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Effect of acute omega 3 supplementation reduces serum tumor necrosis factor-alpha (TNF-a) levels, pain intensity, and maintains muscle strength after high-intensity weight training

El efecto de la suplementación aguda con omega 3 reduce los niveles séricos del factor de necrosis tumoral alfa (TNF-a), la intensidad del dolor y mantiene la fuerza muscular después del entrenamiento con pesas de alta intensidad

*Novadri Ayubi, **Bambang Purwanto, **Purwo Sri Rejeki, *Nining Widyah Kusnanik, **Lilik Herawati, ***Anton Komaini, *Toho Cholik Mutohir, *Nurhasan, *Muchamad Arif Al Ardha, *Awang Firmansyah

*Universitas Negeri Surabaya, (Indonesia), **Universitas Airlangga, (Indonesia), ***Universitas Negeri Padang, (Indonesia)

Abstract This study aims to analyze the acute effect of omega 3 supplementation on serum TNF-α levels, pain intensity, and muscle strength after high-intensity weight training. A total of 20 adult males with a BMI of 18.00-24.99 were enrolled in this study. Subjects were divided into 2 groups, namely (K1) with placebo and (K2) with omega 3 supplementation with a dose of 1000 mg (540 mg EPA and 360 mg DHA). The intervention was carried out 24 hours after high-intensity weight training. The data in this study were taken before and after the intervention. Measurement of serum TNF-α levels using a human ELISA kit, measurement of pain intensity using visual analog scale (VAS), measuring muscle strength using a leg dynamometer. The data analysis technique is used if the data is normally distributed, namely Paired t-test and Independent t-test, if the data has not normally distributed the analysis used is the Wilcoxon signed-rank test. Omega 3 supplementation significantly reduced serum TNF-α levels (p-0.035), significantly reduced pain levels (p-0.007), and did not significantly decrease the strength (p-0.100). Omega 3 supplementations can reduce serum TNF-α levels, pain intensity, and omega 3 supplementations can maintain muscle strength after high-intensity weight training as evidenced by the absence of a significant decrease in muscle strength after high-intensity weight training.

Keywords: Omega 3; TNF-α; Pain Intensity; Muscle Strength.

Resumen Este estudio tiene como objetivo analizar el efecto agudo de la suplementación con omega 3 en los niveles séricos de TNF-α, la intensidad del dolor y la fuerza muscular después del entrenamiento con pesas de alta intensidad. En este estudio se inscribieron un total de 20 hombres adultos con un IMC de 18,00 a 24,99. Los sujetos se dividieron en 2 grupos, a saber, (K1) con placebo y (K2) con suplementos de omega 3 con una dosis de 1000 mg (540 mg EPA y 360 mg DHA). La intervención se llevó a cabo 24 horas después del entrenamiento con pesas de alta intensidad. Los datos de este estudio se tomaron antes y después de la intervención. Medición de los niveles séricos de TNF-α mediante un kit ELISA humano, medición de la intensidad del dolor mediante (VAS), medición de la fuerza muscular mediante un dinamómetro de piernas. La técnica de análisis de datos se utiliza si los datos tienen una distribución normal, es decir, la prueba t pareada y la prueba t independiente; si los datos no tienen una distribución normal, el análisis utilizado es la prueba de rango con signo de Wilcoxon. La suplementación con omega 3 redujo significativamente los niveles séricos de TNF-α (p-0,035), redujo significativamente los niveles de dolor (p-0,007) y no disminuyó significativamente la fuerza (p-0,100). Los suplementos de omega 3 pueden reducir los niveles séricos de TNF-α, la intensidad del dolor y los suplementos de omega 3 pueden mantener la fuerza muscular después del entrenamiento con pesas de alta intensidad, como lo demuestra la ausencia de una disminución significativa en la fuerza muscular después del entrenamiento con pesas de alta intensidad.

Palabras clave: Omega 3; TNF-α; Intensidad del dolor; Fuerza muscular.

Introduction

Weight training with high intensity will cause muscle damage and is characterized by delayed muscle soreness or Delayed Onset Muscle Soreness (DOMS) (Yoon, Lee, and Kim 2020). Muscle pain is caused by an inflammatory process due to increased levels of tumor necrosis factor-alpha (TNF-) in the blood in response to muscle damage (Jieun Kim, Kim, and Lee 2017; Jürgenson et al. 2021). At the right level TNF-a will provide protection and healing. However, at excessive levels it will cause tissue damage (Ayubi et al. 2022).

Post-workout muscle pain according to the US Department of Labor, Bureau of Labor Statistics has an incidence rate of 76.5% (Bumann, Banzer, and Fleckenstein 2020). The management of post-weight training pain has been using pharmacological modalities, it is estimated that 30 million people worldwide taking non-steroidal anti-inflammatory drugs are very prevalent among athletes and people who engage in high physical activity (Schoenfeld 2012; Juhasz et al. 2018). Several types of drugs that are often used are menamat acid, piroxicam and diclofenac sodium (Ayubi et al. 2022). Giving non-

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Anton Komaini
antonkomaini@fik.unp.ac.id

steroidal anti-inflammatory drugs after weight training has the effect of disrupting muscle growth which has an impact on muscle hypertrophy and strength. The results of giving anti-inflammatory drugs will actually negate the results of the exercise performed (Ozaki et al. 2020; 2019; Schoenfeld 2012).

Alternative solutions need to be sought to reduce complaints of pain in muscles, but still do not interfere with the response to muscle growth due to exercise. One of the natural ingredients found in fish oil is omega 3 compounds. Omega 3 can inhibit inflammation by blocking TNF-α signals by activating protein responses in muscles (Khan 2017; Haidari et al. 2019). The anti-inflammatory activity of omega 3 also inhibits the production of pro-inflammatory eicosanoids including prostaglandins and leukotrienes (Williams-Bey et al. 2014; Gonzalez-Jurado et al. 2011). Omega 3 supplementation has also been used by athletes extensively to increase endurance and VO2Max (Żebrowska et al. 2015). Besides omega 3 also increases red blood cells and hemoglobin (Khan 2017). Until now, omega 3 supplementation has not been reported to cause side effects after exercise in athletes, but it has not been tested on the effectiveness of acute effects of omega 3 supplementation to reduce pain due to inflammation after weight training.

Therefore this study was conducted to evaluate the acute effect of omega 3 supplementation on serum TNF-a levels, pain intensity, and muscle strength after high intensity weight training.

Methods

Study Design

This study used a pre and post-test control group design. The taking of research subjects was carried out by purposive sampling and then the subjects were divided into 2 groups. Group 1 (K1) was the control group with a placebo. Group 2 (K2) is the treatment group with omega 3 supplementation.

Subjects

20 adult males participated in this study. The subjects of this study were divided into two groups, namely (K1) with placebo and (K2) with omega 3 supplementation. (Mean ± SD, age K1 23.10±6.13 and K2 27.30±8.21 years, height K1 166.70±3.59 and K2 166.80±4.36 cm, body weight K1 64.00±7.48 and K2 60.45±6.90 kg, BMI K1 22.94±2.46 and K2 21.72±2.69 kg.m-2, systolic K1 107.00±10.59 and K2 117.00±10.59 mmHg, diastolic K1 72.00±6.32 and K2 79.00±8.75 mmHg). The exclusion criteria in this study were under 20 years of age, doing massage, consuming coffee, and using non-steroidal anti-inflammatory drugs. Research subjects received instructions on the procedures to be followed during the study and all subjects gave written consent.

Procedure

1. The procedure before starting the research, the researcher prepares the administration which includes preliminary research, ethical eligibility permit, research permit, facility and infrastructure borrowing permit. Furthermore, preparing research officers, screening respondents who will be subjects in the study based on inclusion criteria, preparing research subjects, and filling in the form of ability to be research subjects (Informed Consent) by the research sample.
2. Carrying out randomization on the subject, so that dividing into two groups, namely group 1 (K1) as the control group and group 2 (K2) as the treatment group. The research subjects were not informed about the treatment to be received. Furthermore, the subjects were given their respective interventions according to their group at the same time. K1 was given a placebo in the form of an empty capsule and K2 was given omega 3 supplementation at a dose of 1000 mg (540 mg EPA and 360 mg DHA). The Intervention was given after taking the pre-test blood sample.
3. Blood sampling to measure serum TNF-a levels is done 2 times (pre-test and post-test) the pre-test is carried out in a range of 12-24 hours after high-intensity weight training and post-test is carried out approximately 24 hours after administration of omega 3 supplementation by officers who are experts in their fields.
4. Measurement of pain intensity is carried out after pre-test and post-test blood sampling using a visual analog scale (VAS) and measurement of muscle strength is carried out using a dynamometer test.
5. Blood samples will be analyzed in the laboratory using the ELISA method with the human catalog number TNF- ELISA kit E-EL-H0109
6. The final stage of this research is data analysis and preparation of research reports, to be presented as the responsibility of the researcher.

Statistical analysis

The data obtained were tested using the Shapiro Wilk method to determine the normality of the data distribution. Data that is normally distributed, then different tests will be carried out with Pairet t-test and Independent t-test. The data were not normally distributed, a different test was performed using the Wilcoxon signed-rank test and the Mann Whitney u-test method. Different tests were performed to

determine TNF-a levels, pain levels, and muscle strength after high intensity weight training in the control group (K1) and the treatment group (K2).

Ethics

This research protocol has been declared ethical in accordance with 7 (seven) WHO 2011 standards, namely 1) social value, 2) scientific value, 3) distribution of burdens and benefits, 4) risk, 5) seduction / exploitation, 6) confidentiality and privacy 7) Approval after explanation, which refers to the 2016 CIOMS guidelines. This is shown by the fulfillment of indicators for each standard. Declaration of ethics was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga with registration number (No.179/EC/KEPK/FKUA/2020.

Results

The research subjects are presented in table 1.

Table 1.
Categories of research subjects

Data	Group	n	x̄±SD	Shapiro-Wilk	P *(Independent t-test)*
Age	K1	10	23.10±6.13	0,105	0,212
	K2	10	27.30±8.21	0,106	
Height	K1	10	166.70±3.59	0,205	0,956
	K2	10	166.80±4.36	0,618	
Weight	K1	10	64.00±7.48	0,200	0,285
	K2	10	60.45±6.90	0,921	
BMI	K1	10	22.94±2.46	0,501	0,306
	K2	10	21.72±2.69	0,553	
Systolic	K1	10	107.00±10.59	0,351	0,059
	K2	10	117.00±10.59	0,111	
Diastolic	K1	10	72.00±6.32	0,212	0,055
	K2	10	79.00±8.75	0,117	

In the independent t-test, there was no significant difference in the characteristics of the research subject, so that if there is a difference at the end of the intervention, it is certain that it is not because of the characteristics of the research subject.

The Acute Effect of Omega 3 Supplementation on Serum TNF-a Levels

Table 2. Mean and standard deviation of TNF-a levels in the two groups

Table 2.
Mean and standard deviation of TNF-α levels in the two groups

Group	n	TNF-α Levels (ng/mL)		
		(pre-test) x̄±SD	*(post-test)* x̄±SD	(Delta) x̄±SD
K1	10	4,50±1,03	4,70±1,54	0,20±0,78
K2	10	5,04±2,66	4,61±2,53	-0,42±0,48

Table 3. Results of the normality test for Serum TNF-a level

Table 3.
Results of the normality test for Serum TNF-α level

Data	Group	Shapiro-Wilk	
		n	P
TNF-α (*Pre-test*)	K1	10	0,004
	K2	10	0,000
TNF-α (*Post-test*)	K1	10	0,002
	K2	10	0,000
Delta TNF-α	K1	10	0,320
	K2	10	0,235

Based on the normality test in Table 3, the data for TNF-a (Pre-test) and TNF-a (Post-test) levels on K1 and K2 shows that the data ($p < 0.05$) means that the data is not normally distributed. The delta data of TNF-a levels on K1 and K2 shows that the data ($p > 0.05$) means that the data is normally distributed.

Table 4. Results of the TNF-a Difference Test

Table 4.
Results of the TNF-α Difference Test

Different Test Method	Group	P
Wilcoxon signed rank tes	K1 (*pre-test and post-test*)	0,646
	K2 (*pre-test and post-test*)	0,035*
Independent t-test	Delta K1 and K2	0,044**

* There was a significant difference in the Wilcoxon signed test ($p < 0.05$)
** There is a significant difference in the Independent t-test ($p < 0.05$)

The Acute Effect of Omega 3 Supplementation on Pain Intensity

Table 5. Mean and Standard Deviation of Pain Intensity in the Two Groups

Table 5.
Mean and Standard Deviation of Pain Intensity in the Two Groups

Group	n	Pain Intensity		
		(pre-test) x̄±SD	*(post-test)* x̄±SD	(Delta) x̄±SD
K1	10	5,90±0,87	5,50±0,70	-0,40±1.07
K2	10	6,00±1,24	3,60±1,83	-2,40±1.71

Table 6. The results of the pain intensity normality test

Table 6.
The results of the pain intensity normality test

Data	Group	Shapiro-Wilk	
		n	p
Pain Intensity (Pre-test)	K1	10	0,017
	K2	10	0,008
Pain Intensity (Post-test)	K1	10	0,002
	K2	10	0,150
Delta Pain Intensity	K1	10	0,090
	K2	10	0,140

Based on the normality test in Table 6, the pain level data (Pre-test) on K1 and K2 and the pain intensity data (Post-test) on K1 shows that the data ($p < 0.05$) means

that the data is not normally distributed. Pain intensity data (Post-test) on K2 and delta pain intensity on K1 and K2 shows that the data (p> 0.05) means that the data is normally distributed.

Table 7. Results of Different Pain Intensity

Table 7.
Results of Different Pain Intensity

Different Test Method	Group	P
Wilcoxon signed rank tes	K1(*pre-test and post-test*)	0,279
	K2(*pre-test and post-test*)	0,007*
Independent t-test	Delta K1 and K2	0,006**

* There was a significant difference in the Wilcoxon signed test (p <0.05)
** There is a significant difference in the Independent t-test (p <0.05)

Effect of Acute Omega 3 Supplementation on Muscle Strength

Table 8. Mean and Standard Deviation of Muscle Strength in the Two Groups

Table 8.
Mean and Standard Deviation of Muscle Strength in the Two Groups

Group	n	Muscle Strength (Kg)		
		(pre-test) $\bar{x}$±SD	*(post-test)* $\bar{x}$±SD	(Delta) $\bar{x}$±SD
K1	10	63,90±16,80	58,90±19,52	-5,00±4,71
K2	10	52,90±18,62	56,04±16,55	3,14±5,40

Table 9. Muscle Strength Normality Test Results

Table 9.
Muscle Strength Normality Test Results

Data	Group	Shapiro-Wilk	
		n	P
Muscle Strength (Pre-test)	K1	10	0,733
	K2	10	0,752
Muscle Strength (*Post-test*)	K1	10	0,405
	K2	10	0,845
Delta Muscle Strength	K1	10	0,036
	K2	10	0,000

Based on the normality test of table 9 muscle strength data (Pre-test) and (Post-test) on K1 and K2, it can be seen that the data (p> 0.05) means that the data is normally distributed. Data delta muscle strength on K1 and K2 shows that the data (p <0.05) which means the data is not normally distributed.

Table 10. Results of Different Tests for Muscle Strength

Table 10.
Results of Different Tests for Muscle Strength

Different Test Method	Group	P
Pairet t-test	K1 (*pre-test and post-test*)	0,009*
	K2 (*pre-test and post-test*)	0,100
Mann Whitney u-test	Delta K1 dan K2	0,002**

* There was a significant difference in the Wilcoxon signed test (p <0.05)
** There was a significant difference in the Mann Whitney u-test (p <0.05).

Discussion

This study was conducted to determine the acute effect of omega 3 supplementation on tumor necrosis factor alpha (TNF-a) levels, pain levels and muscle strength after high intensity weight training.

The Acute Effect of Omega 3 Supplementation on Serum TNF-a Levels

The results of this study proved that K1 with placebo administration did not significantly reduce TNF-a levels after high-intensity weight training, while K2 with omega 3 supplementation could significantly reduce TNF-a levels after high-intensity weight training. This is proven by research (Gutiérrez-Pliego et al. 2018) that the administration of omega 3 supplementation to male rats aged 8 weeks in an inflammatory state proved that there was a significant decrease in TNF-a levels in the omega 3 supplementation group and there was no significant decrease in the placebo control group.

High-intensity weight training causes metabolic stress in the form of energy deficiency and muscle damage, characterized by Delayed Onset Muscle Soreness (DOMS) which will eventually lead to necrosis (de Freitas et al. 2017). The inflammatory response will occur after the morphological damage caused by weight training (Tachtsis, Camera, and Lacham-Kaplan 2018). The cells will release Tissue Factor to stimulate macrophage 1 (M1) in increasing proinflammatory cytokines such as TNF- and IL-6 (Lulińska-Kuklik et al. 2019). Increased TNF-a stimulates macrophages 2 (M2) to increase anti-inflammatory cytokines such as IL-10 which contribute to inflammation control (Mittal and Roche 2015). TNF-a at the right level will provide protection and healing. However, excessive levels will actually cause tissue damage (N Ayubi et al. 2022). One of the efforts that can be done to reduce uncontrolled inflammatory response is by using omega 3 supplementation. Omega 3 has the main content, namely a-linolenic acid (ALA), eicosapentaenoic acid (EPA), and decoshexaenoic acid (DHA) (Corder et al. 2016). Omega 3 especially EPA and DHA is well known for its anti-inflammatory properties (Djuric et al. 2017), omega 3 is able to inhibit inflammation through TNF-a signaling blockade by activating protein response in muscle (Meital et al. 2019). The anti-inflammatory activity of omega 3 also inhibits the production of pro-inflammatory eicosanoids including prostaglandins and leukotrienes (Kyriakidou et al. 2021; Meital et al. 2019).

The Acute Effect of Omega 3 Supplementation on Pain intensity

The results of this study prove that K1 with placebo administration does not significantly reduce pain levels after high-level weight training, on K2 with omega-3 supplementation can significantly reduce post-high intensity weight training pain. This is proven by research (Durán et al. 2019) that before and after giving omega 3 supplementation to 40 volunteers with type 2 diabetes for 3 months can reduce pain intensity significantly. Apart from that research (Jooyoung Kim and Lee 2014) reported that omega 3 intervention can reduce the inflammatory response due to exercise, so it is assumed that omega 3 can decrease DOMS.

DOMS occurs when a person is repeatedly exposed to high eccentric contractions or vigorous exercise. In general, acute structural damage to muscle tissue initiates DOMS and can lead to necrosis (cell death) reaching a peak between 24 and 48 hours after exercise (Jooyoung Kim and Lee 2014; Sari 2016). Muscle pain is caused by an inflammatory process due to increased levels of tumor necrosis factor alpha (TNF-a) in the blood in response to muscle damage (Ayubi et al. 2023). Omega 3 has also been shown to reduce pain and maintain muscle function after muscle damage due to eccentric exercise (Black et al. 2018)

Effect of Acute Omega 3 Supplementation on Muscle Strength

The results of this study proved that K1 with placebo administration significantly decreased muscle strength after high-intensity weight training, in K2 with omega-3 supplementation there was no significant decrease in muscle strength. This is compared to research (Smith et al. 2015) that the provision of long-term omega 3 supplementation to 60 men and women aged 60 to 85 years for 6 months proved that there was an increase in muscle strength due to increased muscle quality and muscle hypertrophy in the omega 3 supplementation treatment group.

Physiologically, muscle strength is the ability of a muscle or group of muscles to perform one maximum contraction against the load. Mechanically muscle strength is defined as the maximum work produced by a muscle or group of muscles (Heiss et al. 2018; Stojanović et al. 2021). DOMS is a major cause of decreased sports performance including muscle strength and range of motion for athletes and non-athletes, and also brings ongoing psychological discomfort (Jooyoung Kim and Lee 2014; Sari 2016). Omega 3 can reduce pain and maintain muscle function after muscle damage (Black et al. 2018). Omega 3 has been shown to have a positive effect on muscle protein synthesis in both the young and the elderly, which in turn promotes muscle hypertrophy (Tachtsis, Camera, and Lacham-Kaplan 2018). Omega 3 helps with muscle hypertrophy so that it has the potential to maintain muscle strength and function due to improved muscle quality (Black et al. 2018).

Thus, the provision of omega 3 supplements can reduce serum TNF-a levels, pain intensity and maintain muscle strength after high-intensity weight training. For more details, see the image below:

Figure 1. Effect of Acute Omega 3 Supplementation Reduces Serum Tumor Necrosis Factor-Alpha (TNF-α) Levels, Pain Intensity and Maintains Muscle Strength After High-Intensity Weight Training

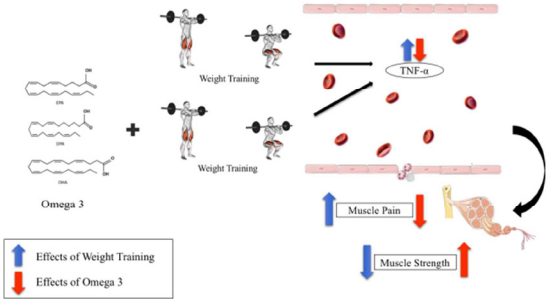

Figure 1. Effect of Acute Omega 3 Supplementation Reduces Serum Tumor Necrosis Factor-Alpha (TNF-α) Levels, Pain Intensity and Maintains Muscle Strength After High-Intensity Weight Training.

Conclusion

Providing omega 3 supplementation can reduce TNF-a levels, pain intensity and maintain muscle strength after high-intensity weight training. Thus, omega 3 supplementation is highly recommended because it can reduce uncontrolled inflammatory response by reducing inflammatory markers, namely serum TNF-a levels caused by high-intensity weight training, so that it can reduce pain intensity and maintain muscle strength which then plays a very important role in supporting performance in running a weight training program so that the maximum training results can be obtained.

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