<td>31.00</td>
<td>0.88</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Greasy Grouper</td>
<td>Hamour</td>
<td>26.85 ± 4.30 76.20 ± 3.70</td>
<td>103.05</td>
<td>0.103</td>
<td>0.087</td>
<td>34.30</td>
<td>0.97</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>Spangled Emperor</td>
<td>Share</td>
<td>8.30 ± 0.64 28.20 ± 3.90</td>
<td>36.50</td>
<td>0.037</td>
<td>0.030</td>
<td>95.50</td>
<td>2.70</td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>White Sardinella</td>
<td>Oom</td>
<td>146.50 ± 20 134 ± 21.70</td>
<td>280.50</td>
<td>0.281</td>
<td>0.239</td>
<td>12.57</td>
<td>0.36</td>
<td>0.125</td>
<td></td>
</tr>
</tbody>
</table>

*Data obtained from Al-Arrayed et al. (1999)

**Based on 0.045 g fish intake per day (Directorate of Fisheries (2005))

Ounce to grams: multiply ounce by 28.3
fatty fish plus oils and foods rich in ALA (flaxseed, canola, soy, walnuts). This comes to ~500 mg/day \(^{58}\). However, the recommendation of the Dietary Reference Intakes for Energy and Macronutrients (AMDR) is to consume two fatty fish meals per week as well as 1 g per day supplement of EPA+DHA for CHD patients, whereas for healthy individuals is to eat at least two fish meals per week mainly fatty fish and to use liquid vegetable oils containing \(\alpha\)-linolenic acid, in addition to 0.3-0.5 g/day EPA+DHA \(^{56,57}\). The latter recommendation seems to be more suitable for Bahrain mainly because the consumption of 1 g/day of EPA+DHA from local marine fish requires eating between 0.36-2.7 kg per day.

### 5 CONCLUSION

In conclusion, our study demonstrated a significant association between low DHA levels and increased incidence of CHD. The results may also indicate a protective effect of DHA against diabetes mellitus (type 2). Finally, the study adds to our knowledge the modest fish consumption in Bahrain in term of n-3 PUFAs. Additional controlled dietary studies are needed to develop effective intervention strategies.

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