Effects of DHA-Rich Fish Oil Supplementation on Lymphocyte Function Before and After a Marathon Race

Vinicius Coneglian Santos, Adriana Cristina Levada-Pires, Sâmia Rocha Alves, Tânia Cristina Pithon-Curi, Rui Curi, and Maria Fernanda Cury-Boaventura

Purpose: To investigate the effects of docosahexaenoic-(DHA)-rich fish oil (FO) supplementation on lymphocyte function before and after a marathon race. Methods: Twenty-one athletes participated in this study. Eight marathon runners were supplemented with 3 g of FO daily for 60 d (FO group), and 13 athletes were not supplemented (C group). The following measures of lymphocytes were taken before and after the marathon: cell proliferation, cytokine production (IL-2, IL-10, TNF-α, and IL-4), and signs of cell death. Results: In the C group, the marathon had no effect on lymphocyte proliferation, DNA fragmentation, or mitochondrial membrane polarization; however, the marathon increased phosphatidylserine externalization (by 2.5-fold), induced a loss of plasma membrane integrity (by 20%), and decreased IL-2, TNF-α, and IL-10 production (by 55%, 95%, and 50%, respectively). FO supplementation did not prevent lymphocyte death induced by the marathon, as indicated by cell viability, DNA fragmentation, and phosphatidylserine externalization. However, FO supplementation increased lymphocyte proliferation before and after the marathon, and before the race, FO supplementation decreased IL-2, TNF-α, and IL-10 production in concanavalin-A-stimulated lymphocytes (by 55%, 95%, and 58%, respectively) compared with cells from the C group. The production of cytokines was not altered before or after the race in the FO group. Conclusions: DHA-rich FO supplementation increased lymphocyte proliferation and prevented a decrease in cytokine production, but it did not prevent lymphocyte death induced by participation in the marathon. Overall, DHA-rich-FO supplementation has beneficial effects in preventing some of the changes in lymphocyte function induced by marathon participation.

Keywords: lipids, leukocyte, exercise, cytokine, apoptosis, immune function

To investigate lymphocyte function after intense exercise, several authors have evaluated lymphocyte proliferation (Gleeson & Bishop, 2005; Kwak, 2006; Levada-Pires et al., 2009) and lymphocyte death (Levada-Pires et al., 2009; Mars, Govender, Weston, Naicker, & Chuturgoon, 1998; Mooren, Lechtermann, & Volker, 2004). Previous studies have demonstrated an inhibition of lymphocyte proliferation capacity in the recovery period after intense exercise (Gleeson & Bishop, 2005; Henson et al., 2004; Kwak, 2006). Some authors have suggested that apoptosis contributes to the loss of lymphocytes in the blood after exercise, possibly via the cell surface’s death-receptor-signaling CD95, resulting in postexercise lymphopenia (Hsu et al., 2002; Levada-Pires et al., 2009; Mars et al., 1998; Mooren et al., 2004).

An increase in the plasma levels of the inflammatory mediators IL-1-ra, IL-6, IL-8, IL-10, and CRP has been observed after a marathon race (Henson et al., 2004; Nieman et al., 2001; Suzuki et al., 2003). In spite of the fact that cytokine production by mononuclear cells has been evaluated after intense physical exercise, this issue is not yet completely understood. Most studies were conducted in animals and/or in vitro with controversial data (Haahr et al., 1991; Hoffman-Goetz, Pervaiz, & Guan, 2009; Hoffman-Goetz, Spagnuolo, & Guan, 2008; Lin, Jan, & Chen, 1993; Radom-Aizik, Leu, Cooper, & Zaldívar, 2007; Sanchina, Hallam, Dias, & Perera, 2009; Starkie, Rolland, Angus, Anderson, & Febbraio, 2001). Moreover, information on cytokine production by lymphocytes after a marathon remains inconclusive.

Adequate levels of long-chain omega-3 polyunsaturated fatty acids (PUFAs), especially those found in fish or fish oils, such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), optimize health and prevent diseases (Kang, 2011). The Western diet is deficient in omega-3 but abundant in omega-6 PUFAs, resulting in a very high omega-6–to–omega-3 fatty-acid ratio. This unbalanced ratio is thought to be associated with a high prevalence of cardiovascular disease, cancer, diabetes, and neurodegenerative diseases. Consequently, government and scientific organizations now recommend an increased dietary intake of omega-3 PUFAs, with doses of 1.5–5 g/day (Kang, 2011).
Fish oil supplementation has beneficial effects on autoimmune and inflammatory disorders, such as psoriasis, rheumatoid arthritis, and diabetes, and strenuous exercise (Simopoulos, 2008). In fact, the EPA and DHA present in fish oil incite anti-inflammatory action and control immune-response regulation through changes in plasma membrane fluidity, fatty-acid composition, production of secondary messengers, activity of transcription factors, and gene expression (Martins de Lima et al., 2007). A previous study in our laboratory demonstrated that DHA-rich fish oil supplementation prevented the increases in IL-6, PGE2, and IL-1ra plasma concentrations that are induced by running in a marathon. In addition, neither the marathon nor the fish oil supplementation affected the plasma levels of TNF-α, IL-8, and IL-10 (Cury-Boaventura et al., unpublished data).

DHA produces less pronounced anti-inflammatory activity than does EPA. Unlike EPA, DHA enhances leukocyte function (Gorjão et al., 2006). Hill et al. (2007) observed no effects of DHA supplementation on cytokine production by T cells during regular physical exercise in patients with risk factors for cardiovascular disease. To our knowledge, there have been no studies in which the effect of DHA-rich fish oil on lymphocyte function was evaluated in athletes.

DHA supplementation may be beneficial to athletes because it has a moderate anti-inflammatory effect that minimizes the exacerbated inflammation induced by the effort of exercise and because it improves leukocyte function. We investigated the effects of chronic supplementation of DHA-rich fish oil on lymphocyte function (proliferation and cytokine production) and death (viability, DNA fragmentation, phosphatidylserine externalization, and mitochondrial membrane depolarization) before and after a marathon.

Materials and Methods

Subjects

Twenty-one male athletes with the following characteristics participated in this study: age 37 ± 2 years, body mass 59 ± 1 kg, height 163 ± 1 cm, body fat 9.0% ± 0.6%, body-mass index (BMI) 22 ± 0.3 kg/m², race duration 186 ± 28 min, training program 136 ± 15 km/week, and race speed 15 ± 0.3 km/hr. The study participants did not take any medications during the study. All human blood donors signed an informed-consent form. The study was approved by the ethical committee of the Cruzeiro do Sul University (protocol number 101/2007).

Experimental Design

Twenty-one male marathon athletes were randomly selected from national competitions. Fish oil supplementation was randomized, and researchers were blind to treatment during data collection and analysis. The fish oil group (8 marathon runners) were supplemented daily with 3 g of fish oil (Omega DHA, Naturalis, São Paulo, Brazil) containing 1.5 g DHA, 0.3 g EPA (5:1 DHA:EPA), and 18 mg α-tocopherol for 60 days. The subjects received exactly 180 capsules with the dates of the beginning and the end of the supplementation period and with instructions from the researchers to take the required dose every day. When asked by researchers, all subjects confirmed that the required doses of PUFA were taken every day for the 60 consecutive days before the race. Thirteen marathon runners were not supplemented (control group). Age, body mass, height, body fat, BMI, race duration, race speed, and training program did not differ between the groups (Table 1). Blood samples (25 ml) were collected from the antecubital vein 3–7 days before the race, when the subjects were at rest (12 hr without exercise), and immediately after the race. The training program of the runners was the same 3–7 days before the marathon competition. The blood volume (25 ml) was too small to have any adverse effect on training 3–7 days before the marathon race. The spread of the collection days/times between the fish oil and control groups was the same.

Dietary intake was monitored using the Food Frequency Questionnaire to identify omega-3 PUFA consumption, and three 24-hr food records were taken on separate days to determine dietary intake per day (analyzed using Dietwin Professional Software, Version 2008, Rio Grande do Sul, Brazil). The average daily intake of the marathon runners, as expressed by $M ± SEM$, was 2,441 ± 217 kcal, 360 ± 70 g of carbohydrate, and 89 ± 14 g of protein. The distribution of macronutrients in total energy intake was of 54% ± 3% carbohydrates, 19% ± 2% protein, and 25% ± 2% lipids. The frequency of fish consumption was less than one fish meal per week.

Limitations of the Study

Vegetable oil capsules were used as a placebo because they are similar in appearance and color to fish oil capsules but do not have the same smell. We chose not to use vegetable oils such as soybean or olive oil that are presented as $M ± SD$ of 8–13 samples. The $p$ value was obtained by nonparametric $t$ tests comparing the C and FO groups.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the Marathon Runners</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C group</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 ± 1.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Race duration (min)</td>
<td>181 ± 31</td>
</tr>
<tr>
<td>Training (km/week)</td>
<td>131 ± 42</td>
</tr>
<tr>
<td>Speed race (km/hr)</td>
<td>14.0 ± 2.3</td>
</tr>
</tbody>
</table>

rich in linoleic and oleic acids because these fatty acids have potent immunomodulatory effects (Martins de Lima et al., 2007) that could confound the study. The lack of a placebo group was a limitation to the study that has to be taken into consideration when interpreting the results.

**Lymphocyte Isolation**

Peripheral blood samples were collected in tubes containing ethylene-diamine tetra-acetic acid (EDTA; 1 mg/ml). Blood was diluted in phosphate-buffer saline (PBS; 1:1), added into a BD Falcon conical tube (San Diego, CA) containing Histopaque-1077, and centrifuged (Harrier 18/80, Sanyo, London, UK) for 30 min at 800 g at 4 °C. Mononuclear cells were collected during interphase and washed once with PBS. Mononuclear cells were isolated from the erythrocytes with a lysis buffer (150 mM NH4Cl, 10 mM NaHCO3, 0.1 mM EDTA, pH 7.4). The mononuclear cells remained in an RPMI 1640 medium for 30 min to allow monocytes to adhere to the plates. Next, the supernatant medium containing lymphocytes was collected to obtain a pure lymphocyte preparation (approximately 98%). The lymphocytes were counted in a Neubauer chamber under an optical microscope (Nikon, Melville, NY).

**Lymphocyte Proliferation Assay**

The lymphocyte proliferation assay was carried out to evaluate lymphocyte function. Lymphocytes (2 × 10⁶ cells/ml) were cultured for 48 hr in the presence of 50 μg/ml of concanavalin A (ConA). The plates were incubated in a humidified atmosphere at 5% CO₂ and 95% air at 37 °C. Afterward, [2-¹⁴C]-thymidine (1 μCi/ml) was added to the medium, and the cells were incubated further for a period of 18 hr. The radioactivity was analyzed using a liquid scintillation counter (Packard TRI-CARB 2100 TR counters, Downers Grove, IL; Dwyer & Johnson, 1981).

**Lymphocyte Cytokine Production**

Lymphocyte cytokine determination was also carried out to evaluate lymphocyte function. Lymphocytes (2 × 10⁶ cells/ml) were incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C in RPMI-1640 medium with fetal bovine serum (10%) and ConA (50 μg/ml), a T lymphocyte mitogen. After 18 hr, lymphocytes were centrifuged for 10 min at 1,200 rpm at 4 °C, and the supernatant was collected. Measurements of IL-2, IL-10, TNF-α, and IL-4 in the supernatant were taken by ELISA using the R&D Systems Quantikine High Sensitivity kit (R&D Systems, Minneapolis, MN). The level of cytokine release from lymphocytes is expressed in pg/ml (2 × 10⁶ cells/ml). The sensitivities were 7.81 pg/ml for IL-2 and TNF-α and 15.62 pg/ml for IL-4 and IL-10.

**Cell Viability Assay**

Lymphocytes (1 × 10⁶ cells/ml) were resuspended in 500 μl of PBS, and 50 μl of propidium iodide (PI, 20 μg/ml in PBS) were added. In cells that have lost membrane integrity, PI binds to DNA by intercalating between the bases with little or no sequence preference. The percentage of viable cells in each sample was determined using a FACSCalibur flow cytometer (Becton Dickinson, San Juan, CA). Fluorescence was measured using the FL2 channel (orange-red fluorescence = 585/42 nm). Ten thousand events were analyzed per experiment. Cells with PI fluorescence were then evaluated using Cell Quest software (Becton Dickinson, San Juan, Puerto Rico).

**DNA Fragmentation**

DNA fragmentation was determined by flow cytometry after DNA staining with PI according to the method described by Nicoletti, Migliorati, Pagliacci, Grignani, and Riccardi (1991) to characterize apoptotic cells. Cells were resuspended in a solution containing detergents to allow the prompt incorporation of the dye into the DNA. Briefly, cells were centrifuged at 1,000 g for 15 min at 4 °C. The pellet was gently resuspended in 300 μl of a hypotonic solution containing 50 μg/ml PI, 0.1% sodium citrate, and 0.1% Triton X-100. The cells were then incubated for 2 hr at room temperature. Fluorescence was determined by flow cytometry as described for cell viability.

**Phosphatidylserine Externalization**

Phosphatidylserine externalization was analyzed by flow cytometry after staining with Annexin V-FITC, according to the method described by Veronesi, Haanen, Steffen-Nakken, and Reutelingsperger (1995), to characterize apoptotic cells. Cells (1 × 10⁶) were washed twice with cold PBS and resuspended in 100 μl of binding buffer (10 mM Heps/NaOH, 140 mM NaCl, 2.5 mM CaCl₂); then 5 μl of fluorescein-conjugated annexin V (annexin V-FITC) were added. The cells were gently agitated and incubated for 15 min in the dark at room temperature (20–25 °C). Next, 10 μl of PI solution and 400 μl of binding buffer were added and the cells were analyzed by flow cytometry. Annexin V-FITC fluorescence was measured in the FL1 channel (green fluorescence, 530/30 nm), and PI was measured in the FL2 channel (orange-red fluorescence, 585/42 nm) of a flow cytometer, as described previously.

**Mitochondrial Membrane Potential**

The mitochondrial membrane potential evaluates apoptosis induced by intrinsic pathway. Cells were centrifuged at 1,000 g for 15 min at 4 °C, and the pellet was resuspended in 1,000 μl of PBS. Rhodamine 123 is a cell-permeable, cationic, fluorescent dye that is readily sequestered by active mitochondria without inducing toxic effects. Rhodamine 123 (5 μM) was added and the cells were then incubated for 15 min at 37 °C in the dark. Cells were washed twice with cold PBS and incubated for 30 min at 30 °C in the dark. Fluorescence was determined using the FL1 channel (green fluorescence, 530/30 nm) of a flow cytometer as described previously (Darzynkiewicz, Staiano-Coico, & Melamed, 1981).
Statistical Analysis

Data were analyzed using a two-way analysis of variance (ANOVA) and Tukey’s post hoc test. The statistical analyses of the marathon runners’ characteristics, such as age, body mass, height, body fat, BMI, race duration, training program, and race speed, were performed using nonparametric t tests. The results were considered statistically significant when \( p < .05 \). GraphPad Prism 5 software (Graph Pad Software, Inc., San Diego, CA) was used for statistical analysis.

Results

Lymphocyte Function

Lymphocyte proliferation activity increased in the fish oil group compared with the control group before (7,264 ± 282 and 3,805 ± 124 counts/min, respectively) and after the competition (4,348 ± 314 and 2,919 ± 317 counts/min, respectively; Figure 1). Lymphocyte proliferation activity decreased after the race in the fish oil group but remained above the values of the control group (Figure 1).

The marathon led to a decrease of IL-2, TNF-\( \alpha \), and IL-10 production in ConA-stimulated lymphocytes by 80%, 75%, and 50%, respectively (56 ± 13, 822 ± 179, and 566 ± 96 pg/ml, respectively) compared with the values before the race (342 ± 71, 3,230 ± 1,022, and 1,073 ± 130 pg/ml, respectively; Table 2). Fish oil supplementation induced a decrease in IL-2, TNF-\( \alpha \), and IL-10 production in stimulated lymphocytes by 55%, 95%, and 58%, respectively (151 ± 53, 121 ± 59, and 452 ± 192 pg/ml, respectively) compared with the values of the control group before the race (Table 2). IL-2, TNF-\( \alpha \), and IL-10 production were not altered after the race in the fish oil group. IL-4 production was detected before but not after the marathon race in both groups (Table 2).

Lymphocyte Death

The marathon race had no effect on cell DNA fragmentation (Figure 2[A]), but it induced an increase in the percentage of lymphocytes with phosphatidylserine externalization (by 2.5-fold; Figure 2[C]) and a decrease in the percentage of lymphocytes with loss of plasma membrane integrity in both the control and fish oil groups (95% ± 1% to 86% ± 3% and 95% ± 1% to 77% ± 2%, respectively; Figure 2[B]). Fish oil supplementation did not prevent marathon-induced lymphocyte death (Figures 2[A], 2[B], and 2[C]). The marathon also led to a slight increase in the lymphocyte mitochondrial membrane potential in both groups, but this effect was significant only in cells from the fish oil group (Figure 2[D]).

Discussion

The marathon led to increased lymphocyte phosphatidylserine externalization, a loss of plasma membrane integrity, and decreased IL-2, TNF-\( \alpha \), and IL-10 production. DHA-rich fish oil supplementation increased lymphocyte proliferation and decreased IL-2, TNF-\( \alpha \), and IL-10 production in ConA-stimulated lymphocytes before the race compared with the control group, indicating an improvement in lymphocyte function in the fish oil group.

Leukocyte function is modulated by \( \omega -3 \) PUFA through different pathways such as proliferation control, cytokine and anti-inflammatory mediator (eicosanoids, resolvins, protectins, and maresins) generation, adhesion molecule expression, and cell death (Akhtar Khan, 2010; Gorjão et al., 2009; Martins de Lima et al., 2007). Fish oils containing DHA-rich fish oil (FO) supplementation on lymphocyte proliferation activity in athletes before and after a marathon. Lymphocytes were plated and cultured for a period of 48 hr in the presence of 50 \( \mu \)g/ml concanavalin A. The values are presented as \( M ± SEM \) of 8–13 samples. *\( p < .05 \) and **\( p < .001 \) when comparing the control (C) group with the FO group. *\( p < .05 \) when comparing athletes before and after the race.

Figure 1 — Effect of DHA-rich fish oil (FO) supplementation on lymphocyte proliferation activity in athletes before and after a marathon. Lymphocytes were plated and cultured for a period of 48 hr in the presence of 50 \( \mu \)g/ml concanavalin A. The values are presented as \( M ± SEM \) of 8–13 samples. *\( p < .05 \) and **\( p < .001 \) when comparing the control (C) group with the FO group. *\( p < .05 \) when comparing athletes before and after the race.
Table 2  Cytokine Production by Lymphocytes Stimulated With Concanavalin A (50 μg/ml)

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Control group</th>
<th>Fish oil group</th>
<th>Control group</th>
<th>Fish oil group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>342 ± 71</td>
<td>151 ± 53#</td>
<td>56 ± 13***</td>
<td>25 ± 12</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3,230 ± 1,022</td>
<td>121 ± 59##</td>
<td>822 ± 179*</td>
<td>144 ± 62</td>
</tr>
<tr>
<td>IL-10</td>
<td>1,073 ± 130</td>
<td>452 ± 192#</td>
<td>566 ± 96*</td>
<td>391 ± 64</td>
</tr>
<tr>
<td>IL-4</td>
<td>12 ± 10</td>
<td>14 ± 4</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note. ND = not detected. Values are presented as M ± SEM of 8–13 samples. The production of cytokines by lymphocytes was measured in pg/ml of supernatant obtained from 2 × 10^6 cells/ml cultured lymphocytes.

*p < .05, **p < .01 when comparing athletes before and after the race. #p < .05, ##p < .01 when comparing the control and fish oil groups.

different compositions of EPA and DHA can exert divergent effects on immune responses. DHA promotes less pronounced anti-inflammatory activity than EPA. EPA-rich fish oil (2:1, EPA:DHA), which is the most widely available type commercially, suppresses leukocyte function, thus decreasing lymphocyte proliferation and immunity, whereas DHA-rich fish oil (5:1, DHA:EPA) stimulates several aspects of immune function, including lymphocyte proliferation, thus improving acquired immunity (Gorjão et al., 2009). DHA supplementation may be beneficial to athletes because it has a moderate anti-inflammatory effect that minimizes the exacerbated inflammation induced by exercise with no marked effect on leukocyte function. Thus, the selection of DHA- or EPA-rich fish oils must be carefully considered before being used to control inflammatory responses induced by exercise. DHA-rich fish oil supplementation has an anti-inflammatory effect and contributes to the improvement of lymphocyte function in marathon runners.

Immune function is maintained due to the balance between cell death and cell proliferation. Nieman et al. (1995) observed that the proliferative activity of lymphocytes did not differ between runners who had been training for marathon race events and sedentary controls. However, in this study we observed an impairment of lymphocyte proliferation during the periods before and after the competition in the control group compared with the fish oil supplementation group. In the fish oil group, we observed impairment in lymphocyte proliferation after the race compared with at rest, but this was an improvement compared with the control group.

Many studies have shown an inhibition of lymphocyte proliferation capacity in the recovery period after intense exercise, but not compared with nonathletes (Gleeson & Bishop, 2005; Henson et al., 2004; Kwak, 2006). Levada-Pires et al. (2009) demonstrated that lymphocyte proliferation is reduced in triathletes at rest, as well as after triathlon competitions, suggesting that it is altered by the exercise training itself, as indicated in our current study. Moreover, MacNeil, Hoffman-Goetz, Kendall, Houston, and Arumugam (1991) reported a reduction in lymphocyte proliferation capacity due to physical effort in the recovery period after an exercise session. A previous study from our laboratory demonstrated an improvement in ConA-stimulated proliferation of lymphocytes from healthy volunteers after 2 months of DHA-rich fish oil supplementation. In the current study, DHA-rich fish oil supplementation promoted an augmentation of the ConA-induced lymphocyte proliferative capacity in marathon runners before and after the competition. These results suggest that athletes supplemented with fish oil have better acquired immunity and less susceptibility to pathogen infections than nonsupplemented athletes. In spite of the marathon race’s decreasing lymphocyte proliferation in the fish oil group, this function remained better than the control group after the race. Andrade, Ribeiro, Bozza, Costa Rosa, and Tavares do Carmo (2007) also observed an increase of lymphocyte proliferation in elite swimmers after EPA-rich fish oil supplementation. In contrast, Kew et al. (2004) found that DHA supplementation decreases T-lymphocyte activation. It is noteworthy that Kew et al. employed higher doses of DHA (4.91 g/day) than the dose used in the current study (1.5 g/day), which may have affected the results of the experiments. In fact, a previous in vitro study demonstrated that treatment with DHA induces human lymphocyte proliferation at lower concentrations (<50 μM) and leads to its reduction at higher concentrations (>100 μM; Gorjão, Cury-Boaventura, de Lima, & Cury, 2007). The discrepancy between the studies may also be explained by the different approaches used by the authors: While Kew et al. evaluated CD69 expression, we assessed cell division in our study.

The release of IL-2 and TNF-α by lymphocytes is crucial to activating other immune cells of acquired immunity and preventing pathogen infections. The production of IL-10 plays a central role in limiting host immune responses to pathogens, thereby preventing damage to the host and the risk of developing many autoimmune diseases (Iyer & Cheng, 2012). Production of TNF-α, IL-2, and IL-10 was markedly decreased after the race, deregulating acquired immunity. In rats, the production of IL-2 in spleen lymphocytes stimulated by ConA was significantly lower in an exercise group than in a sedentary group (Lin et al., 1993). Haahr et al. (1991) found that concentric bicycle
Figure 2 — Effect of DHA-rich fish oil (FO) supplementation on (A) DNA fragmentation, (B) cell viability, (C) phosphatidylserine externalization, and (D) mitochondrial membrane potential in lymphocytes from athletes before and after a marathon. DNA fragmentation, cell viability, phosphatidylserine externalization, and mitochondrial membrane potential were determined using a FACSCalibur flow cytometer. Fluorescence was measured in FL3 (>670 nm) and FL2 (585/42 nm) channels for propidium iodide or the FL1 channel (530/30 nm) for Annexin V-FITC and rhodamine 123. A total of 10,000 events were evaluated per experiment. The values are presented as $M \pm SEM$ of 8–13 samples. *$p < .05$ when comparing athletes before and after the race.
exercise did not alter IL-2 production by lymphocytes in vitro. Moderate exercise (three times a week) also did not alter IL-2 production by monocytes in patients at risk for cardiovascular disease (Hill et al., 2007). The treatment of T cells (Jurkat) with a serum obtained from healthy volunteers after 30 min of exercise led to a reduction in IL-2 and TNF-α production, but it had no effect on TGF-β1 and IL1-α production compared with treatment with a serum obtained before exercise (Radom-Aizik et al., 2007). Hoffman-Goetz et al. (2009; 2008) also found a decrease in TNF-α expression in mouse intestinal lymphocytes immediately after treadmill exercise, and Senchina et al. (2009) observed a reduction in TNF-α and IL-1β but not in IL-10 production by mononuclear cells after strenuous exercise. In contrast, another study showed no change in TNF-α production and a decrease in IL-1α and IL-6 synthesis by monocytes after prolonged running (Starkie et al., 2001).

Low-dose fish oil supplementation (<750 mg of EPA and/or DHA) did not affect the production of IL-6, TNF-α, IL-1β, and IL-10 by mononuclear cells in healthy human subjects (Treble et al., 2003; Wallace, Miles, & Calder, 2003). Nevertheless, an inhibitory effect on the production of these cytokines may occur due to daily intake of high doses of EPA and DHA, even after a prolonged period of treatment at low doses (Yaquob & Calder, 1995). A high intake of DHA (>1,500 mg) has been shown to lead to decreased production of IFN-γ, IL-2, IL-10, and TNF-α by lymphocytes, monocytes, and macrophages in human volunteers (Endres, Meydani, & Dinarello, 1991; Verlengia et al., 2004a, 2004b; Yaquob & Calder, 1995). The supplementation of DHA-rich fish oil reduced IL-2, IL-0, and TNF-α generation by lymphocytes before the race compared with the control group, indicating an anti-inflammatory effect.

The high occurrence of lymphocyte apoptosis has been associated with postexercise immunosuppression (Mars et al., 1998), and high-intensity exercise and/or prolonged exercise increases the percentage of apoptotic lymphocytes (Hsu et al., 2002; Mars et al., 1998; Mooren, Blomming, Lechtermann, Lerch, & Volker, 2002; Mooren et al., 2004; Steensberg, Morrow, Toft, Bruunsgaard, & Pedersen, 2002). An exercise intensity threshold between 40% and 60% of VO$_{2}$max induced an increase in lymphocyte apoptosis (Navalta et al., 2009; Navalta, Mohamed, El-Baz, McFarlin, & Lyons, 2010; Navalta, Sedlock, & Park, 2007). Levada-Pires et al. (2009) have reported that the duration of high-intensity exercise is correlated with lymphocyte death in elite athletes based on different mechanisms. A long-duration triathlon competition did not induce lymphocyte apoptosis, but it led to an augmentation of the proportion of cells with membrane integrity loss, suggesting that these cells were dying by necrosis (Levada-Pires et al., 2009). Likewise, Peters, Van Eden, Tyler, Ramautar, and Chuturgoon (2006) and Mooren et al. (2004) did not find DNA damage or phosphatidylserine externalization in lymphocytes obtained from well-trained endurance athletes after either a 2.5-hr trial or a marathon competition, but those studies did not examine plasma membrane integrity. In our study, the marathon had no effect on lymphocyte DNA fragmentation, but it led to an increase in the proportion of lymphocytes with phosphatidylserine externalization, which is indicative of an increase in lymphocyte apoptosis.

Marfe et al. (2010) have shown that endurance efforts by amateur runners attenuate the extent of apoptosis and that HSP 70, BCL-2, and HSP32 play an antiapoptotic role in modulating caspase and DNA fragmentation, providing physiological protection against apoptosis. Fish oil supplementation suppressed the expression of proapoptotic genes such as Caspases 3, 7, and 2 and led to an increase in HSP 70 mRNA expression, protecting against apoptosis (Gorjão et al., 2006). However, EPA and DHA can also decrease MAPK and Akt pathways involved in IL-2 production and lymphocyte survival, indicating a proapoptotic effect (Gorjão, Hirabara, de Lima, Cury-Boaventura, & Curi, 2007). In this study, fish oil supplementation did not alter marathon-induced lymphocyte death. Previous studies have shown that intense exercise diminishes lymphocyte glutathione content and subsequently enhances oxidative-stress-induced apoptosis (Levada-Pires et al., 2009; Wang & Huang, 2005). Accumulation of reactive oxygen species readily modifies the activity of a new class of proteins called sirtuins. In particular, sirtuin-1 can modulate cellular stress response and survival through the regulation of p53, NF-kB-signaling, and FOXO transcription factors (Marfe et al., 2010) that may not be modulated by DHA and EPA. The mechanisms of lymphocyte death induced by exercise and the possible pro- or antiapoptotic effect of DHA and EPA have yet to be elucidated.

In conclusion, DHA-rich fish oil supplementation has immunomodulatory effects reducing pro- and anti-inflammatory cytokine production by lymphocytes, attenuating the exacerbation of immune stimulation and/or immune suppression. The balance in the production of pro- and anti-inflammatory cytokines may contribute to the repair of muscle injury induced by physical exercise. Although fish oil did not prevent lymphocyte apoptosis, it improved the responsiveness of the cells to ConA stimulation. These results suggest that athletes supplemented with fish oil have better acquired immunity and less susceptibility to pathogen infections than nonsupplemented athletes. Thus, DHA-rich fish oil supplementation is beneficial for lymphocyte function in marathon runners.

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