EFFECTS OF DIFFERENT PUFA SUPPLEMENTATION ON INFLAMMATORY RESPONSE MARKERS IN YOUNG SOCCER PLAYERS

Kristina Radoman[1], Vesna Vucic[2], Aleksandra Arsic[2], Dejan Cubrilo[3], Nevena Jeremic[4], Jovana Jeremic[4], Vladimir Jakovljevic[5]
[1]College of Health Studies, Podgorica
[2]Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Serbia
[3]Faculty of Sport and Tourism, TIMS, EDUCONS University, Novi Sad
[4]Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[5]Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

EVALUACIJA EFEKATA RAZLICITIH REZIMA ISHRANE NA INFLAMATORNI ODGOVOR KOD MLADIH FUDBALERA

Kristina Radoman[1], Vesna Vučić[2], Aleksandra Arsić[2], Dejan Čubrilo[3], Nevena Jeremić[4], Jovana Jeremić[4], Vladimir Jakovljević[5]
[1] Visa medicinska skola, Podgorica, Crna Gora
[2] Centar izuzetnih vrednosti za istraživanja u oblasti ishrane i metabolizma, Institut za medicinska istraživanja, Univerzitet u Beogradu, Beograd, Srbija
[3] Fakultet za sport i turizam, Tims, Educons Univerzitet, Novi Sad
[4] Katedra za farmaciju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[5] Katedra za fiziologiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija

Received / Primljen: 29.09.2015. Accepted / Prihvaćen: 17.10.2015.

ABSTRACT

Considering the limited knowledge regarding the effects of n-3 and n-6 PUFAs on the inflammatory response during physical activity, we aimed to evaluate the level of pro- and anti-inflammatory cytokines in young soccer players before and after a maximal physical load test at the beginning and end of a two-month training process. The study included 75 young footballers from Football School "Kragujevac," who were followed during the two-month training programme. The subjects were divided into the following groups: 1) control group (consumed a standard diet); 2) group that consumed fish oil (2500 mg of n-3 PUFAs per day); 3) group that consumed nutritional sunflower oil (2500 mg of n-6 PUFAs daily). The maximal progressive exercise test was performed using a treadmill belt. Venous blood samples were drawn 4 times for the determination of cytokine levels (IL-6 and TNF-α): before and after the exercise load test before the two-month training programme (initial measurement) and immediately before and after the exercise load test after the two-month training programme (control measurement). Supplementation with fish oil (n-3) has been associated with reduced levels of IL-6 compared with the initial values. After an acute bout of exercise, n-3 PUFAs did not show a significant effect on inflammatory marker dynamics, whereas n-6 PUFAs slightly stimulated the production of TNF-α.

Key words: *polyunsaturated fatty acids, cytokines, inflammation, young soccer players.*

SAŽETAK

S` obzirom na ograničeno znanje o efektima polinezasićenih masnih kiselina (n-3 i n-6 PUFA-Polyunsaturated Fatty Acids), cilj ovog istraživanja bio je da se procene vrednosti pro- i anti- inflamatornih medijatora u miru i nakon maksimalnog testa opterećenja pre i nakon dvomesečnog trenažnog programa. U studiji je učestvovalo 75 mladih fudbalera omladinske fudbalske škole "Kragujevac", starosti od 18 - 19 godina, koji su bili praćeni tokom dva meseca. Oni su bili podeljeni u tri grupe: 1. kontrolna grupa (standardna ishrana), 2. grupa koja je konzumirala riblje ulje (2500mg n-3 PUFA dnevno), 3. grupa koja je konzumirala suncokretovo ulje (2500mg n-6 PUFA dnevno). Venski uzorci krvi za određivanje nivoa citokina (IL-6, TNF- α) uzimani su 4 puta: u miru i nakon testa opterećenja, pre i posle dvomesečnog trenažnog programa. Svakodnevna suplementacija ribljim uljem povezana je sa smanjenim vrednostima IL-6 u odnosu na njegove početne vrednosti. Upotreba različitih vrsta polinezasiđenih masnih kiselina nije bitnije uticala na proizvodnju citokina, neposredno nakon akutnog nastupa sportske aktivnosti (testa opterećenja).

Ključne reči: *polinezasićene masne kiseline, citokini, upala, mladi fudbaleri*

ABBREVIATIONS

ALA - α-linolenic acid
EPA - eicosapentaenoic acid
DHA - docosahexaenoic acid
IL-6 - interleukin-6
LA - linoleic acid
LTE4 - leukotriene E4
PGE2 - prostaglandin E2
PUFA - polyunsaturated fatty acid
TNF-α - tumour necrosis factor alpha
TXB2- thromboxane B2

DE GRUYTER OPEN

UDK: 613.2:796.322 / Ser J Exp Clin Res 2015; 16 (4): 305-311
DOI: 10.1515/SJECR-2015-0055

Corresponding author:
Professor Vladimir Lj. Jakovljevic, MD, PhD,
Phone: +381 34 342944, Fax: +381 34 306800/ext 112, E-mail: drvladakgbg@yahoo.com

INTRODUCTION

Although physical activity has numerous beneficial effects on health and especially cardiovascular diseases (1, 2), in recent years, there has been growing concern about the molecular basis of its deleterious impact. Namely, intense and prolonged exercise has been associated with the onset of a condition known as "calm" inflammation, primarily of muscle tissue (3, 4). This finding is based on strenuous exercise provoking the inflammation of muscle fibres, leading to damage, fatigue and a reduction in muscle performance (3-5).

Most of the inflammatory reactions are mediated by inflammatory cytokines. Therefore, to assess muscle inflammation, the greatest attention has been paid to particularly two cytokines: interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) (6). These cytokines produce mainly inflammatory cells, such as T lymphocytes and monocytes (6). The physiological roles of these cytokines are numerous. It has been shown that IL-6 is one of the most potent stimulators of prostaglandin E2 (PGE2) production, which is directly involved in the progress of inflammatory reactions (6). However, according to the latest findings, IL-6, depending on the situation, can act as both a pro- or as an anti-inflammatory cytokine (7). By contrast, TNF-α, possesses clear pro-inflammatory potential, promoting an inflammatory response that includes muscle damage (7).

Given that polyunsaturated fatty acids (PUFAs) are essential for the overall health of athletes. In the past decade, studies have investigated the effect of PUFAs on the degree of inflammation and muscle damage. There are two classes of polyunsaturated fatty acids (PUFAs), n-6 (omega 6 fatty acids) and n-3 (omega 3 fatty acids) (8). The main representative of the group of omega-6 PUFAs is linoleic acid (LA), and that of the group of omega-3 PUFAs is α-linolenic acid (ALA) (9). Although it is known that these two types of fatty acids have opposite effects in the body (10), data regarding the specific role of n-3 or n-6 inflammatory processes are poor and controversial.

There is evidence that fish oil, which contains a high concentration of EPA eicosapentaenoic acid (EPA, n-3) and docosahexaenoic acid (DHA, n-3), can be useful in preventing or mitigating the symptoms of muscle inflammation that often occur with intense physical activity (11). The literature data show that supplementation with n-3 fatty acids indicate arterial vasodilatation, whereas n-6 fatty acids do not lead to these effects (12). Investigations on animal models have shown that fish oil may reduce the initial production of cytokines after physical activities (13). Research in the human population have confirmed this hypothesis, pointing out that supplementation with n-3 fats leads to a reduced production of IL-6 and TNF-α in healthy volunteers (14).

Considering the limited knowledge regarding the effects of n-3 and n-6 PUFAs on the inflammatory response during physical activity, the aim of this study was to evaluate the level of pro- and anti-inflammatory cytokines in young athletes before and after a maximal physical load test at the beginning and end of a two-month training process.

MATERIALS AND METHODS

Subjects

The study included 75 young soccer players from Football School "Kragujevac". All of the players were male, aged 18-19 years, well trained with a minimum sports experience of 5 years and 12 hours of training a week.

Study design

All of the participants were followed during the two-month training programme. Before the start of the study, all the players were subjected to a standard sports medical examination. The study involved only subjects who were absolutely healthy without a history of disease, any special eating habits or the use of any medications and supplements. Participants were not included in the study if routine laboratory analyses showed that they have indications of acute inflammatory processes (increased white cell counts, sedimentation, and C-reactive protein levels). The study was approved by the ethics committee of the Faculty of Medical Sciences, University of Kragujevac.

The subjects were divided into the following groups:

1. a group that, during the two-month training program, consumed a standard diet—the control group (n = 25),
2. a group that, during a two-month training program, consumed fish oil (2500 mg of n-3 PUFAs per day)—the n-3 PUFA group (n = 25),
3. a group that, during the two-month training program, consumed nutrient-rich, cold-pressed sunflower oil (2500 mg of n-6 PUFA daily)—the n-6 PUFA group (n = 25).

All of the subjects were informed about the nature, purpose, duration, expected effects and risks of research, and they and their parents provided written consent for participation in the study.

Protocol

Testing was carried out during the regular sports and medical health examination. Each participant was subjected to the measurement of body composition and the progressive, continuous maximum physical load test. The measurement of body composition was performed using an apparatus for bioelectrical impedance analysis, the Tanita BC-418 system (Tokyo, Japan), whose validity was previously confirmed (15). The maximal progressive exercise test was performed on a treadmill belt (treadmill ECG9230K; POWERJOG, Japan). The athletes were familiarized to the testing procedure. The test was performed according to the protocol by Ellestad (16) and lasted until

voluntary exhaustion. The athletes stated their subjective feeling of exhaustion using a Borg's CR10 exhaustion scale of at least 8 (17). During the test, athletes breathed through a two-way mouthpiece (Hans Rudolph, Kansas City, USA). Maximal oxygen consumption (VO_2max) and the heart rate were monitored by an automated cardiopulmonary exercise system (FitMate Pro, COSMED, Italy) whose validity, reliability, and accuracy were previously reported (18). We considered that VO_2max was reached when the oxygen consumption reached its plateau (when an increase in the workload cannot induce an increase in oxygen consumption) (18).

At the same time, venous blood samples were drawn at rest and immediately after the load to analyse the parameters of inflammation such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). At the end of the two-month training programme and diet supplementation, all of the participants were subjected to an identical sports medical examination as the initial examination. The study was conducted during the competition mesocycle (Table 1).

Biochemical assays

Venous blood samples, for the analysis of biochemical parameters, were drawn before and immediately after the test load. The venous blood samples (9 ml) were drawn 4 times: before and after the exercise load test before the two-month training programme (initial measurement) and immediately before and after the exercise load test after the two-month training programme (control measurement). Blood samples were drawn from antecubital veins into Vacutainer test tubes containing sodium citrate anticoagulant. The blood samples were processed and stored immediately. Blood was centrifuged to separate plasma and red blood cells (RBCs).

Measurement of the serum levels of cytokines (TNF-α and IL-6)

The cytokine concentrations in the serum were determined using ELISA assays specific for human cytokines (Human IL-6 DUOSET ELISA Development kit, R & D Systems, USA; Human TNF-α/TNFSF1A DUOSET ELISA Development kit, R & D Systems, USA) (19, 20).

Table 1: Example of a microcycle when the match is played on Saturdays

Day	Training	Duration
Monday	REST DAY	
Tuesday	High-Intensity Training	90 min
Wednesday	High-Intensity Training	90 min
Thursday	Medium-Intensity Training	75 min
Friday	Medium-Intensity Training	60 min
Saturday	MATCH	
Sunday	Players who played: Regeneration Training	45 min
	Players who did not play: High-Intensity Training	75-90 min

The standards were dissolved before use in phosphate-buffered saline (PBS) (pH 7.2) until an initial concentration of 2,000 pg/ml. Stock solutions were serially diluted 7 times in a solvent twice (Eng. Reagent Diluent-in (1% BSA in PBS) to obtain a standard curve of 7 points. Next, 100 µl of capture antibody was added to the wells of microtitre plates (MTPs). The plates were taped with ELISA plate sealers and left overnight at room temperature, after which the wells were washed with wash buffer in an automatic washing machine for MTF. In all of the wells, block buffer was added to a final volume of 300 µl, and the MTPs were incubated for a minimum of 1 hour at room temperature. Then, the MTPs were washed with wash buffer. All of the samples were previously diluted 10 times in deionized water. The diluted samples and standards were added to the wells of the MTPs, which were covered with plate sealers and allowed to incubate for 2 hours at room temperature. After the incubation and washing of the MTPs, 100 µl of detection antibody was added to each well, and the plates were again covered with plate sealers and incubated for 2 hours at room temperature. MTPs were washed again, and 100 µl of streptavidin horseradish peroxidase was added to all of the wells. Incubation at room temperature was stopped after 20 min by washing the MTPs. In the next step, 100 µl of substrate solution (Colour reagent A + Colour reagent B, 1:1) was added. After 20 min, 50 ml of stop solution (2 N H_2SO_4) was added, and the optical density was directly measured in each well using a Microplate Reader (ZENYTH, Anthos, UK) set at 450 nm (19, 20).

All of the measured values were reduced for the absorbance values of the blanks (deionized water). A standard curve was made based on the measured values of the standards, and it was calculated as values for each individual sample. All of the samples were measured in triplicate.

Biochemical analyses of cytokine concentrations were carried out at the Laboratory of Immunology, Faculty of Medical Sciences in Kragujevac. The measurements were performed on the apparatus ZENYTH, Anthos, United Kingdom.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 for Windows. The results are expressed as the means ± standard deviation of the mean (SD). Data distribution was checked using the Kolmogorov-Smirnov or Shapiro-Wilk test and, depending on the results, the appropriate parametric or nonparametric test was used. The differences between the values of the means from two related samples were assessed by paired t-test or Wilcoxon's test. The alpha level for significance was set at $P<0.05$.

RESULTS

The levels of IL-6 in the blood of the subjects from different experimental groups at the initial (before two-

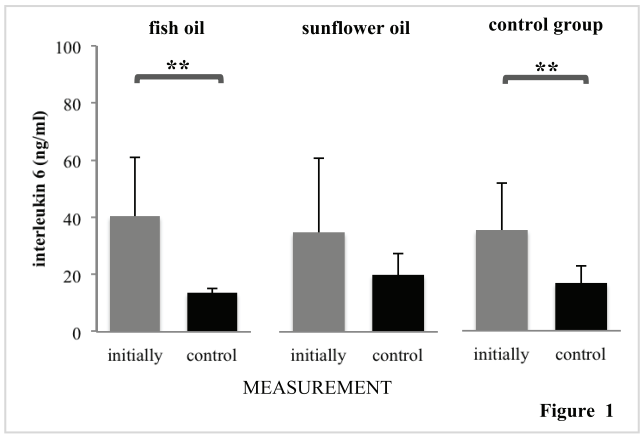

Figure 1. Level of IL-6 in the blood of subjects from different experimental groups at the initial and control measurements. The values are expressed as X ± SE; *p<0.05, **p<0.01.

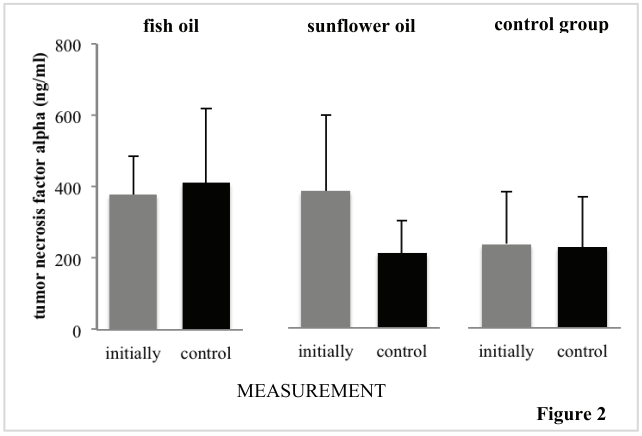

Figure 2. Level of TNF-α in the blood of subjects from different experimental groups at the initial and control measurements. The values are expressed as X ± SE; *p<0.05, **p<0.01.

month follow up period) and control measurements (after the two-month follow-up period) are shown in Figure 1. There was a significant decrease in the levels of IL-6 in the group that consumed fish oil (p<0.01) and in the control group (p<0.01). However, this reduction was more prominent in the group with fish oil supplementation (Fig 1).

The levels of TNF-α in the blood of the subjects from different experimental groups at the initial and control measurements are shown in Figure 2. None of the groups differed in the levels of this cytokine after the two-month follow-up period.

Changes in the levels of IL-6 in the blood of subjects from different experimental groups induced by physical load test (PLT) are presented in Figure 3. The levels of this inflammatory factor were not statistically changed in any group, after the physical load test, at the initial or control measurement (Fig 3).

Changes in the levels of TNF-α in the blood of subjects from different experimental groups as measured by the physical load test (PLT) are presented in Figure 4. The values of TNF-α were significantly increased after the physical load test in the control measurement in subjects who consumed sunflower oil (p<0.05) (Fig 4).

DISCUSSION

The concept of "silent inflammation" in sports has been established to note the potential dangers of an incorrectly dosed training process and overtraining that could lead to muscle damage and reduction in sports performance (21, 22). A key role in intercellular communication during the development and progression of inflammation is attributed to cytokines (23). Although some studies have suggested that TNF-α and IL-6 have pro-inflammatory effects (24, 25), others have emphasized their opposite character (26). There are several possible explanations for the different actions of these cytokines during physical activity (27). First, the type of physical activity, as well as the intensity and duration of exercise, can extremely affect the cytokine profile. Northoff and Berg were the first who noticed that IL-6 might be involved in the acute phase of inflammation after exercise (28-30).

On the other hand, the number of studies that have examined the impact of physical activity on the production of cytokines (inflammation) in young athletes-adolescents is negligible. Most children with a sports experience duration of more than five years may represent a potential critical group for the development of inflammation and its consequences for health.

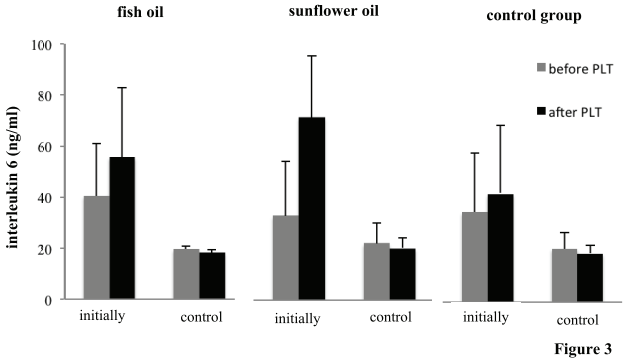

Figure 3. Levels of IL-6 in the blood of subjects from different experimental groups according to the physical load test (PLT). The values are expressed as X ± SE; *p<0.05, **p<0.01.

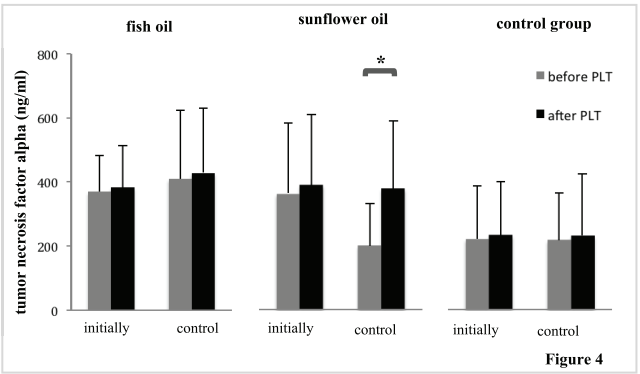

Figure 4. Levels of TNF-α in the blood of subjects from different experimental groups according to the physical load test (PLT). The values are expressed as X ± SE; *p<0.05, **p<0.01.

Additionally, supplementation of omega-3 and omega-6 fatty acids has become a trend only in recent years, and there are almost no studies on the effect of fish or sunflower oil on the production of inflammatory molecules. Because of this, the leading hypothesis of the present study was related to the potential mutual positive effect of a properly dosed training process and PUFA supplementation on the markers of inflammation in young athletes.

Indeed, after a two-month experimental period, the group that consumed fish oil had a significantly reduced level of IL-6 (Fig 1) compared with the initial measurement. Although, in the control group, we also found a decrease in the values of these cytokines, the decrease was greater in the athletes on fish oil supplementation (Fig 1). By contrast, the levels of TNF-α were not significantly changed between the groups (Fig 2). These results are in correlation with previously studies (28-30) and also show that IL-6 is probably the most sensitive marker of silent inflammation in athletes. These results also suggest that fish oil presumably has positive effects on inflammation by decreasing the levels of IL-6.

Only a few studies have examined the effects of PUFAs (mainly fish oil) on the production of cytokines. Using an animal model in rats, Robinson and Field noted that n-3 PUFAs have no significant effect on the activity of inflammatory cells in rats subjected to physical exercise (31). Tartibian and coworkers recorded that fish oil supplementation in recreational athletes prevented the occurrence of muscle fatigue and muscle damage probably by reducing the production of inflammatory cytokines (32). Mickleborough and colleagues have shown that n-3 PUFAs may reduce bronchoconstriction, which often occurs as a result of hard training in elite athletes (marathoners, triathletes), because of the increased generation of cytokines (33).

Supplementation with n-3 PUFAs (EPA and DHA), which are found in fish oils, leads to an increase in the concentration of these fatty acids in lipid membranes of inflammatory cells, thereby replacing part of the AA (34). Less substrate for the synthesis of eicosanoids from AA can result from the impaired production of its pro-inflammatory molecules: prostaglandins (PGE2) (35), thromboxane B2 (TXB2) (36), and leukotrienes (LTE4) (37). Fish oil reduces the production of cytokines from monocytes or endothelial cells also. (38). Because PGE2 and LTE4 are involved in the control of cytokine synthesis (IL-6 and TNF-α), it is assumed that one possible mechanism by which EPA and DHA reduce the production of IL-6 is related to the potential interactions of n-3 PUFAs with PGE2 and leukotrienes (39).

In our study, only the group of subjects that consumed sunflower oil experienced a significant increase in the level of TNF-α after the physical load test (Figure 4). The other experimental groups did not differ in the levels of cytokines before or after exercise load, even at the initial or control measurement (Figs 3, 4). Based on these findings, it seems that the effects of PUFAs on cytokine production after an acute bout of exercise depend on the PUFA type. Although n-3 PUFAs did not significantly affect the dynamics of the estimated inflammatory markers, n-6 PUFAs slightly stimulated it (Fig 4). On the other hand, as noted earlier, the length and type of physical activity can strongly affect the level of cytokines. Therefore, studies with protocols in which the length of the exercise test lasted longer than in our study have recorded an increase in the value of this cytokine (40, 41).

Previous studies have shown that an acute bout of strenuous exercise may lead to increased production of IL-6, which, in turn, intensifies the damage of muscle fibres (the release of creatine kinase (CK)) (40). Tofta et al and associates examined the impact of supplementation with fish oil on the cytokine synthesis in athletes for 6 weeks (42). The study was performed on marathon runners, and blood samples from the antecubital vein were collected the week before and immediately after the end of the marathon race. The results showed that the use of fish oil during this period did not induce significant changes in the production of IL-6 and TNF-α in response to the acute stress test.

Most of the research regarding the effects of n-6 fatty acids on inflammation markers were conducted in animals. For twelve weeks, Bhattacharya and colleagues treated mice with corn oil and safflower oil, which (similar to sunflower oil) contains a high proportion of LA. During the same experimental period, the animals were subjected to the treadmill test and determination of TNF-α. The results showed that the group treated with safflower oil had increased levels of TNF-α in response to the stress test (43).

One of the possible explanations for this result is that n-6 fatty acid chains, in response to the rapid onset of exercise load, can enter into potential interactions with the lipid membranes of macrophages, which normally generate TNF-α, thus potentiating its increased production (44). Nevertheless, to confirm these assumptions, more complex studies are needed that could combine the functional, histological and immunohistochemical analyses of muscles and other tissues.

CONCLUSION

Supplementation with fish oil (n-3) has been associated with reduced levels of IL-6 compared with the initial values. This oil can have a beneficial effect in preventing the acute phase of inflammation that occurs in young athletes. In this study design, the effects of PUFAs on cytokine production after an acute bout of exercise depend on the PUFA type. Although n-3 PUFAs did not have a significant effect on inflammatory marker dynamics, n-6 PUFAs slightly stimulated the production of TNF-α. This result could be of interest in determining an algorithm of PUFA supplementation in athletes in the training process or during the competitive season.

Acknowledgements

This work was supported by Junior Project No. 09/11 from the Faculty of Medical Sciences, University of Kragujevac, Serbia.

Conflict of Interest

All of the authors declare no conflict of interest.

REFERENCES

1. Hills AP, Street SJ, Byrne NM. Physical Activity and Health. What is Old is New Again. Adv Food Nutr Res. 2015; 75:77-95.
2. Maddison R, Jiang Y, Foley L, Scragg R, Direito A, Olds T. J Sci Med Sport. 2015; doi: 10.1016/j.jsams.2015.08.001. [Epub ahead of print].
3. Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. Mediators Inflamm 2008; 2008: 109502.
4. Suzuki K, Takahashi M, Li CY, Lin SP, Tomari M, Shing CM, Fang SH. The acute effects of green tea and carbohydrate coingestion on systemic inflammation and oxidative stress during sprint cycling. Appl Physiol Nutr Metab 2015; 10: 1-7.
5. MacIntyre DL, Reid WD, Lyster DM, Szasz IJ, and McKenzie DC. Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. J Appl Physiol 1996; 80: 1006-13.
6. La Gerche A, Inder WJ, Roberts TJ, Brosnan MJ, Heidbuchel H, Prior DL. Relationship between Inflammatory Cytokines and Indices of Cardiac Dysfunction following Intense Endurance Exercise. PLoS One. 2015 12;10(6):e0130031.
7. Ulven SM, Foss SS, Skjølsvik AM, Stadheim HK, Myhrstad MC, Raael E, Sandvik M, Narverud I, Andersen LF, Jensen J, Holven KB. An acute bout of exercise modulate the inflammatory response in peripheral blood mononuclear cells in healthy young men. Arch Physiol Biochem. 2015; 121(2): 41-9.
8. Vucic V. The role of dietary polyunsaturated fatty acids in inflammation. Ser J Exp Clin Res 2013; 14 (3): 93-9.
9. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. Mol Nutr Food Res 2008; 52(8): 885-97.
10. Zuliani G1, Galvani M, Leitersdorf E, Volpato S, Cavalieri M, Fellin R. The role of polyunsaturated fatty acids (PUFA) in the treatment of dyslipidemias. Curr Pharm Des. 2009; 15(36): 4087-93.
11. Bruunsgaard H. Physical activity and modulation of systemic low-level inflammation. J Leukoc Biol 2005; 78(4): 819-35.
12. Tian HH, Ong WS, Tan CL. Nutritional supplement use among university athletes in Singapore. Singapore Med J 2009; 50: 165-72.
13. Park Y, Moon HJ, Kim SH. N-3 polyunsaturated fatty acid consumption produces neurobiological effects associated with prevention of depression in rats after the forced swimming test. J Nutr Biochem. 2012; 23(8): 924-8.
14. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun. 2011; 25(8): 1725-34.
15. Mally K, Trentmann J, Heller M, Dittmar M. Reliability and accuracy of segmental bioelectrical impedance analysis for assessing muscle and fat mass in older Europeans: a comparison with dual-energy X-ray absorptiometry. Eur J Appl Physiol 2011; 111(8): 1879-87.
16. Evans CH, White RD (2009). Exercise testing for primary care and sports medicine physicians. New York: Springer. 49-50.
17. Howley ET, Bassett DR, Welch HG. Criteria for maximal oxygen uptake: review and commentary. Med Sci Sport Exer 1995; 27: 1292-301.
18. Nieman DC, Austin MD, Benezra L et al. Validation of cosmed's FitMate in measuring oxygen consumption and estimating resting metabolic rate. Res Sport Med 2006; 14: 89-96.
19. Radosavljevic G, Jovanovic I, Majstorovic I, Mitrovic M, Lisnic VJ, Arsenijevic N, Jonjic S, Lukic ML. Deletion of galectin-3 in the host attenuates metastasis of murine melanoma by modulating tumor adhesion and NK cell activity. Clin Exp Metastasis 2011; 28: 451-62.
20. Crowther JR. ELISA. Theory and practice. Methods Mol Biol 1995; 42: 1-218.
21. Arsic A, Vucic V, Tepsic J, Mazic S, Djelic M, Glibetic M. Altered plasma and erythrocyte phospholipid fatty acid profile in elite female water polo and football players. Appl Physiol Nutr Metab 2012; 37(1): 40-7.
22. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 2005; 98(4): 1154-62.
23. Tepsic J, Vucic V, Arsic A, Blazencic-Mladenovic V, Mazic S, Glibetic M. Plasma and erythrocyte phospholipid fatty acid profile in professional basketball and football players. Eur J Appl Physiol 2009; 107(3): 359-65.
24. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Gyori G, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int J Obes (Lond) 2007; 31(9) :1420-8.
25. Pedersen BK, Fischer CP. Physiological roles of muscle-derived interleukin-6 in response to exercise. Curr Opin Clin Nutr Metab Care 2007; 10(3): 265-71.
26. Barton BE. IL-6: Insights into novel biological activities. Clin Immunol Immunopathol 1997; 85: 16-20.
27. Pedersen BK, Ostrowski K, Rohde T, Bruunsgaard H. The cytokine response to strenuous exercise. Can. J. Physiol. Pharmacol. 1998; 76: 505-11.

28. Tepsic J, Vucic V, Arsic A, Mazic S, Djelic M, Glibetic M. Unfavourable plasma and erythrocyte phospholipid fatty acid profile in elite amateur boxers. Eur J Sport Sci 2013; 13(4): 414-21.
29. Castell LM, Poortmans JR, Leclercq R, Brasseur M, Duchateau J, Newsholme EA. Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. Eur J Appl Physiol 1997; 75: 47-53.
30. Rohde T, MacLean DA, Richter EA, Kiens B, Pedersen BK. Prolonged submaximal eccentric exercise is associated with increased levels of plasma IL-6. Am J Physiol 1997; 273: 85-91.
31. Robinson EL, Field JC. Dietary Long-Chain (n-3) Fatty Acids Facilitate Immune Cell Activation in Sedentary, but not Exercise-Trained Rats. J Nutr 1998; 128: 498-504.
32. Tartibian B, Maleki BH, Abbasi A. The effects of ingestion of omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained men. Clinical Journal of Sport Medicine 2009; 19: 115-9.
33. Mickleborough DT, Murray LR, Ionescu AA, Lindley MR. Fish Oil Supplementation Reduces Severity of Exercise-induced Bronchoconstriction in Elite Athletes. Am. J. Respir. Crit Care Med 2003; 168: 1181-9.
34. Ristić-Medić D, Vučić V, Takić M, Karadžić I, Glibetić M. Polyunsaturated fatty acids in health and disease. J Serb Chem Soc 2013; 78: 1-21.
35. Trebble TM, Wootton SA, et al. Prostaglandin E_2 production and T-cell function after fish-oil supplementation: response to antioxidant co-supplementation Am. J. Clin Nutr 2003; 78: 376-82.
36. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor α and interleukin 1β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am J Clin Nutr 1996; 63: 116-22.
37. Wardhana, Surachmanto ES, Datau EA. The role of omega-3 fatty acids contained in olive oil on chronic inflammation. Acta Med Indones 2011; 43(2): 138-43.
38. Vanden Berghe W, Vermeulen L, Delerive P, De Bosscher K, Staels B, Haegeman G. A paradigm for gene regulation: inflammation, NF-kappaB and PPAR. Adv Exp Med Biol 2003; 544: 181-96.
39. Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids 2006; 75: 197-202.
40. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA, and Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol (Lond) 1997; 499: 833-41.
41. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen BK. A trauma-like elevation in plasma cytokines in humans in response to treadmill running. J Physiol (Lond.) 1998; 508: 949-53.
42. Toft AD, Thorn M, Ostrowski K, Asp S, et al. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. J Appl Physiol 2000; 89: 2401-6.
43. Bhattacharya A, Rahman MM, Sun D. The combination of dietary conjugated linoleic acid and treadmill exercise lowers gain in body fat mass and enhances lean body mass in high fat–fed male balb/C mice. J Nutr 2005; 135: 1124-30.
44. Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 1998; 53: 45-53.