

Research article

Effects of Omega-3 Supplementation on the Delayed Onset Muscle Soreness after Cycling High Intensity Interval Training in Overweight or Obese Males

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Abstract

People with overweight or obesity preferred high-intensity interval training (HIIT) due to the time-efficiency and pleasure. However, HIIT leads to delayed onset muscle soreness (DOMS). The present study aimed to investigate the effects of omega-3 supplementation on DOMS, muscle damage, and acute inflammatory markers induced by cycling HIIT in untrained males with overweight or obesity. A randomized, double-blinded study was used in the present study. Twenty-four males with a sedentary lifestyle were randomly assigned to either receive omega-3 (O3) (4 g fish oil) or placebo (Con). Subjects consumed the capsules for 4 weeks and performed cycling HIIT at the 4th week. After 4 weeks-intervention, the omega-3 index of O3 group increased by 52.51% compared to the baseline. All subjects performed HIIT at 4th week. The plasma creatine kinase (CK) level of Con group increased throughout 48h after HIIT. While the CK level of O3 group increased only immediately and 24h after HIIT and decreased at 48h after HIIT. The white blood cell count (WBC) of Con group increased immediately after the HIIT, while O3 group did not show such increase. There was no change of CRP in both groups. O3 group had a higher reduction of calf pain score compared to Con group. O3 group also showed a recovery of leg strength faster than Con group. Omega-3 supplementation for 4 weeks lower increased CK level, reduced calf pain score, and recovery leg strength, DOMS markers after cycling HIIT.

Key words: Delayed onset muscle soreness, fish oil, high intensity interval training, muscle damage, obesity.

Introduction

Obesity is a growing health problem worldwide (Sae-Tan et al., 2014), leading to many chronic diseases. A traditional strategy for alleviating obesity is lifestyle intervention including diet and exercise. Moderate-intensity continuous exercise (MICE) is traditionally recommended for the general population. However, the barriers to exercise in adults are lack of willpower, time, and enjoyment (Astorino et al., 2019). HIIT was reported on the time efficiency in adults with obesity (Sawyer et al., 2016). Previous studies reported that HIIT was more pleasant and enjoyable compared to MICE and vigorous-intensity continuous exercise (Jung et al., 2014; Oliveira et al., 2018). A meta-analysis reported that HIIT was a more effective and time-efficient intervention for improving blood pressure and aerobic capacity levels in youth with obesity in comparison to other types of exercise (García-Hermoso et al., 2016). Short-term MICE and HIIT were reported to have similar effects on body composition improvement in sub-

jects with obesity (Wewege et al., 2017). HIIT reduced total, abdominal, trunk and visceral fat and increased fat free mass in young males with overweight (Heydari et al., 2012). Long-term HIIT also improved cardiorespiratory fitness, cardiometabolic health, endothelial function (Farias-Junior et al., 2019), and improved body composition in subjects with obesity (Gremeaux et al., 2012). Therefore, HIIT seems to be an alternative approach to MICE to improve adults' health and fitness.

Unaccustomed exercise causes muscle soreness (Matsumoto et al., 2009). This symptom is known as delayed onset muscle soreness (DOMS) since it does not happen right away. Soreness is typically described as discomfort, stiffness, or incapacitating pain that limits movement (Kenney et al., 2012). Muscle actions can be classified as eccentric, concentric, and isometric. Eccentric motions usually involve the greatest amount of tension (Lieber and Friden, 2002). Several theories have been proposed to explain DOMS including connective tissue damage, muscle damage, enzyme efflux, and inflammation. The following was proposed as the integration of those theories to explain DOMS. First, high tension forces during eccentric muscle activity cause disruption of structural proteins in muscle fiber (connective tissue damage theory and muscle damage theory). Second, damage to the sarcolemma leads to the accumulation of calcium, which disturbs calcium homeostasis. This activates calcium-dependent proteolytic enzymes that degrade the z-line of sarcomeres, troponin, and tropomyosin (enzyme efflux theory). This also promotes the breakdown of muscle proteins and increasing cell permeability, which allows some cell contents to enter the bloodstream. The cell contents released from myocytes into the bloodstream include creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobins (Matsumoto et al., 2009). After a few hours, neutrophils are significantly increase, while intracellular components and markers of connective tissue damage and muscle damage diffuse into the plasma and interstitium. These substances attract monocytes and convert to macrophages, which is called acute inflammation. Upon exposure to inflammatory environment, macrophages produce prostaglandin E2 (Cheung et al., 2003; Smith, 1991). General progression of DOMS usually initiates symptoms between 24 and 48 h, sore around 24 to 72 h, and subsiding withing 5 to 7 days (Deo et al., 2017). Many studies reported that eccentric exercise resulted in a significant increase in CK levels 24 to 48 h after the exercise and this may peak between 3 to 6 days, depending on the nature of exercise. The appearance of CK in serum was

interpreted as indicator of increased permeability or breakdown of the membrane of muscle cells (Lieber and Friden, 2002). The magnitude of DOMS after exercise also impacts the exercise adherence (Farias-Junior et al., 2019). Moreover, a previous study reported that % body fat (%BF) influenced the level of HIIT-induced muscle damage (Yoon and Kim, 2020). Males with %BF ≥ 20 had higher CK and myoglobin during the recovery period after HIIT compared to males with %BF $\leq 15\%$. Therefore, the study on DOMS reduction gains more attention for people with overweight or obesity because it may help to continue the exercise.

Most of the unaccustomed high-intensity physical activity caused exercise-induced muscle damage was reported in a large eccentric component like weight training and running, while no report about DOMS from a main concentric component exercise like cycling. However, running is a high-impact activity that could lead to the development of joint degeneration, especially in people with overweight or obesity (Bijker et al., 2002; Kotler et al., 2016). Therefore, cycling was chosen for the present study since it is less impact and safer for subjects with overweight or obesity. With the benefits of HIIT mentioned above, the present study aimed to implement HIIT in the form of cycling for subjects with overweight or obesity. Given that most untrained people with overweight and obesity do not engage in vigorous physical activity on a daily basis, determining the magnitude of DOMS after a cycling HIIT session in untrained subjects could provide useful information for strength and conditioning professionals when prescribing HIIT to novice people with overweight and obesity.

Many strategies have been performed to alleviate the severity of DOMS and to restore muscle function as soon as possible. Omega-3 polyunsaturated fatty acids have been shown to increase athletic performance, and reduce oxidative stress, muscle damage, and inflammatory markers in athletes (Gligor, 2016). Several studies showed that omega-3 supplementation reduced DOMS markers including soreness based on the weighted and fully extended measures (Jouris et al., 2011), perceived pain and knee range motion at 48h (Tartibian et al., 2009), TNF- α , prostaglandin E2, interleukin (IL)-6, CK and myoglobin (Tartibian et al., 2011), plasma malondialdehyde (Atashak et al., 2013) and C-reactive protein (CRP) (Lembke et al., 2014). All previous studies were done in weight training or other kinds of resistance training, which are eccentric muscle exercises. A recent study also showed that omega-3 supplementation blunted IL-6 levels in healthy subjects after running-induced muscle damage (Kyriakidou et al., 2021). The effects of omega-3 supplementation on cycling HIIT-induced DOMS and muscle damage are scarce. Herein, the present study aimed to investigate the effects of omega-3 supplementation on DOMS in untrained males with overweight or obesity who performed cycling HIIT.

Methods

Subjects

Twenty-four males (18 - 40 years old), BMI ≥ 23 kg/m² to < 30 kg/m², criteria for Asian population (Jih et al., 2014),

with a sedentary lifestyle or having standard physical activity < 2 times/week (< 20 min/time with self-report) were included in the study. The sample size was computed regarding a prior study (Heydari et al., 2012), under a statistical power of 0.80, alpha error probability of 0.05, and effect size of 2.1 using G*power (version 3.1.9.4, University Kiel, Germany), which yielded 8 subjects per group. To account for the subjects' dropout and a higher statistical power, an additional 4 subjects (50% more) were added to each group which resulted in 12 subjects per group. Twelve subjects per group were required and 50% dropout was allowed. Those, who have a history of type 2 diabetes or cardiovascular disease, dieting or any restrictive dietary habits, smoking, or excessive alcohol consumption (> 3 times/week with 3 drinks/time), allergy to fish or seafood products, consumption of fish oil supplement (> 200 mg/day) and regular consumption of fish (> 2 fish meals/week) were excluded. All subjects were asked to complete a validated semi-quantitative food frequency questionnaire for the assessment of habitual food intake over the preceding 12 months for screening.

The subjects who volunteered to be the participants in the study were invited to attend the orientation at the faculty of Sports Science, Kasetsart University. They were briefed on the details of the study including the purpose, procedure, benefits, potential risks, and duration of the study. The investigator addressed questions and concerns that the subjects had. They were asked to complete Physical Activity Readiness Questionnaire and Health (Ferguson, 2014). All subjects had a physical examination by a medical doctor. All subjects read and signed an informed consent form, which had been approved by the ethics committee of Kasetsart University (KUREC-HS 60/016) to participate in this study, according to the ethical standards of the latest version of the Declaration of Helsinki, 2013.

Experimental design and oral omega-3 supplementation

A randomized, double-blinded, repeated measures study was used in the present study. All subjects were randomly assigned to either placebo group (Con) or Omega-3 (O3) group. The subjects in Con group received placebo capsules and the subjects in O3 group received omega-3 capsules. The subjects were asked to consume 8 capsules/day for 4 weeks and during 48h after the cycling HIIT. The subjects in O3 group received 4 g fish oil (PronovaPure® 500:200TG, Newtrition, BASF, Singapore) containing 2000 mg EPA and 800 mg DHA n-3 fatty acids per day for 4 weeks (Lembke et al., 2014). Placebo capsules contained 100% soybean oil. Both omega-3 and placebo capsules were similar in appearance, texture, and taste. Subjects were asked to return the supplement package every two weeks during the study for compliance. Line mobile application was used to remind the subjects to take the supplement during the study.

Cycling high-intensity interval training (HIIT) protocol

After 4 weeks of omega-3 supplementation, all subjects completed a cycling HIIT session (Heydari et al., 2012).

The subjects were advised to avoid strenuous activity for 72h prior to the test and caffeine consumption for 24h prior to testing. The cycling HIIT session consists of 8s of high-intensity cycling followed by 12s of slow cycling recovery continuously throughout a 20 min session on Monark ergometric (894E peak bike, Sweden). The cycling HIIT workload was set at 80 - 90% of each subject's heart rate (HR) peak at a cadence between 120 and 130 rpm and the recovery period was set at that same amount of resistance but at a cadence of 40 rpm. Thus, before beginning the program, the participant was required to assess the incremental load and sustain a cadence ranging from 120 to 130 rpm. The load of each subject was recorded and used during exercise (2 - 2.5 kg). HR was also monitored, and the load was adjusted to maintain the subjects' heart rate at 80-90% of their peak HR. HR was measured by a heart rate monitor (Polar, Finland). The subjects were instructed to keep their exercise intensity at a level necessary to produce a heart rate between 80-90% HR peak level. Cycling HIIT coordinates with a prerecorded compact disc counting down each sprint in a 3-2-1 manner. The subjects performed a 5-min warm-up and cool-down on the bike prior to and after the exercise session.

Omega-3 index determination

Omega-3 index was determined by collecting blood drop onto PUFAcoat (Xerion, Brighton, Australia) and analyzed at Lipomic laboratory (New Delhi, India). Briefly, blood drops were collected and spotted on the collection card. The cards were kept in a dry and cool place before analysis. The fatty acids in the phospholipid fraction of dried blood spot-on were analyzed using gas chromatography (Liu et al., 2014).

Blood biochemistry analysis

Ten-hour fasting blood samples were collected via venepuncture procedure. Blood was collected into a tube containing EDTA. Blood samples were taken before cycling HIIT and immediately (0h), 24h, and 48h after cycling HIIT. Subjects' blood samples were sent to the BK Lab Health Center (Nakhonpathom, Thailand) for plasma CK, C-reactive protein (CRP) and WBC analysis with a kinetic enzymatic method, chemiluminescence immunoassay, and light scattering method, respectively.

Measurements of delayed onset muscle soreness (DOMS)

The DOMS was measured as muscle soreness, strength, and flexibility. The same investigator recorded these measurements throughout the study. All markers of DOMS were measured before cycling HIIT, and immediately (0h), 24h, and 48h after cycling HIIT. Muscle soreness was rated on a numeric pain rating scale (0 -10; 0 means no pain and 10 means worst possible pain). Perceived pain in the anterior thigh, posterior thigh, and calf region on a non-dominant leg during a stepping up and down from a 40-cm high box were self-reported immediately after the task (Chen et al., 2007). Strength was indirectly determined by 1RM of leg press test with indirect method (NSCA, 2016). The flexibility was measured by using sit and reach box (Heyward, 2005).

Statistical Analysis

All data was presented as mean \pm standard deviation (SD). Statistical comparison of participant characteristics was performed using independent sample t-test. Statistical comparison of other parameters between groups and between time points were carried out by two-way repeated measures ANOVA by IBM SPSS Statistics Version 28.0 (Thaisoftup Co., Ltd., Thailand). When ANOVA found a significant difference, Tukey's post hoc test was used to detect the differences between each time point. The significance level was set at $P < 0.05$.

Results

Characteristics of participants

Demographical data of Con and O3 group at baseline were shown in Table 1 and there were no significant differences between groups. The omega-3 index at the baseline of Con group and O3 group were not significantly different. After 4 weeks of omega-3 intervention, the omega-3 index of O3 group was significantly increased and significantly higher compared to Con group, while the omega-3 index of Con group did not change after intervention. Nutrition intake of each group did not change after 4 weeks of intervention and between groups (Table 1). This indicated that omega-3 intervention for 4 weeks increased the omega-3 index in the subjects.

Table 1. Characteristics of participants. Data are means \pm SD.

Characteristics	Participants	
	Con (n=12)	O3 (n=12)
Age (yrs)	21.17 \pm 4.17	21.17 \pm 3.33
Height (cm)	175.67 \pm 6.39	177.42 \pm 6.30
Weight (kg)	88.06 \pm 10.11	87.33 \pm 9.24
BMI (kg/m ²)	28.38 \pm 1.98	27.55 \pm 2.10
Omega-3 index (%)		
- Baseline	7.26 \pm 1.07	7.37 \pm 1.16
- After 4 weeks	6.18 \pm 0.82	11.24 \pm 1.18 #,*
Nutrition intake		
- Baseline	2216.3 \pm 499.3	2384.5 \pm 360.4
- After 4 weeks	2142.6 \pm 420.4	2444.9 \pm 392.6

means different from exercise group ($P < 0.05$). * means different from baseline ($P < 0.05$).

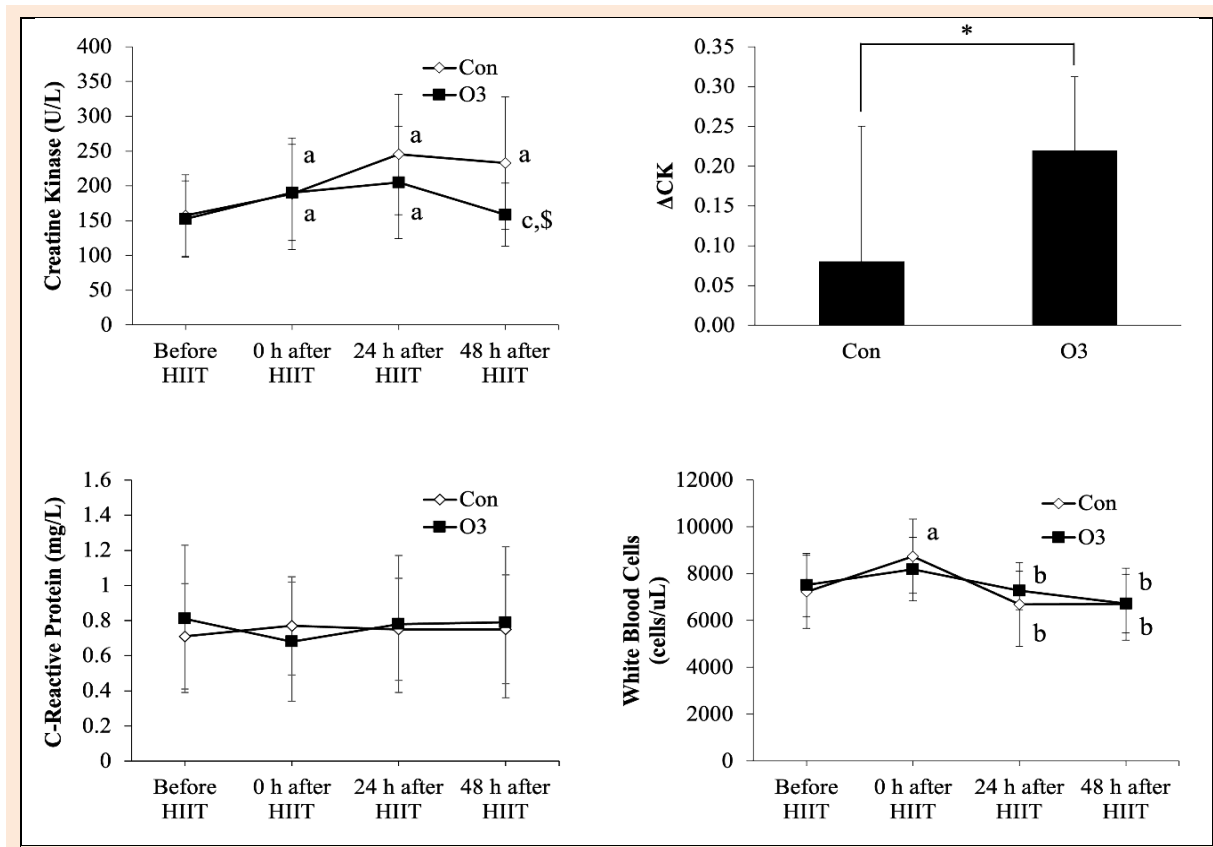


Figure 1. Comparison of creatine kinase (CK) (A), CK reduction (B), C-reactive protein (CRP) (C) and white blood cells (WBC) (D) between Con group and O3 group of untrained males with overweight and obesity at before the HIIT, immediately (0h), 24 hours and 48 hours after the HIIT. CK reduction = $(CK \text{ at } 24h - CK \text{ at } 48h) / CK \text{ at } 24h$. Values are mean \pm SD. Two-way repeated measure ANOVA and Tukey's *post hoc* test was used. * $P < 0.05$, ^a different from before HIIT, ^b different from 0h after HIIT, ^c different from 24h after HIIT. [§] different from Con group at that time point.

Effects of omega-3 intervention on CK, CRP, and WBC after cycling HIIT

The present study showed that the CK level significantly increased after the cycling HIIT session. CK levels increased immediately after exercise (0h) and remained increased at 24h after the cycling HIIT session in both groups (Figure 1A). This confirmed that the cycling HIIT session in the present study induced muscle damage. The CK levels of Con group at 0h, 24h and 48h after the cycling HIIT session were significantly higher compared to the level before the cycling HIIT session, and no significant difference between 0h, 24h and 48h after the HIIT session in Con group. The CK levels of O3 group at 0h and 24h after the cycling HIIT session were also significantly higher compared to the CK level before the cycling HIIT session. Interestingly, the CK level of O3 group at 48h after the cycling HIIT session was significantly lower compared to the CK level at 24h after the cycling HIIT session and this level was as low as the level before the exercise. The CK level at only 48h after the cycling HIIT session of O3 group was also significantly lower compared to the that of Con group. This suggested that a 4-week intervention of omega-3 reduced the elevated plasma CK level after the cycling HIIT session. To compare the recovery between groups, CK reduction was calculated, and the results showed that the CK reduction of O3 group was significantly more than the CK reduction of Con group (Figure. 1B). This suggested that omega-3 intervention for 4 weeks efficiently reduced the

cycling HIIT-induced CK.

There was no significant change in CRP in both groups throughout 48 h after the cycling HIIT session (Figure 1C).

Here we found that immediately after the cycling HIIT session, the WBC of Con group significantly increased, while the WBC of O3 group was unchanged compared to the WBC before the cycling HIIT session (Figure 1D). There were no differences in WBC in both groups at all time points. However, the levels of WBC at 24 and 48 h after the cycling HIIT session of both Con and O3 group significantly lowered compared to the WBC at 0 h after the cycling HIIT session. This indicated that 4-week omega-3 intervention delayed the increase of WBC induced by cycling HIIT.

Effects of omega-3 intervention on muscle soreness, flexibility, and strength after cycling HIIT

The present study evaluated the muscle soreness of subjects in a scoring system. Although there was no significant difference in pain scores of the anterior thigh, posterior thigh, and calf between Con and O3 group at each time point, there were differences between time points (Figure 2A, 2B, and 2C). The results showed that pain scores at the anterior thigh and posterior thigh but not the calf of Con and O3 group at 24h after the cycling HIIT session were significantly higher than the pain scores before and 0h after the HIIT session. The pain scores of the anterior thigh and

posterior thigh at 48h after the cycling HIIT session were significantly lower than the pain scores at 24h after the cycling HIIT session in both groups. This indicated that painfulness induced by cycling HIIT was significantly decreased at 48h after the HIIT session in both groups. However, there was no significant difference between pain score of anterior and posterior thigh between Con and O3 group. When comparing pain score reduction between groups, the results showed that the calf pain score reduction of O3 group was significantly higher than the calf pain score reduction of Con group (Figure 2D). This indicated that 4-

week omega-3 intervention efficiently reduced the cycling HIIT-induced calf pain better than placebo.

There was no change of flexibility in both groups (Figure 3A). Immediately after the cycling HIIT session, the strength of both groups significantly reduced compared to the strength before the cycling HIIT session. The strength of O3 group was significantly increased since 24 h after the HIIT session, while the strength of Con group was significantly increased at 48 h after the HIIT session (Figure 3B). This indicated that 4-week omega-3 intervention improved the strength faster than placebo.

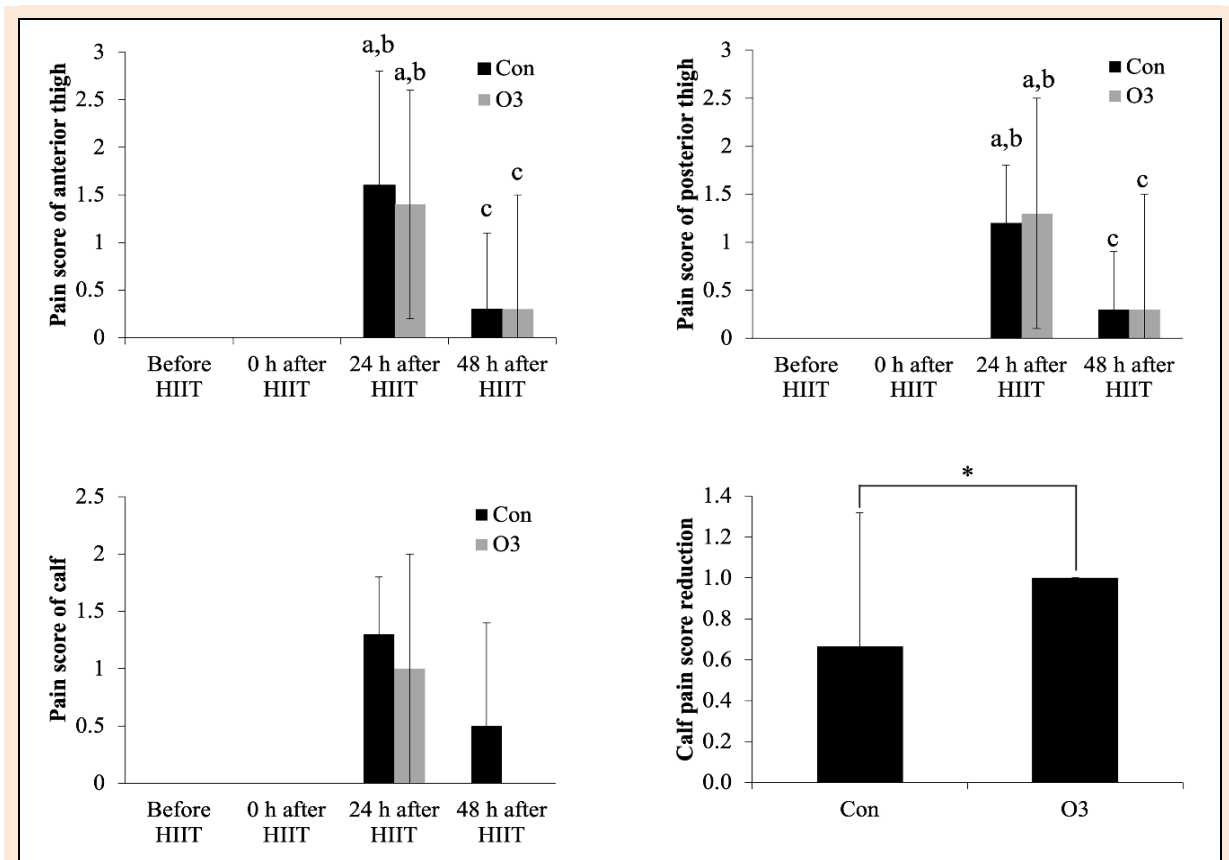


Figure 2. Comparison of mean variations of pain scores of anterior thigh (A), posterior thigh (B) and calf (C) and calf pain score reduction (D) in Con group and O3 group of untrained males with overweight and obesity at before the HIIT, immediately (0h), 24 hours and 48 hours after the HIIT. The calf pain score reduction = (calf pain score at 24h - calf pain score at 48h)/calf pain score at 24 h). Values are mean \pm SD. Two-way repeated measure ANOVA and Tukey's post hoc test was used. * $P < 0.05$, ^a different from before HIIT, ^b different from 0h after HIIT, ^c different from 24h after HIIT.

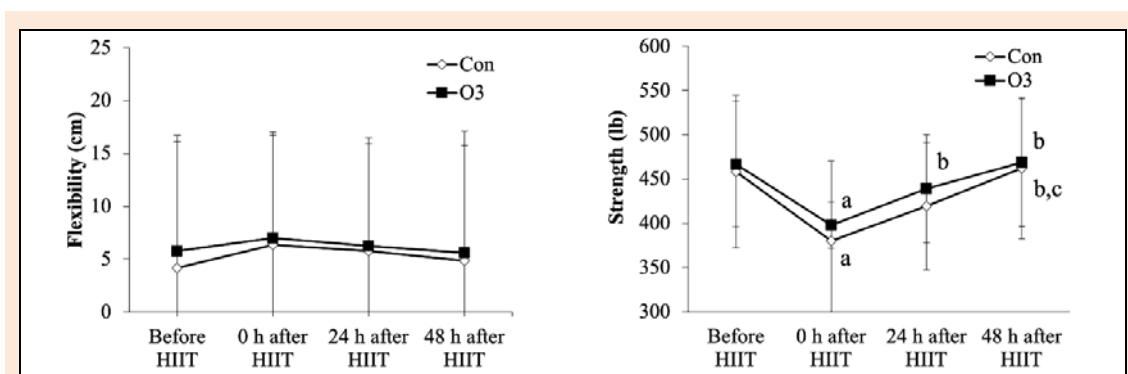


Figure 3. Comparison of mean variations of flexibility (A) and strength (B) in Con group and O3 group of untrained males with overweight and obesity at before the HIIT, immediately (0h), 24 hours and 48 hours after the HIIT. Values are mean \pm SD. Two-way repeated measure ANOVA and Tukey's post hoc test was used. ^a different from before HIIT, ^b different from 0h after HIIT, ^c different from 24h after HIIT.

Discussion

HIIT is preferred due to the enjoyment, pleasantness, and time efficiency. However, unaccustomed exercise could lead to DOMS and several theories have been proposed to explain DOMS including muscle damage and acute inflammation. Therefore, the present study aimed to investigate the effects of omega-3 intervention on cycling HIIT-induced DOMS, muscle damage, and inflammatory responses in untrained males with overweight or obesity. Muscle damage is characterized by the release of CK due to increased cell permeability. The increased CK level begins around 24 h, peaks around 3 to 6 days, and has been used as a marker of muscle damage. Damaged tissue may progress to neutrophil accumulation and acute inflammation (Cheung et al., 2003; Tartibian et al., 2009). Although previous studies showed that omega-3 reduced DOMS (Jouris et al., 2011; Kyriakidou et al., 2021; Lembke et al., 2014; Tartibian et al., 2009; Tartibian et al., 2011), these benefits were studied only in eccentric exercise including weight training and running.

Cycling is considered a more concentric exercise (Bijker et al., 2002), which is suitable for subjects with overweight or obesity due to less impact and less risk of injury to the subjects (Messier, 2010). Therefore, this study applied cycling HIIT to untrained males with overweight or obesity and investigating the effects of omega-3 on cycling HIIT-induced DOMS, muscle damage, and acute inflammatory responses. The present study showed that cycling HIIT induced DOMS as shown with increased muscle pain score, low flexibility, and low strength. Cycling HIIT also increased CK and WBC. CK is generally considered to be an indirect marker of muscle damage due to the increased cell permeability after exercise (Baird et al., 2012). The previous study showed that CK level of healthy young male volunteers significantly increased at 3h after the cycling session and gradually increased after that until day 3 after cycling (Totsuka et al., 2002). Calf rising exercise also increased CK levels at 48h after exercise in untrained men (da Silva et al., 2018). The increased CK in the present study was consistent with the previous study showing that males with obesity had high CK levels after HIIT (Yoon and Kim, 2020). However, the increased CK level induced by cycling HIIT in this study was much lower compared to the previous study using an elbow flexor, which is an eccentric exercise (Yoon and Kim, 2020). This indicated that cycling HIIT resulted in less CK level. This may infer that cycling HIIT may be suitable for the general population since it is likely to cause less muscle damage. However, the increased CK and WBC from the present study cannot be neglected because it could lead to discontinuing the exercise. The present result on CRP was consistent with the previous study showing that the CRP levels of subjects were not changed after isokinetic contraction (Franklin et al., 1992). They explained that this may be due to the isokinetic contraction did not cause acute inflammation, CRP was not always the marker of acute inflammation, and the radial immunodiffusion method that they used to detect CRP was not sensitive enough. This agreed with CK's results that cycling HIIT did not cause a sudden and steady increase. This indicated there was no muscle injury

after cycling HIIT.

The present study showed that the painfulness reduction and strength recovery of muscle in O3 group were quicker than those in Con group. This indicated that omega-3 intervention for 4 weeks effectively reduced DOMS after the cycling HIIT session. Although the difference in pain scores between groups in each area was significant, the differences were minimal, and this may be insignificant in practice. The same trends were observed in flexibility and strength. This may be because cycling HIIT induced less muscle damage, as shown by the lower CK compared to other studies. It is possible that other markers may have peaked prior to the 24 h after exercise, while there was no measurement in that period in this study.

Although the effects of omega-3 intervention on exercise-induced DOMS have been studied, those exercises were eccentric exercise, and the results were not conclusive. Previous studies showed that ingestion of omega-3 ameliorated eccentric exercise-induced DOMS in untrained subjects (Lembke et al., 2014; Tartibian et al., 2009). However, omega-3 intervention did not reduce DOMS induced by isokinetic eccentric elbow flexion contractions (Lenn et al., 2002). A recent study in healthy males also showed that omega-3 supplementation tended to decrease muscle damage-induced running, however, no significant improvement was found (Kyriakidou et al., 2021). The differences between studies may be due to the different dose of omega-3 and subject conditions.

One of the theories to explain the pain associated with DOMS is muscle damage (Cheung et al., 2003). The present study showed that omega-3 intervention for 4 weeks attenuated some HIIT-induced muscle damage marker as shown by the reduction of CK. The present study also showed that O3 group had a faster reduction of CK level after exercise compared to Con group and no increase of WBC after exercise. However, the CRP levels of both groups did not change over time. This inferred that cycling HIIT could induce an increased CK but not progress to muscle injury. The reduction of CK with omega-3 intervention also contributed to the reduction of DOMS as shown by the great reduction of calf pains. However, the present study did not find a significant change in IL-6 and TNF- α in both groups (data not shown), this may be because macrophage infiltration to damaged tissues and pro-inflammatory cytokines secretion occurs after acute inflammation (Cheung et al., 2003), while the present study did not cause inflammation and muscle injury.

Omega-3 intervention possibly has beneficial effects on cycling HIIT-induced DOMS in two ways. Firstly, omega-3 fatty acids such as EPA and DHA reduce a variety of eicosanoids including thromboxane, leukotrienes, and prostaglandins (Healy et al., 2000). These eicosanoids are involved in the breakdown of damaged muscle fibers and connective tissue (Cheung et al., 2003). The reduction of eicosanoids results in the reduction of DOMS. Secondly, omega-3 has beneficial effects on muscle recovery. After exercise or competition, all athletes experience fatigue and challenge muscle recovery. Although protein has been well recognized as the main component of muscle protein synthesis for the muscle recovery process, omega-3 has been reported to facilitate the remodeling process of muscle pro-

tein synthesis (Heaton et al., 2017). Omega-3 was reported that synergistically works with ingested protein in increasing protein utilization for muscle protein synthesis (Huang et al., 2020; Malta et al., 2019).

The strength of this present study are (1) cycling HIIT is suitable for subjects with overweight or obesity due to less impact and less risk of injury to people with overweight and obesity (Messier, 2010), (2) cycling is considered more concentric exercise and the present study investigated DOMS induced by concentric exercise, while many previous studies focused on eccentric exercise, and (3) the use of omega-3 supplement with high bioavailability so that we were able to increase omega-3 index after 4-week intervention.

However, the present study had a few limitations. First, the participants in this study are untrained healthy males with overweight or obesity, so the data cannot be generalized to males with overweight or obesity with chronic diseases or well-trained males with overweight or obesity. The BMI cut points that were applied in the present study were specific to the Asian population. Second, we did not measure self-efficacy or future intention to exercise, which are important factors of willingness to engage in long-term exercise (Astorino et al., 2019). Third, we did not measure antioxidant status, which could be used to explain the effects of omega-3 supplementation. The supplementation with linseed oil, high in polyunsaturated fatty acids, benefited pro- and anti-oxidant balance in obesity-induced oxidative stress in high-fat diet-fed rats (Groussard et al., 2021). Therefore, the aspect of anti-oxidant mechanism of omega-3 remains as a hypothesis. These limitations should be addressed in future studies.

Conclusion

Ingestion of omega-3 4g/d for 4 weeks increased the omega-3 index of males with overweight or obesity. Omega-3 intervention for 4 weeks lowered a marker of muscle damage (CK) and reduced pain score and increased muscle strength after the cycling HIIT more rapidly than placebo. This indicated the reduction of HIIT-induced DOMS in males with overweight or obesity by omega-3 supplementation.

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Key points

- Omega-3 supplementation 4g/d for 4 weeks increased omega-3 index in males with overweight or obesity.
- Omega-3 supplementation reduced creatine kinase and pain scores, while increased muscle strength after the first cycling HIIT more rapidly than placebo.
- Omega-3 supplementation could reduce DOMS and inflammatory markers induced by cycling HIIT.
- The present results can be applied to people with overweight or obesity who desired to exercise for weight management but encounter DOMS after exercise.

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