Omega-3 fatty acid effect in cardiovascular disease risk factors

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Abstract

Background: One of the leading causes of death around the globe is cardiovascular disease (CVD). Nutrition plays a vital role in the prevention of CVD. However, the effects of margarines, rich in n-3 and n-6 fatty acids, compared to butter, are still controversial in plasma lipid levels.

Method: A single blind parallel study was carried out. Twelve (n=12) healthy UAE University female students were recruited from age 19-24 years old and were randomly allocated in 2 groups. The participants were asked to consume either 25g of margarine or 25g of butter for 1 month and to retain their normal daily dietary habits. Fasting blood samples were collected before the beginning and at the end of the dietary periods for the assessment of lipid profile.

Result: Triglycerides decreased by 12% within the intervention group, total cholesterol, and glucose levels were decreased in both groups, but it was not statistically significant within and between the margarine and butter group.

Conclusion: The present study showed no statistical differences in the effects of butter and margarine, enriched with n-3 and n-6 fatty acid, on cholesterol, triglycerides, and glucose. Further research is necessary with bigger sample size and longer duration.

Key words: Cardiovascular diseases, plasma lipids, butter, margarine, saturated fatty acids, polyunsaturated fatty acids
Introduction

One of the leading causes of death around the globe is cardiovascular disease (CVD), with low- and middle-income countries to be disproportionately affected: in particular, over 80% of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women (WHO, 2011). Cardiovascular diseases are predicted to remain the single leading cause of death and mortality from CVD - mainly from heart disease and stroke - has been projected to reach 23.3 million by 2030 (Mathers & Loncar 2006; WHO, 2011). Factors such as unhealthy diet and physical inactivity contribute to CVD by indicating high blood lipids, high blood glucose, high blood pressure, and overweight and obesity. These “intermediate risks factors” can be measured in primary care facilities and indicate an increased risk of developing a heart attack, stroke, heart failure and other complications (WHO, 2008).

Several studies have shown that n–3 fatty acids reduce the rates of death from cardiac causes and sudden death, the hypothesis that n–3 fatty acids work largely by preventing life-threatening cardiac arrhythmias gained increasing attention. In addition, n-6 is suggested to have a lowering effect on total cholesterol (TC), and LDL cholesterol (LDL). The distinct functions of these two families make the balance between dietary n-6 and n-3 fatty acids an important consideration for decrease cardiovascular disease risk factor (Kang & Leaf, 1994; Billman et al. 1997; McLennan, 2001; Raffaele De Caterina, 2011). In particular, since 1978, Dyerberg and colleagues (1978) hypothesized that marine n–3 fatty acids might provide protection against atherosclerosis and thrombosis, and they began research on the potential effects of n–3 fatty acids in the prevention and treatment of vascular disease. N–3 fatty acids may affect several intermediate determinants of cardiovascular risk. At doses of 3 g per day or more, these substances generally reduce hypertriglyceridemia in humans (Von Schacky, 2006; Sadovsky & Kris-Etherton, 2006) without changing cholesterol levels significantly (Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico, 1999). The n–3
fatty acids are also associated with decreased levels of markers and mediators of inflammation such as the cytokines interleukin-1β and tumor necrosis factor α (Endres et al. 1989; Calder, 2007). Studies have shown that increased intake of n–3 fatty acids is associated with a small reduction in blood pressure (approximately 2 to 3 mmHg systolic and 1 to 2 mmHg diastolic) (Geleijnse et al. 2002) and with a reduction in the resting heart rate (approximately 3 beats per minute) (Mozaffarian, 2007). A short course of n–3 fatty acid supplementation has been reported to improve endothelial function (Goodfellow et al. 2000) and to reduce features of inflammatory atherosclerotic plaque (Thies et al. 2003); at present low-dose n-3 fatty acids (1 g/day) are recommended for patients with coronary disease in many countries (Ann C Skulas-Ray et al. 2007; Schacky & Haris 2007; Marik & Varon, 2009). However, although the effects of n-6 and n-3 fatty acids are for long considered to be anti-atherogenic, the effects of margarines compared to butter remain unclear. Therefore, the aim of the present preliminary study was to investigate the effect of margarine (with 2g of omega-3 fatty) acid intake in lipid profile.

Methodology

Participants

The present study is parallel single-blinded, of sample that include twelve (n=12) healthy UAE University female students were recruited (age 19-24 years old). The exclusion criteria were any female who has any chronic disease such as CVD, hypertension, renal failure and liver diseases; or taking omega-3 fatty supplements. They should not exercise vigorously and they should not be on any diet. Background dietary habits should resemble a typical “westernized” diet pattern. Ethics approval was granted by the Ethics Committee of the United Arab Emirates University.
24-hr Dietary Recall

A 24-hr food recall collected from each study participants before they entered the study. The dates and times of these recalls were scheduled and participants have been asked to not change their normal eating pattern. The participants asked if they used butter in there usually food, most of them confirmed that they don’t, except two of them were eaten butter at breakfast with croissant in campus, and since the winter break started they stated that they don’t eat it at home as breakfast. None of the participants have ever used Flora margarine at home before, so they introduced it to their daily life for one month. Participants were recommended to not change their dietary intake, and the habitual physical activity levels.

Dietary intervention

The participants were asked to consume either 25 g of margarine or 25 g of butter for 1 month, without changing the rest of their dietary habits. They were divided into two groups, first group was given margarine (Flora, Unilever) to consume daily 25 g = 1 g n-3) of the margarine for one month. On the other hand, the control group consumed 25 g butter (Lurpak). The 25 g of margarine or butter could be consumed throw out the day, which is equal to \(1\frac{1}{2}\) Tbsp., and all of the participants were shown how to measure the \(1\frac{1}{2}\) tablespoon to consume it. The participants were followed to ensure that they maintained there usual diet and adhered to the protocol, by creating group conference in the application “WhatsApp” that is available in all smartphone for free, every day a message is being send as group or private message as reminder.

Blood Test and Anthropometric Measurements

Blood samples were collected from the volunteers at fasting state, before the beginning and at the end of the study. Fasting blood samples were collected from each subject into 10 ml vacutainer tube. The tubes were centrifuged 3000 rpm, and kept there for 15 minutes, from
each vacutainer tube plasma drawn to two eppendorfe 2ml tube each one has 1.5 ml of plasma and were stored in the freezer (−80°C) for further analysis of TG, TC, and glucose (Glu) in the blood using the “Cobas c111” biochemical analyzer (Roche, Ltd).

The anthropometric measurement were taken twice as will, at the beginning and at the end of one month of the study, to see if there is any changes in the BMI, in the in both groups. Body height and body weight were measured using a stadiometer and standard scales in the Laboratory of Human Nutrition, of the Department of Nutrition and Health.

Statistical analysis

SPSS version 21 used to analyze the differences within and between the groups. T-test used to see the effects on TG, TC, and Glu by the product given. P-values of < 0.05 were considered significant. Office Open XML (Microsoft Excel) used to calculate the percentage and mean different between pre and post blood test for both intervention and control group.

Results

The flow of the participants across the study and their dietary habits are given in Figure 1 and Table 1 respectively. Body weight and Body Mass Index are given in Table 2.

**Figure 1.** Flow chart of the recruitment
Table 1. Dietary intakes of the subjects in the beginning and at the end of the intervention periods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Margarine Pre mean value</th>
<th>Margarine Post mean value</th>
<th>P mean value</th>
<th>Butter Pre mean value</th>
<th>Butter Post mean value</th>
<th>P mean value</th>
<th>Both Pre mean value</th>
<th>Both Post mean value</th>
<th>P mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/day)</td>
<td>1771.5</td>
<td>1864.5</td>
<td>0.001</td>
<td>1419</td>
<td>1572.2</td>
<td>0.001</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Total fat (g/day)</td>
<td>57</td>
<td>50</td>
<td>NS</td>
<td>37</td>
<td>47</td>
<td>0.01</td>
<td>NS</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>SF (g/day)</td>
<td>8</td>
<td>9</td>
<td>NS</td>
<td>10</td>
<td>14</td>
<td>0.05</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Omega-3 (mg/day)</td>
<td>112</td>
<td>117</td>
<td>0.001</td>
<td>62</td>
<td>71</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Omega-6 (mg/day)</td>
<td>77</td>
<td>87</td>
<td>0.04</td>
<td>57</td>
<td>57</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>CHO (g/day)</td>
<td>244.3</td>
<td>234.3</td>
<td>NS</td>
<td>188.6</td>
<td>158.6</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>87.6</td>
<td>86.6</td>
<td>NS</td>
<td>65.4</td>
<td>55.4</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.8</td>
</tr>
</tbody>
</table>

24-hr recalls that were taken twice, before the first blood test and after 1 month before the second blood test. From the analysis of the 24-hr recalls it was observed that in the Margarine and Butter groups that there was significant increase in the Energy intake due to the intake of 1 ½ tablespoon of margarine or butter, without a statistical significant difference between the groups. Saturated fat and total fat intakes were significantly increased in the butter group and there was a significant increase in n-3 and n-6 fatty acid intakes in the margarine group. It is noteworthy that although carbohydrate and protein intakes were different between the 2 study groups, intakes did not change during the interventions.

Table 2. Body weight and Body mass index following the two interventions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Margarine Pre mean value</th>
<th>Margarine Post mean value</th>
<th>P mean value</th>
<th>Butter Pre mean value</th>
<th>Butter Post mean value</th>
<th>P mean value</th>
<th>Both Pre mean value</th>
<th>Both Post mean value</th>
<th>P mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>52</td>
<td>51</td>
<td>NS</td>
<td>54</td>
<td>53</td>
<td>0.5</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20</td>
<td>20</td>
<td>0.06</td>
<td>21</td>
<td>21</td>
<td>0.5</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The weight in the margarine group participant’s decreased, but it was not statistically significant (P= 0.1). On the other hand the butter group had a significant change in body weight compared to the margarine group (P= 0.03). For the BMI, there was a significant difference between the two interventions. However, from the values themselves, it is obvious
that these differences have no clinical significance.

Blood lipid and glucose levels following the 2 interventions are given in Table 3. In TG levels for the Margarine and Butter groups, there was a decrease in total TG, but it was not significant (margarine P=0.2, butter P=0.28) and there was no significant difference between the two groups either (P=0.4). Moreover, similar results were observed with the TC, where there was a decrease in both groups but it was not significant, (margarine P=0.3, butter P=0.1). For the glucose there was a decrease in both groups but not significant difference between the groups either.

**Table 3.** Blood lipid and glucose levels following the 2 interventions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Margarine</th>
<th></th>
<th></th>
<th>Butter</th>
<th></th>
<th></th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>P</td>
<td>Pre</td>
<td>Post</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>mean value</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>63.9</td>
<td>55.9</td>
<td>NS</td>
<td>66.7</td>
<td>53.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>161.8</td>
<td>145.7</td>
<td>NS</td>
<td>184.2</td>
<td>154.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>91.1</td>
<td>83.3</td>
<td>NS</td>
<td>91.1</td>
<td>85.5</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Discussion**

The main result in this small preliminary study is that a one-month intervention with either butter or margarine has no effect on plasma lipid levels.

In more detail, 24-hr recalls that were conducted twice, showed in the Margarine and Butter groups that there was a significant increase in the energy intake a significant increase in Saturated Fat and total fat on the butter group (SF P= 0.01, TF P=0.08) was observed. On the other hand, there was also a significant increase in n-3 and n-6 fatty acids in the margarine group (N-3 P=0.0, N-6 P=0.04). No differences in the intake of carbohydrate and protein were observed.
Following one-month consumption of the margarine, a decrease in the total TG, from 63.9 to 55.9 mmg/dL was observed. However, this difference was not statistically significant (P =0.2). Following the butter intervention, a decrease of total TG was observed from 66.7 to 53.3 mg/dL but this difference was not statistically significant either. When a comparison between the two interventions was carried out, a 12% decrease in fasting TG levels in the margarine group and a 22% decrease in fasting TG levels in the butter group were observed but no statistical difference was observed between the two interventions. Therefore, the margarine compared to butter didn't affect significantly total TG levels. It is of interest that although n-3 fatty acids rom fish have a profound hypotriglyceridemic effect, the present study does confirm other findings. However, it has been suggested that it needs at least 3 gr of n-3 fatty acids daily to produce a decrease in plasma TG in short term interventions (Von Schacky, 2006; Sadovsky & Kris-Etherton, 2006).

For the cholesterol, one-month consumption of the margarine, had as a result a decrease in the total TC, from 161.8 to 145.7 mg/dL but this difference was not statistically significant. Following the butter intervention, a decrease of total TC was observed from 184.2 to 154.2 mg/dL but this difference was not statistically significant either. When a comparison between the two interventions was carried out a 9% decrease in fasting serum TCs changed from baseline in margarine group and a 16% decrease in butter group were observed respectively but no statistical difference was observed between the two interventions. Therefore, the margarine compared to butter didn't affect significantly total cholesterol levels. These results were unexpected. It is widely known that saturated fatty acids, which are present in butter, increase plasma lipid levels and n-6 fatty acids, which are present in the margarine have a lowering effect. Therefore, a bigger study with more volunteers and possibly longer duration is needed.
The result for the Glucose level, for the margarine group showed a decrease in the total glucose, from 91.1 to 83.8 mg/dL. However, this difference was not statistically significant. Following the butter intervention, a decrease of total glucose was observed from 91.1 to 85.5 mg/dL, but this difference was not statistically significant either. When a comparison between the two interventions was carried out, a 7% decrease in fasting glucose changed from baseline in margarine group and a 6% decrease in butter group were observed respectively but no statistical difference was observed between the two interventions. Therefore, the margarine compared to butter didn't affect significantly total glucose levels.

**Conclusion**

The findings of the present study are that TC, TG, and glucose levels are affected similarly following a 1-month intervention of either margarine or butter in young female volunteers. However, the limited time of the intervention, but most importantly, the small sample size cannot generalize the results.

**References**


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