Acute effects of n-3 polyunsaturated fatty acids (PUFAs) reducing tumor necrosis factor-alpha (TNF-α) levels and not lowering malondialdehyde (MDA) levels after anaerobic exercise

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This study aimed to analyze the acute effect of n-3 PUFA on TNF-α and MDA levels after anaerobic exercise. This experimental study involved 16 healthy adult men randomly selected with a BMI range of 19.00-24.00, and age ranged from 20-30 years. Subjects were selected randomly and then divided into 2 groups: group K1 using placebo and group K2 using n-3 PUFAs at a dose of 540 mg EPA and 360 DHA. The intervention was carried out 24 hours after the research subjects completed the anaerobic exercise. The data in this study were TNF-α levels measured using a human ELISA kit, and MDA was measured using a spectrophotometer taken before and after the intervention. The test of different levels of TNF-α in K2 ($p<0.05$) p=0.027, K1 ($p>0.05$) p=0.327, so that n-3 PUFAs significantly reduced TNF-α levels and the test of different levels of MDA in the group K2 ($p> 0.05$) p = 0.511, K2 ($p> 0.05$) p = 0.541 so that n-3 PUFAs did not significantly reduce MDA levels. Thus, it can be concluded that the administration of n-3 PUFAs has been shown to reduce TNF-α levels without a decrease in Malondialdehyde (MDA) levels after anaerobic exercise.

Anaerobic exercise with high intensity causes metabolic stress in the form of energy deficiency and muscle damage called Exercise Injured Muscle Damage (EIMD) (1). Uncontrolled muscle damage in the recovery phase causes Delayed Onset Muscle Soreness (DOMS) (2). DOMS will trigger the inflammatory process so that tumor necrosis factor-alpha TNF-α in the blood will increase because the response has occurred muscle damage (3). In addition, physical exercise triggers an increase in oxygen consumption by up to 10 times compared to at rest (4). As a result of metabolic processes, cells will produce free radicals or reactive oxygen species (ROS). Free radicals are characterized by increased malondialdehyde (MDA) (5). In addition to aerobic exercise, which generally triggers free radicals, anaerobic exercise that involves less oxygen circulation can also cause oxidative stress that triggers the increased production of free radicals through the xanthine oxidase and NADPH oxidase pathways, ischemia-reperfusion, increased purine oxidation, damage to iron-containing proteins, impaired Ca2+

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homeostasis and catecholamine autooxidation (5). Increased production of free radicals will also cause muscle damage (4).

The management of post-exercise DOMS uses pharmacological modalities; an estimated 30 million people worldwide who take non-steroidal anti-inflammatory drugs are very common in athletes and people who do high physical activity (6); this will certainly have a major impact on health and muscle growth (7). In addition, the increase in free radicals can lead to degenerative diseases such as cancer, diabetes mellitus and atherosclerosis, which are the causes of heart disease. It is estimated that as many as 17 million people worldwide die each year due to degenerative diseases. Several countries in the world, such as Brazil, Canada, China, India, Nigeria, Pakistan, Russia, England and Tanzania, have an incidence of death from degenerative diseases reaching 80% (5).

Solutions need to be found to overcome these problems; one way to reduce TNF-α production and excessive free radical production is by consuming one of the natural products derived from fish oil, namely n-3 Polyunsaturated Fatty Acids (PUFAs). The ingredients contained in n-3 PUFAs are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (7). n-3 PUFAs are also known for their anti-inflammatory properties (7).

Role of n-3 PUFAs in inhibiting inflammation through blockade of TNF-α signaling by activating protein responses in muscle (3). on the other hand, n-3 PUFAs play a role in suppressing pro-oxidant activity by increasing heme oxygenase 1 (HO-1) and glutathione peroxidase (GPx) genes (8).

Based on this, the study was designed to investigate the acute effect of n-3 PUFA on TNF-α and MDA after anaerobic exercise. It is hoped that this study can be a guideline to overcome the production of uncontrolled levels of TNF-α and MDA levels after anaerobic exercise.

MATERIAL AND METHODS

Participants and procedure

This research uses experimental research with pre and post control group design. This study involved 16 healthy adult men who were randomly selected with a BMI range of 19.00-24.00, age ranged from 20-30 years, and not currently taking non-steroidal anti-inflammatory drugs. The following procedure was used in the study:

Informed consent was filled out by the research subjects.

A randomized sample was created by dividing the participants into two groups: group 1 (K1) as a placebo group and group 2 (K2) as a group of n-3 PUFAs.

Subjects did anaerobic exercise (weight training) with high intensity ranging from 80-90% of maximum ability, performed 4 sets, 10 repetitions and recovery between sets of approximately 60 seconds.

Twenty-four hours after anaerobic exercise, pre-test blood samples were taken for analysis in the laboratory; then intervention was given to the subject. The intervention was given according to the group at the same time. K1 was given a placebo, and K2 was given n-3 PUFA with a dose of 540 mg EPA and 360 DHA.

Twenty-four hours after the intervention, a post-test blood sample was taken again, which would later be analyzed in the laboratory.

Instruments

Measurement of serum TNF-α levels were obtained using the Human TNF-α ELISA kit and the measurement of serum MDA levels using a spectrophotometer.

Statistical Analysis

Statistical analysis was carried out using SPSS application with Paired t-test and Independent t-test method.

RESULTS

In the Mann Whitney U difference test, there were no significant differences in the characteristics of the research subjects between the two research groups, so if there were differences at the end of the intervention, it could be ascertained that they were not due to the characteristics of the study.

Based on the normality test (Table II and III), data on the levels of TNF-α (Pre-test) and TNF-α (Post-test) in K1 and K2 showed data ($p<0.05$), meaning that the data was not normal. The delta data of TNF-α levels in K1 and K2 showed ($p>0.05$) so that the data were normally distributed. Then, based on the normality test of the data on the MDA (pre-test), (post-test), the deltas on K1 and K2 show ($p>0.05$)

so that the distribution is normal.

The test of different levels of TNF-α in K2 ($p<0.05$) $p=0.027$, K1 ($p>0.05$) $p=0.327$, so that n-3 PUFAs significantly reduced TNF-α levels and the test of different levels of MDA in the group K2 ($p> 0.05$) $p = 0.511$, K2 ($p> 0.05$) $p = 0.541$ so that n-3 PUFAs did not significantly reduce MDA levels. Thus, it can be concluded that the administration of n-3 PUFAs

Table I. *Characteristics of research subjects*

Characteristics	Group	n	x̄±SD	Shapiro-Wilk	p *(Mann Whitney u-test)*
Age	K1	8	21.63±4.20	0.000	0.251
	K2	8	24.63±6.67	0.009	
Height	K1	8	166.50±3.81	0.003	0.164
	K2	8	168.13±3.72	0.497	
Weight	K1	8	61.63±5.50	0.242	0.864
	K2	8	61.06±7.61	0.878	
BMI	K1	8	22.18±1.92	0.382	0.640
	K2	8	21.58±2.98	0.347	
Systolic	K1	8	107.50±8.86	0.054	0.242
	K2	8	112.50±7.07	0.056	
Diastolic	K1	8	71.52±6.40	0.037	0.424
	K1	8	73.75±5.17	0.000	

Table II. *Mean and standard deviation of TNF-α and MDA level in both groups*

Data	Group	n	Levels (ng/mL) *(pre-test)* x̄±SD	*(post-test)* x̄±SD	(Delta) x̄±SD
TNF-α	K1	8	5.24±2.98	4.67±2.84	0.56±0.44
	K2	8	4.45±1.16	4.81±1.71	0.64±0.54
MDA	K1	8	292.43±12.58	296.33±12.37	13.32±10.45
	K2	8	255.77±27.22	249.66±19.00	18.33±16.80

Table III. *Results of the normality test for TNF-α and MDA levels*

Data	Group	n	Shapiro-Wilk P
TNF-α (*Pre-test*)	K1	8	0.001
	K2	8	0.004
TNF-α (*Post-test*)	K1	8	0.000
	K2	8	0.011
Delta TNF-α	K1	8	0.375
	K2	8	0.287
MDA (*Pre-test*)	K1	8	0.196
	K2	8	0.315
MDA (*Post-test*)	K1	8	0.874
	K2	8	0.392
Delta MDA	K1	8	0.634
	K2	8	0.077

Table IV. *Results of Different Tests for TNF-α and MDA Levels*

Data	Different Test Method	Group	P
TNF-α	Wilcoxon Signed Ranks Test	K1 (pre-test and post-test)	0.327*
		K2 (pre-test and post-test)	0.027
	Independent t-test	Delta K1 and K2	0.768*
MDA	Pairet t-test	K1 (pre-test and post-test)	0.541*
		K2 (pre-test and post-test)	0.511*
	Independent t-test	Delta K1 and K2	0.468*

*$p>0.05$

has been shown to reduce TNF-α levels without a decrease in Malondialdehyde (MDA) levels after anaerobic exercise (Table IV).

DISCUSSION

The results of this study proved that K1 with placebo administration did not significantly reduce TNF-α levels after anaerobic exercise, while K2 with n-3 PUFAs administration could significantly reduce TNF-α levels after anaerobic exercise; this is confirmed by research carried out by Hedayatpour, Izanloo Falla, Kawamura and Muraoka, 2018 (1) that the administration of omega 3 (n-3 PUFAs) in 54 hemodialysis patients for 6 consecutive months can reduce TNF-α levels.

Anaerobic exercise with high intensity causes metabolic stress in the form of energy deficiency and muscle damage (1). Uncontrolled muscle breakdown in the recovery phase causes DOMS (2). DOMS can occur with many complex factors combined after exercise-induced muscle damage. The inflammatory response will occur after the morphological damage caused by the eccentric contraction. Cytokines are released in the damaged muscle, when inflammatory cells such as neutrophils and macrophages are more active. Inflammatory mediators are released, namely proinflammatory cytokines such as TNF-α and IL-6 and anti-inflammatory cytokines such as IL-10. Increased TNF-α in the blood will trigger an inflammatory process that will cause muscle pain in response to muscle damage. One of the efforts to reduce TNF-α overproduction) is giving n-3 PUFAs. n-3 PUFAs are known for their anti-inflammatory properties (9). n-3 PUFAs can inhibit inflammation by blocking TNF-α signaling by activating protein responses in muscle (10).

The results of this study proved that K1 with placebo administration and K2 with n-3 PUFAs did not significantly reduce malondialdehyde (MDA) levels after anaerobic exercise. Free radicals are characterized by increased MDA production; uncontrolled free radicals will cause muscle damage (2).

There are pros and cons to several studies of the effects of n-3 PUFAs on MDA levels. On research, Ateya et al., 2017 (8) reported that administration of n-3 PUFAs in 49 pediatric patients with kidney disease for 6 months at a dose of 1 gram for 6 months reduced markers of oxidative stress such as MDA. In addition, research Pooya et al., 2010 (11) reported that administration of n-3 PUFAs in 81 type-2 diabetic patients for 2 months only reduced hemocysteine levels without a decrease in MDA and C-Reactive protein (CRP). Research Vaagenes et al., 1998 (12) reported that administration of n-3 PUFAs at low doses (250 mg/day) in rats had no effect on decreasing MDA levels. The findings in this study reported that acute administration of n-3 PUFAs at high doses (1000 mg) did not decrease MDA levels after anaerobic exercise.

Acute effect of n-3 PUFAs was shown to reduce the level of TNF-α without a decrease in the level of MDA after anaerobic exercise.

Ethics statement:

This research was approved by the ethics committee of Universitas Airlangga with registration number No. 179/EC/KEPK/FKUA/2020.

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