Omega 3 fatty acids-supplementation to competition athletes: impact on the biochemical indicators related to the lipid metabolism*

Priscila de Mattos Machado Andrade, Beatriz Gonçalves Ribeiro and Maria das Graças Tavares do Carmo

ABSTRACT

Purpose: To assess the effects of omega 3 fatty acid supplementation to swimmers on biochemical indicators. Methods: Male elite swimmers (n = 14) were assessed in a placebo-controlled randomized study over a 6-week (45-day) experimental period. The placebo group (GP) received mineral oil (n = 10). The supplementation was in capsules, with the supplementation group (GOP) receiving fish oil (n = 8) containing a total of 950 mg of eicosapentaenoic acid and 500 mg of docosapentaenoic acid. Immediately before starting the supplementation (T0), as well as 15 (T15), 30 (T30) and 45 days (T45) after that point, blood samples were collected and analyzed by gas chromatography for fatty acids composition, and by specific commercial kits for plasmatic lipoproteins. Results: The results showed that the diets of the swimmers were unbalanced regarding the macronutrient ingestion/body weight ratio (g/kg). The analysis of the consumption frequency questionnaire showed that (1) the swimmers have not regularly ingested omega 3 dietary sources and (2) the fish consumption was below once a week for 85% of the sample. The plasmatic fatty acids profile presented an increase in omega 3 polyunsaturated fatty acids (p < 0.05) and decrease in arachidonic fatty acid in the supplemented group (p < 0.05). The fish oil supplementation led to a hypcholesterolemic effect, with a decrease in VLDL, LDL and total cholesterol blood levels. The HDL levels presented no significant differences between the groups in any moment of the study (p > 0.05). Conclusion: N-3 fatty acids supplementation to swimmers alters the biochemical indicators of the lipid metabolism, with an influence in the decrease of the cholesterol-rich plasmatic lipoproteins, so preventing cardiovascular diseases.

INTRODUCTION

In the latest years, massive interest has been raised by the scientific community about the Omega 3 polyunsaturated fatty acids, especially EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), found in fish and fish oils. The grounding of this interest, through the dietetic ingestion of EPA and DHA, comes from population and epidemiological studies which have shown that fish consumption is associated with decrease of the morbidity coefficients by the cardiovascular diseases[1]. The Omega 3 polyunsaturated fatty acids (AGPI N-3) need to be in the human body in an adequate proportion in order to present these benefits. The dietetic ratio of AGPI N-6/N-3 should be 3:1 to 5:1[2]. The western diets have high amount of AGPI n-6, establishing the ratio between of 10 to 25:1[3-4]. Moreover, currently higher consumption of AGPI N-3 is recommended due to the low ingestion of these fatty acids by the population. Actually, a decrease in the fish consumption, which is the main source of AGPI N-3, and higher production of animal food such as hen and captivity fish, rich in AGPI N-6, are observed[2]. In short, the modern agriculture promoted in the latest years reduction in the AGPI N-3 amount in several food items such as eggs, vegetables and meats[2].

Keywords: Swimmers. Fish oil supplementation. Lipoproteins.

METHODOLOGY

Subjects – Fourteen male swimmers were volunteers for this study. The athletes’ selection was conducted in a swimming club of Rio de Janeiro, RJ through a personal interview. As inclusion criterion, a minimum of two year-regular training was required. Moreover, the swimmers should not be smokers, drink alcohol, use anabolic steroids or similar medication and not have metabolic diseases history either. All volunteers were informed and signed clarified consent form according to the Ethics Commission in Research of the Clementino Fraga Filho University Hospital of the Federal University of Rio de Janeiro (no. 098/03 from 04.09.2003).

Analysis of the dietary ingestion – The three-day food register was applied for the pre-supplementation quantitative analysis. The qualitative analysis of the Omega 3 fatty acids was evaluated through the selective consumption frequency questionnaire for dietary sources of Omega 3 fatty acids. The NUTWIN Program of Nutrition Aid, version 1.5/2002 was applied for the energy, carbohydrates, lipids and proteins analysis. The routine diet, presented in the results section (table 1), was adjusted so that the athletes could ingest an energetic value of 50 kcal per kg of body weight and of proteins, carbohydrates and lipids, respectively 1.8 g, 8 g and 1.2 g in grams per kg of body weight based on recommendations of the Brazilian Society of Sports Medicine[5]. The athletes were instructed to ingest fish oil, totaling 2.5 g per day of oil, containing 1.8 g of Omega 3 fatty acids (n = 10), or placebo (mineral oil) (n = 10). The supplementation was in capsules, with the athletes ingesting 5 units of 500 mg per day during breakfast.
Anthropometrical evaluation - The anthropometrical measures were always taken in the morning before the training session by a trained professional. All the measures were taken with the athletes wearing the least clothes and being barefoot. The measures were taken on the right side of the body. The thickness of the skin folds were evaluated (chest, medium underarm, triceps, biceps, sub scapular, supra-iliac, abdominal, thigh and calf) using LANGE skin-fold calipers. The thickness of the skin folds were evaluated using the method by Jackson and Pollock(9).

Study outline - Such study was randomized and placebo-controlled. The athletes were divided in two groups by random raffle: fish oil group (n = 10, 21,17 ± 2 years of age; 76,78 ± 1,89 kg) and placebo group (n = 10, 20,5 ± 1 year of age; 77,13 ± 0,86 kg). The groups received the supplementation for a period of 45 days, and blood samples were collected immediately before (T0), at fifteen (T15), at thirty (T30) and at forty-five (T45) days of supplementation.

Physical activity protocol - The athletes trained six days per week in two periods; morning and afternoon, an average of 5 hours per day. The training consisted of water circuits which totalize an average of 50 km/week. Besides that, the swimmers performed at alternated days, at least 1 hour of muscular resistance training.

Samples collection - The blood samples were taken in the morning, at the club, with the aid of a skilled professional, through punct of the antecubital vein with sterile needles and syringes. After the serum or plasma separation, post previous 12-hour fasting, this material was taken to the Nutritional Biochemistry Laboratory of UFRJ for later analysis of the plasmatic fatty acids and lipoproteins.

Biochemical Analyses - The plasmatic concentrations of TC (total cholesterol), HDL and TG were determined through enzymatic kits provided by Katal Biotecnológica Ind. Com. Ltda., Belo Horizonte, M/G/Brazil. The HDL-C measuring in the serum was determined through precipitation technique with phosphotungstic acid and magnesium chloride. The LDL-C concentration was calculated using the equation by Friedewald et al.(10). The plasmatic fatty acids profile was determined by gas chromatography. The lipidic extraction, saponification and plasmatic methyl fatty acids were collected according to the method by Lepage and Roy(11). The methyl esters were quantified through gas chromatography using Perkin Elmer auto system XL chromatographer skilled with ionized flame detector and software Turbochrom (Perkin Elmer, USA). The methyl fatty acids were sorted with capillary column (SP-2560 biscyanopropyl-polysiloxane, 100 m x 0,25 millimeters of diameter and 0,20 micron of film thickness, Supelco, Bellefonte, PA). The running temperature was set to begin at 175°C for 8 minutes with increase of 1°C/minute up to 195°C; from that time on, with increase of 3°C until reaching 240°C, keeping this temperature for 5 minutes. The total running time was 58 minutes. The injector and detector temperatures were respectively, 250°C and 270°C. The hydrogen was used as sliding gas in 28 Psi pressure. The split ratio was 1:40. The esters were identified through comparison with its retention time with known patterns (Sigma, Supelco and Nuchek).

The results were expressed over the total fatty acids percentage.

Statistical analysis - The times results (T0, T15, T30, T45) are presented in the figures in average index ± standard deviation. The study of the consecutive measurements of the considered variables was performed through the analysis of repeated measures by the Friedman' test for non-parametric data. The comparison among groups was through Student “t” test. All the statistical conclusions were performed at the 5% significance index. The tests were performed through the SPSS 11.0 statistical program.

RESULTS

Dietetic profile - Table 1 presents the usual dietetic profile of the swimmers. The results demonstrated an imbalance in the athletes’ diet considering the g/kg of body weight ingestion of the macronutrients.

The analysis of the consumption frequency questionnaire showed that the athletes did not regularly ingest Omega 3 dietetic sources and that the fish consumption in 85% of the sample was below or equal to once a week (figure 1).

Anthropometrical profile - The anthropometrical features of the recruited athletes in this study are found in table 2. The results are presented as average ± standard error. The average age was 21,17 ± 2 years. The data statistical analysis indicated lower values with statistical significance (P = 0,042) for the sum of the skin folds after the supplementation with fish oil period. The remaining analyzed parameters did not present any significant difference.

Biochemical parameters - For the biochemical data analysis, sample “n” of 6 subjects from the supplemented group and 8 from the placebo group was used, considering that 6 individuals (4 from the supplemented group and 2 from the placebo group) did not show up in the last blood collections (30 and 45 days of the study).

Plasmatic fatty acids concentration - The plasmatic fatty acids profile is described in table 3, being the indices presented as average ± average standard error (ASE). The plasmatic analysis of the saturated fatty acids, for both groups, initially shows decrease in the percentage indices followed by increase until the end of the study, being this oscillation statistically different only for the fish oil group (P = 0,047). In the supplemented group the percentage...
Palmitic (C16:0) 23.57 ± 1.19 25.21 ± 0.85 26.94 ± 0.53 24.92 ± 0.83 25.77 ± 0.82 28.24 ± 0.32** 25.81 ± 0.58 25.94 ± 1.09 0.06 ns 0 0.041 s
DHA (C22:6) 01.76 ± 0.19 02.70 ± 0.45 01.29 ± 0.32 **..3.84 ± 0.67** 02.99 ± 0.40 2.55 ± 0.38 00 0 1.87 ± 0.18 004.07 ± 0.72** 0.092 ns 0.01 s 00
Linolenic (C18:3) 0,217 ± 0.3 00 0,24 ± 0.02 00,39 ± 0.07 0,597 ± 0.11 00,48 ± 0.07 0,17 ± 0.04** 00,34 ± 0.07 0,133 ns 0,018 s 0
N 3
Saturated N = 6 N = 8 N = 6 N = 8 N = 6 N = 8 N = 6 N = 8
Linoleic (C18:2) 25.31 ± 1.88 24.03 ± 1.44 29.32 ± 1.59 023.05 ± 2.03* 29.85 ± 1.15 23.95 ± 1.28** 0 23.26 ± 1.40 22.56 ± 1.42 0,013 s 0,256 ns
Arachidonic (C20:4) 4.69 ± 0.63 4.04 ± 0.41 5.00 ± 0.36 4.102 ± 0.16 5.108 ± 0.98 4.21 ± 0.76 0 5.108 ± 0.98 4.21 ± 0.76 0,048 s 0,008 s
Total 30.14 ± 2.47 28.27 ± 1.89 35.59 ± 2.15 27.95 ± 2.46 35.40 ± 1.79 29.1 ± 1.48 29.92 ± 1.79 27.96 ± 2.07 0,041 s 0,510 ns
N 3
Linoleic (C18:3) 0,217 ± 0,3 0,24 ± 0,02 0,39 ± 0,07 0,597 ± 0,11 0,48 ± 0,07 0,17 ± 0,04** 0,34 ± 0,07 0,48 ± 0,12 0,133 ns 0,018 s
EPA (C20:5) 0,47 ± 0,17 0,50 ± 0,19 0,26 ± 0,02 0,19 ± 0,27 0,27 ± 0,07 0,14 ± 0,29** 0,23 ± 0,04 0,14 ± 0,51 0,362 ns 0,002 s
DHA (C22:6) 1,76 ± 0,19 2,70 ± 0,45 1,29 ± 0,32 3,84 ± 0,67** 1,97 ± 0,18 4,07 ± 0,72** 0,992 ns 0,01 s
Total 3,17 ± 0,42 4,39 ± 0,55 2,39 ± 0,41 5,69 ± 0,90 4,03 ± 0,54 3,8 ± 0,53 2,81 ± 0,296 6,41 ± 0,94 0,178 ns 0,092 ns
(18:3 N-3) fatty acid (linolenic acid) were steady in the placebo group throughout the study, while the arachidonic percentage presented significant lower indices in the fish oil group at days 30 and 45.
Significant increase of EPA N-3 (p = 0.002) and DHA (p = 0.01) polyunsaturated fatty acids is observed throughout the study in the group supplemented with fish oil. The percentage indices of the 18:3 N-3 fatty acid (linolenic acid) were steady in the placebo group throughout the study, while in the fish oil group the indices showed oscillation (initially increasing at day 15 followed by decrease at day 30 and increasing again at day 45, with statistically significant difference in the analysis as time passes by (p = 0.018).
Comparatively with the placebo group, the EPA and DHA percentage indices were higher in the groups supplemented with fish oil throughout the entire study, presenting significant difference at day 30 for the EPA (p = 0.0009) and at days 15 and 45 for the DHA, respectively, p = 0.0093 and 0.0019.
Concerning the AGPI N-6/N-3 plasmatic relation, it is observed in table 3 that the group supplemented with fish oil presented this relation comparatively lower than the placebo group; however, with statistic significance only at day 30 of the study (P < 0.05).
Lipoprotein profile – The plasmatic lipoprotein profile is described in table 4. Concerning the HDL plasmatic concentrations, no significant difference is observed, neither as time passes by, nor due to fish oil supplementation. The plasmatic indices found for the LDL showed tendency to decrease as time passes by (p = 0.06) for the supplemented group, which did not occur with the control group. Individually comparing the time between groups, the difference reaches significance index with values of p < 0.01 (p = 0.0031) at day 45 between the placebo and supplemented groups.
The Omega 3 polyunsaturated fatty acids (AGPI N-3) have been calling attention of countless researchers for their role in stimulating the lipidic metabolism and lipoproteins turnover[12]. Moreover, higher ingestion of AGPI N-3 follows an increase of these fatty acids concentration in the plasma, which, may be important to the immunological dynamics of the individual[13] and the protection against cardiovascular events[14].

Within this context, our aim in this work was to investigate the action of these fatty acids in biochemical indicators in swimmers, associated with the lipidic metabolism, using as nutritional intervention model the supplementation of Omega 3 fatty acids with fish oil capsules administered in the 45 days prior to the competition day.

After the supplementation with fish oil capsules, a decrease in the plasmatic concentrations of eicosapentaenoic N-3 (EPA) and docosapentaenoic (DHA) fatty acids was observed Conversely, reduction in the n-6 polyunsaturated fatty acids concentration is observed, specially of arachidonic acid (AA) and linoleic acid. Thus, it shows that the supplementation model proposed in the study reflected the dietetic ingestion, being able to derive compatible Omega 3 fatty acids indices in the blood in order to verify physiological and biochemical alterations in the body.

Similarly, Foulon et al.[15] also showed, after N-3 supplementation (1,8 g/day) in healthy individuals for three weeks, decrease in the plasmatic indices of n-6 polyunsaturated fatty acids and increase in the n-3 plasmatic concentrations. Nonetheless, no significant alterations in the plasmatic concentrations of Total Cholesterol, LDL and TG were observed; contrary to our results, which showed a decrease in these indicators. These differences could be partly explained by the supplementation time adopted in both studies, one that in our study supplementation was longer (6 weeks).

In the present study, the plasmatic saturated AG percentage increased in the group supplemented with fish oil as time passes by (table 3). When the two groups were compared, it is observed that the increase was significant in the supplemented group (p = 0.05; p = 0.047) in relation to the placebo one. Opposite behavior was found for the monounsaturated fatty acids whose indices were significantly lower in the fish oil group as time passes by, as well as in relation to the placebo at day 30. These results show that, in general, exercises of high intensity, the monounsaturated fatty acids are preferably picked by the tissues, especially the muscular, in order to restore their intramuscular TG stocks (IMTG) as well as to provide energy. Some researchers suggest that the training does not increase the plasma derived-AO oxidation, but probably increases the IMTG stock[16]. Another possible explanation for these alterations observed in the fish oil group is derived from the lower desaturation of the stearic (C18:0 n-9) and palmitic (C16:0 n-7) fatty acids resulting in the decrease of the oleic synthesis (C18:1 n-9) and palmitoleic (C16:1 n-7) synthesis respectively, and because of that, lower proportions of these fatty acids in the plasma and increased indices of their precursors.

The desaturation and elongating enzymes may act not only on the series of N-3 and N-6 polyunsaturated fatty acids, but also on the N-9 and N-7 fatty acids, actually with competition between the substrates. Besides that, the desaturation and elongating velocities differ between the series, decreasing in the n-3 > n-6 > n-9 > n-7 order. Thus, higher concentrations of N-3 fatty acids in the diet may inhibit the desaturation of other series of fatty acids such as n-9 and n-7[17].

The TG alone is not a structural component of the atherosclerotic lesion; however, high concentrations of plasma TG may exhibit an adverse effect due to its promotion of atherogenic remaining kilomcrons[18]. Moreover, high concentrations of TG may induce the increase of the amount of cholesterol esters and cholesterol esters in the HDL by the action of the CETP enzyme (cholesterol ester transfer protein), interfering in the beneficial effect that the HDL would have in the cholesterol metabolism[19]. Consequently, therapies that improve the lipoprotein metabolism would decrease the occurrence of dyslipemias which are associated with the risk for coronary arterial disease.

The results of the present study demonstrated that the Omega 3 fatty acids supplementation is an important indicator of the lipoproteins metabolism, supporting the hypothesis that the AGPI N-3 consumption would favorably influence the entire dynamics of the lipoproteins in the body in competitive athletes. Although the HDL-C concentrations in the serum did not reach statistical significance, probably due to the concise sample size, when the LDL:HDL and the CT:HDL-C relations are evaluated though, no significant decrease was observed (table 4).

Studies with humans show that the GPI N-3 causes consistent hypotriglyceridemic effect. In a review by Harris[19], it was showed that 72 controlled studies by placebo were supplemented from 1 to 7 daily grams of EPA and DHA, for at least 2 weeks and the TG plasmatic concentrations were usually decreased from 25 to 30%. The supplementation duration is another relevant factor, since low doses of AGPI N-3 supplemented for an extensive period of time would have a hypotriglyceridemic effect similar to supplementations for shorter periods and with high doses. The supplementation of 1 g of AGPI N-3 for 12 weeks significantly decreased the fasting TG concentrations in 21%[18]. The importance of the supplementation duration is confirmed by the results of other studies.

### TABLE 4

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Before (zero)</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Fish oil</td>
<td>Placebo</td>
<td>Fish oil</td>
</tr>
<tr>
<td>mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>46,94 ± 7,14</td>
<td>42,49 ± 4,62</td>
<td>43,08 ± 3,73</td>
<td>38,33 ± 2,68</td>
</tr>
<tr>
<td>VLDL</td>
<td>17,66 ± 1,77</td>
<td>17,78 ± 1,60</td>
<td>20,52 ± 2,71</td>
<td>17,22 ± 1,86</td>
</tr>
<tr>
<td>LDL</td>
<td>117,5 ± 16,6</td>
<td>111,9 ± 13,7</td>
<td>95,29 ± 10,62</td>
<td>78,88 ± 5,4</td>
</tr>
<tr>
<td>CHOL</td>
<td>182,1 ± 15,5</td>
<td>172,2 ± 6,37</td>
<td>158,9 ± 11,66</td>
<td>134,4 ± 4,34*</td>
</tr>
<tr>
<td>TG</td>
<td>88,29 ± 8,04</td>
<td>88,92 ± 8,02</td>
<td>102,59 ± 13,5</td>
<td>86,08 ± 9,3</td>
</tr>
<tr>
<td>CHOL/HDL</td>
<td>4,32 ± 0,64</td>
<td>4,27 ± 0,48</td>
<td>3,73 ± 0,22</td>
<td>3,65 ± 0,29</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2,90 ± 0,56</td>
<td>2,81 ± 0,41</td>
<td>2,23 ± 0,22</td>
<td>2,16 ± 0,24</td>
</tr>
</tbody>
</table>

* P < 0,05; ** P < 0,01; *** P < 0,001; Difference between the placebo and supplement groups at the same day, evaluated through the Student t-test. e Statistical analysis through the Friedman Test as time passes by in each study group. S: significant (P < 0,05) and NS non-significant (P > 0,05).
Schmidt et al. demonstrated that the amount of 4 g of AGPI N-3 for 9 months also decreased the TG indices in the plasma. The works conducted by Saynor and Gillott showed a continuous decrease when the supplementation occurs for periods longer than 4 years. In the present study it was verified that daily doses of 1,8 gr for 6 weeks are already efficient in promoting a decrease of the plasmatic triglycerides. In any event, studies relating the supplementation with Omega 3 fatty acids in swimmers and the lipoprotein profile are scarce, making any comparative analysis difficult between our results and the ones from the literature.

Evidence shows that N-3 fatty acids decrease the TG concentrations through the reduction in the endogenous production of VLDL. Studies conducted with animals show that the AGPI N-3 inhibit the TG synthesis (through the inhibition of the 1,2 diacylglyceroltransferase enzyme) and the synthesis and secretion of the VLDL. However, the precise biochemical mechanisms involved in this process still need further elucidation. Another possible explanation for this Omega 3 hypotriglyceridemic effect would be increase in the degradation of the kilomicrons derived from alteration in the metabolism of the lipase lipoprotein enzyme concerning the enzymatic concentration, activity or affinity.

The hypcholesterolemic effect of the N-3 fatty acids may be attributed to the decrease of the index of LDL formation, since the EPA as well as the DHA would be efficient in stimulating the activity of its receptor in the hepatic tissue, contributing even more for the reduction of the LDL concentrations. Moreover, this effect can be attributed to the suppression of the HMG-CoA enzyme activity reductase in the hepatic tissue.

REFERENCES


Finally, the fish oil supplementation rich in Omega 3 fatty acids is important for the maintenance of the suitable lipoproteins profile, besides promoting a stimulus for the oxidation of fatty acids. Although beneficial effects in the lipoproteins profile are pointed in the literature after supplementation with N-3, works with elite athletes in Brazil are still scarce. This study with swimmers demonstrates the supplementation of AGPI N-3 effect in increasing the plasmatic concentrations of EPA and DHA fatty acids. Thus, the described model for the fish oil capsules supplementation in competitive athletes would be an important therapy for the maintenance of the suitable lipoprotein profile in this population. Yet, further studies with other swimmers and other sports athletes are still needed in order to acknowledge the real potential of the Omega-3 fatty acids and confirm these observations. As future proposal, it would be interesting as well to investigate the dose-response effect attributed to dietetic supplementation of Omega 3 fatty acids in athletes.

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