Effect of Virgin Coconut Oil Supplementation on Immune Function in a Rat Model of Physical Exercise

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Track Record Article

Revised: 09 January 2026
Accepted: 04 June 2026
Published: 30 June 2026

How to cite :
Sinaga, F. A., Sinaga, R. N., Ginting, A. A., & Elvana, A. (2026). Effect of Virgin Coconut Oil Supplementation on Immune Function in a Rat Model of Physical Exercise. *Contagion: Scientific Periodical Journal of Public Health and Coastal Health*, *8*(2), 63–73.

Abstract

Physical exercise can induce physiological stress that triggers inflammatory responses, marked by increased levels of proinflammatory cytokines and acute-phase proteins. Virgin Coconut Oil (VCO) contains medium-chain fatty acids that are known to possess anti-inflammatory and immunomodulatory properties, which may help regulate immune responses caused by physical activity. This study aimed to evaluate the effect of VCO supplementation on immune function by examining serum levels of Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and C-Reactive Protein (CRP) in a rat model subjected to physical exercise. An experimental approach was conducted using rats divided into four groups, consisting of a control group and several VCO-treated groups undergoing physical exercise. VCO was administered orally at varying doses throughout the exercise intervention period. At the end of the treatment, blood samples were collected, and serum concentrations of IL-6, TNF-α, and CRP were analyzed using immunoassay techniques. The data were evaluated descriptively to observe trends among the treatment groups. The findings demonstrated a consistent decrease in IL-6, TNF-α, and CRP levels in rats receiving VCO supplementation compared to the control group. The most pronounced reduction in all inflammatory markers was observed in the group receiving the highest dose of VCO, indicating a dose-dependent effect. These results suggest that VCO supplementation effectively attenuated exercise-induced inflammatory responses. Overall, VCO supplementation shows potential immunomodulatory and anti-inflammatory effects in rats exposed to physical exercise, as evidenced by reduced levels of IL-6, TNF-α, and CRP. VCO may therefore be considered a functional nutritional supplement to support immune balance during physical activity. However, further studies are needed to confirm these findings and to clarify the underlying biological mechanisms involved

Keywords: ***Virgin Coconut Oil, Physical Exercise, Immune Function, Nutraceuticals, Experimental Animal Models, Immunomodulation, Rat Model***

INTRODUCTION

High-intensity physical activity or exercise to the point of exhaustion often triggers a systemic inflammatory response and severe oxidative stress, which paradoxically suppresses the immune system (Cerqueira et al., 2020; Leandro & Silva, 2020). Prolonged strenuous exercise is strongly associated with an increased risk of respiratory tract infections and impaired immune cell function due to heavy training loads (Cerqueira et al., 2020; Suzuki, 2021). Mechanistically, this condition triggers the excessive and uncontrolled release of pro-inflammatory cytokines, commonly referred to as a “cytokine storm”, which acts as a primary factor causing tissue damage and multi-organ failure (Basheer et al., 2022; Filgueira et al., 2021). The catastrophic impact of this hyperinflammatory pathway has been widely highlighted during the COVID-19 pandemic, where non-surviving patients exhibited

significantly higher plasma levels of Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Tumor Necrosis Factor-alpha (TNF-α) compared to recovered individuals (Chen et al., 2020; Huang et al., 2020; Ruan et al., 2020). Additionally, elevated serum C-Reactive Protein (CRP > 5 mg/L) levels have been established as a crucial clinical predictor of extensive lung tissue damage and subsequent fibrosis (Luo et al., 2020; Saputro et al., 2022). Therefore, identifying immunomodulatory strategies that can suppress this harmful inflammatory cascade while protecting tissue integrity is a matter of great urgency in modern functional nutrition (Suzuki, 2021; Suzuki & Wu, 2025).

In this specific context, Virgin Coconut Oil (VCO) has emerged as a promising candidate for targeted functional nutrition aimed at reducing exercise-induced systemic stress. The therapeutic potential of VCO is primarily attributed to its unique composition of medium-chain fatty acids (MCFAs), such as lauric acid, which exerts potent immunomodulatory effects by enhancing macrophage phagocytosis and optimizing antibody titer formation. (Sebayang et al., 2021; Widianingrum & Salasia, 2021). Unlike long-chain saturated fats, the MCFAs in VCO are rapidly absorbed and efficiently metabolized via beta-oxidation to support immediate cellular energy production and maintain mitochondrial biogenesis (Wang et al., 2018). This biochemical pathway theoretically protects the immune system from debilitating oxidative stress and metabolic fatigue triggered by intense physical activity (Suzuki & Wu, 2025; Wang et al., 2018). However, while other nutritional interventions, such as a cocoa-rich diet, have been shown to maintain certain baseline immune parameters in animal models, the specific efficacy of VCO fatty acids in maintaining homeostasis during extreme physical exhaustion remains poorly understood (Suzuki, 2021). Current evidence suggests that while MCFA supplementation can modulate general metabolism, empirical and mechanistic data evaluating how VCO prophylaxis specifically alters post-exercise pro-inflammatory cytokine dynamics and immune cell recovery in animal models remain very limited (Sinaga et al., 2024).

To address this critical empirical gap, further investigation is urgently needed to validate the precise mechanisms of VCO action under acute physical stress. Understanding whether VCO-based nutritional pre-treatment can actively restore immune homeostasis disrupted by physical fatigue holds vital clinical and practical relevance. Therefore, this study aims to evaluate specific changes in the profile of pro-inflammatory cytokines and analyze the dynamics of immune cell populations in a mouse model receiving VCO supplementation prior to undergoing a strenuous physical exercise protocol. We systematically test the hypothesis that prophylactic VCO intervention can successfully attenuate detrimental systemic inflammatory responses and accelerate post-exercise immune recovery.

METHODS

This study used a randomized controlled experimental laboratory design. The experimental protocol was reviewed and officially approved by the Health Research Ethics Committee of the University of North Sumatra under registration number 0220/KEPH-FMIPA/2023. The production and formulation of Virgin Coconut Oil (VCO) were conducted at the Formulation Laboratory, Faculty of Pharmacy, University of North Sumatra (USU). The in vivo laboratory phase (Alpha and Beta testing) was conducted at the USU Pharmacology Laboratory and the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra, Medan, Indonesia, between March and October 2023.

A total of 48 healthy male Wistar rats (*Rattus norvegicus*), aged approximately 8–10 weeks and weighing between 180 and 220 grams, were used in this study. Prior to the commencement of the experimental period, all animals were acclimatized to laboratory conditions for 7 days to eliminate stress. The rats were housed in standard polycarbonate cages (3–4 rats per cage) under a controlled environment with a 12-hour light/dark cycle, an ambient temperature maintained at 22 ± 2°C, and a relative humidity of 50% ± 10%. The animals had ad libitum access to a standard commercial pellet diet and clean drinking water throughout the study. All procedures regarding animal handling and care strictly adhered to international guidelines for the care and use of laboratory animals.

The formulation of VCO followed a controlled fermentation method. Fresh coconut flesh was removed, grated, and mixed with hot water (70°C) at a strict 2:1 ratio (w/v). The mixture was squeezed and filtered to extract the coconut milk. The extract was transferred into a large transparent vessel and allowed to settle for 2–3 hours until a distinct separation into two layers occurred: the upper cream layer and the lower skim layer. To initiate controlled fermentation, the cream layer was isolated, and 0.1 g of *Saccharomyces cerevisiae* (Fermipan) dissolved in 10 mL of warm water was uniformly incorporated. The mixture was then divided into 350 mL fermentation bottles and incubated at room temperature (26-28°C) under varying time blocks of 14, 16, 18, 20, 22, and 24 hours. The bottles were sealed to prevent dust and external contaminants. Following incubation, the resulting three distinct layers VCO, gelondo (protein mass), and water were observed. The pure VCO fraction was separated from the galendo using standard analytical filter paper and collected for supplementation.

The 48 Wistar rats were randomly allocated into four experimental groups (n = 12 per group) using a computer-generated randomization sequence:

Group 1 (Control / No Exercise): Received standard diet + distilled water vehicle.

Group 2 (Exercise Only): Subjected to the exercise protocol + distilled water vehicle.

Group 3 (VCO Only / No Exercise): Received oral VCO supplementation without exercise.
Group 4 (Exercise + VCO Supplementation): Subjected to the exercise protocol and received oral VCO supplementation.

VCO was administered daily via oral gavage at a therapeutic dose of 2 mL/200 g body weight (BW) for a consecutive duration of 6 weeks.

At the end of the 6-week supplementation period, rats in Group 2 and Group 4 were subjected to an acute, high-intensity exhaustive exercise protocol using a standardized Forced Swimming Test (FST). The swimming test was conducted in a cylindrical acrylic tank (diameter: 50 cm, height: 80 cm) filled with water to a depth of 50 cm to prevent the rats from supporting themselves with their tails or paws touching the bottom. The water temperature was strictly maintained at a thermoneutral range of 25±1°C. To standardize the exercise intensity and accelerate exhaustion, a constant physical load equivalent to 5% of the rat's individual body weight was securely attached to the base of the tail using a lead weight. The animals swam continuously until they reached a state of complete physiological exhaustion. Exhaustion was operationally defined using strict criteria: the point at which the rat remained submerged beneath the water surface for 10 consecutive seconds without attempting to surface or show coordinated swimming movements. Upon reaching exhaustion, the rats were immediately removed from the water, gently dried with a warm towel, and prepared for biofluid collection.

Immediately following the exhaustive exercise protocol, all rats were deeply anesthetized. Whole blood samples were collected via cardiac puncture using a sterile 21-gauge needle attached to a 5 mL syringe. The collected blood was immediately transferred into two types of tubes: tubes containing ethylenediaminetetraacetic acid (EDTA) for whole blood analysis and non-coagulant tubes for serum separation. To isolate the serum and plasma, the blood samples underwent immediate centrifugation at 3,000 rpm for 15 minutes at 4°C. The clear supernatant fractions (serum and plasma) were carefully aliquoted into sterile microcentrifuge tubes. Samples intended for delayed analysis were immediately frozen and stored at -80°C to preserve protein stability and prevent the degradation of inflammatory markers.

The 48 male rats were divided into 4 groups randomly, with 12 in each. Group-1: No Exercise; Group-2: Exercise; Group-3: No Exercise + VCO Supplementation; Group 4: Exercise + VCO Supplementation. VCO administration is given daily at a dose of 2ml/200 g BB for 6 weeks. After 6 weeks, all the rats were told to do maximum physical activity by forcing them to swim. Next, IL-6 levels, CRP levels, and TNF-α levels were measured.

Hematological examination

Blood plus the anticoagulants EDTA, sodium citrate, and heparin. If the blood sample testing is delayed, the separated plasma and serum should be stored at -80°C until the sample is analyzed for anti-inflammation.

RESULT

Effect of VCO Administration on Interleukin-6 Levels

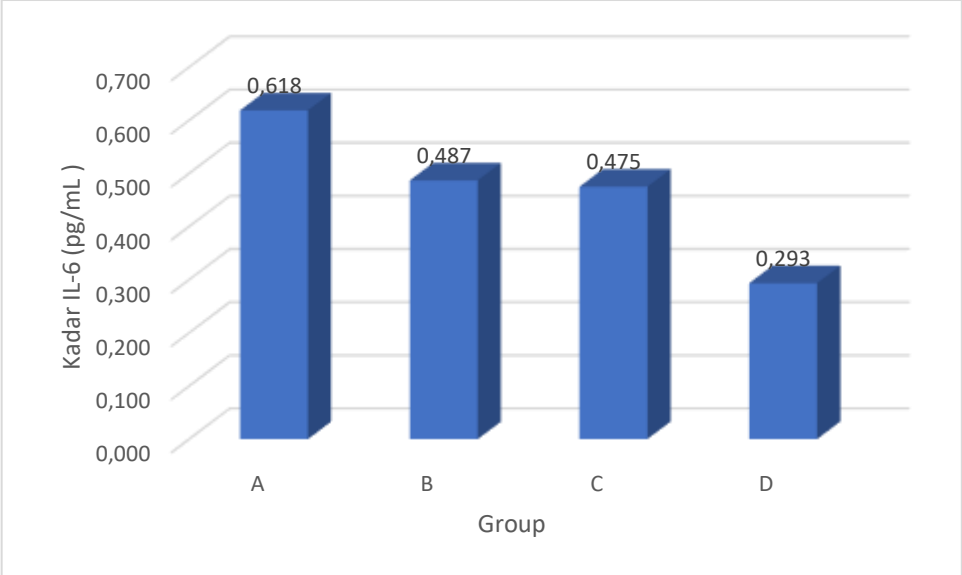

Figure 1. Effect of VCO Administration on IL-6 Levels

Description:
A : Non-Practice Group
B : Practice Group
C : Non-Practice Group + VCO
D : Training Group + VCO

The results of measuring interleukin-6 (IL-6) levels in mice who performed physical activity showed a difference in mean values between treatment groups. Group A had the highest levels of IL-6 with an average value of 0.618, followed by Group B at 0.487, and Group C at 0.475. Meanwhile, the lowest levels of IL-6 were found in Group D with an average value of 0.293.

Descriptively, there is a trend of decreasing IL-6 levels along with the administration of Virgin Coconut Oil (VCO) to rats undergoing physical activity. The group receiving VCO treatment showed lower levels of IL-6 than the group without or with minimal treatment, indicating the potential immunomodulatory and anti-inflammatory effects of VCO in responding to physiological stress due to physical activity.

This decrease in IL-6 levels reflects that VCO supplementation has the potential to suppress the inflammatory response that is generally enhanced by intensive physical exercise. This suggests that VCO administration may play a role in maintaining a balance of the immune

response in mice experiencing exercise stress, as shown by a decrease in the inflammatory mediator IL-6.

Effect of VCO Administration on CRP Levels

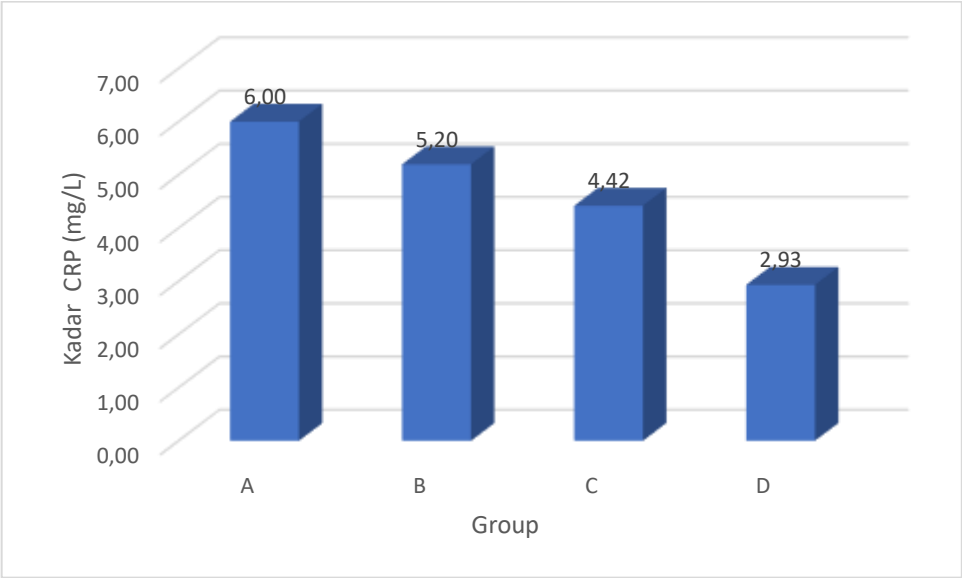

Figure 2. The Effect of VCO Administration on CRP Levels

Description:
A : Non-Practice Group
B : Practice Group
C : Non-Practice Group + VCO
D : Training Group + VCO

Measurements of C-Reactive Protein (CRP) levels in mice undergoing physical activity showed variations in values between treatment groups. Group A showed the highest CRP level with an average value of 6.00, followed by Group B at 5.20, and Group C at 4.42. In contrast, Group D showed the lowest CRP level, which was 2.93.

The average difference illustrates a progressive decrease in CRP levels in the group that received Virgin Coconut Oil (VCO) treatment. This pattern suggests that the administration of VCO is associated with a decrease in systemic inflammatory responses arising from physical activity. CRP as a marker of acute -phase inflammation appears to be more pronounced in the group with the VCO intervention compared to the control group.

Biologically, low CRP levels in the treatment group indicate that VCO has the potential to play a role in suppressing inflammation due to exercise stress, thereby supporting physiological adaptation processes during physical activity. These findings show that VCO supplementation is not only associated with modulating the immune response but also with the control of systemic inflammation in physically active mice.

Effect of VCO Administration on TNF-α Levels

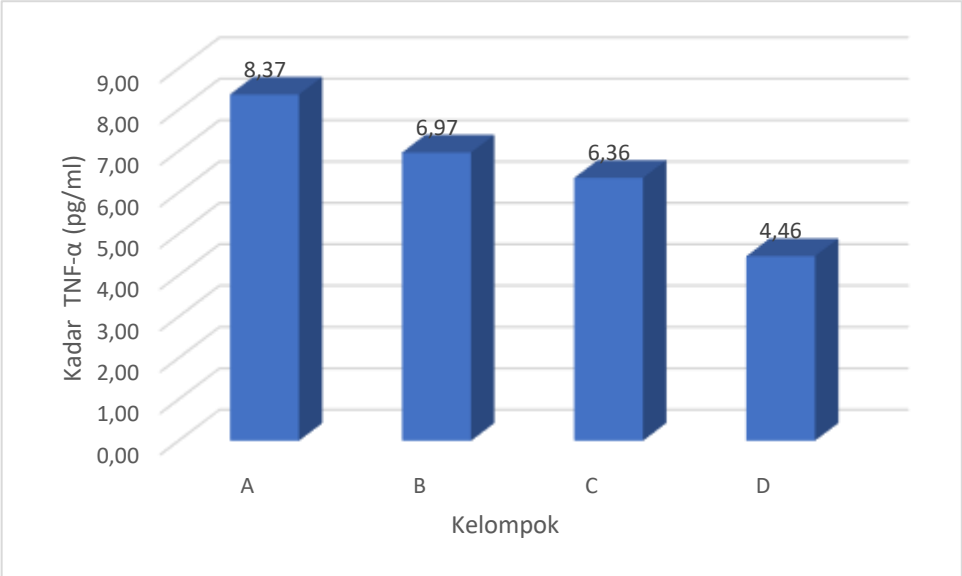

Figure 3. Effect of VCO Administration on TNF-α Levels

Description:
A : Non-Practice Group
B : Practice Group
C : Non-Practice Group + VCO
D : Training Group + VCO

The results of the analysis of Tumor Necrosis Factor-α (TNF-α) levels in mice who performed physical activity showed differences in average values between treatment groups. Group A showed the highest levels of TNF-α with an average value of 8.37 pg/mL, followed by Group B of 6.97 pg/mL and Group C of 6.36 pg/mL. The lowest value was found in Group D with an average level of 4.46 pg/mL.

The distribution of these values showed a decrease in TNF-α concentration in the group of rats that received Virgin Coconut Oil (VCO). This decrease reflects a reduced activation of proinflammatory cytokines that generally increase in response to physical activity. Compared to the non-intervention group, the group with VCO supplementation showed lower levels of TNF-α, indicating the protective effect of VCO on the inflammatory response due to exercise.

Overall, these findings indicate that VCO administration has the potential to support the regulation of inflammatory responses in physically active mice, as reflected in the decrease in TNF-α inflammatory mediators.

DISCUSSION

Physical activity is known to trigger systemic inflammatory responses as part of physiological adaptations to the mechanical and metabolic stress experienced by body tissues (Gleeson, 2013). This inflammatory response is generally characterized by an increase in

proinflammatory cytokines and acute phase proteins that serve as mediators between tissue damage and the recovery process (Walsh et al., 2011). Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and C-Reactive Protein (CRP) are key biomarkers often used to evaluate the inflammatory dynamics of physical activity (Wunderle et al., 2025).

IL-6 is a pleiotropic cytokine released by skeletal muscle during contraction and plays a role in the regulation of energy metabolism and immune response during exercise (Pedersen & Febbraio, 2008). Although IL-6 plays a role in physiological adaptation, excessive increases in IL-6 levels can contribute to systemic inflammation and an imbalance of the immune response. A decrease in IL-6 levels in a group of mice receiving Virgin Coconut Oil (VCO) in this study showed a modulation of the inflammatory response due to exercise stress (Nurdiana et al., 2025).

TNF-α is a major pro-inflammatory cytokine that plays a role in immune cell activation and amplification of inflammatory responses in tissue damage conditions (Bradley, 2008). Intensive physical activity can increase TNF-α production in response to muscle microtrauma and macrophage activation. Decreased levels of TNF-α in the group receiving VCO indicated that this supplementation has the potential to suppress the primary inflammatory pathways activated during exercise stress (Calder, 2011).

CRP is an acute-phase protein synthesized in the liver in response to increased proinflammatory cytokines, especially IL-6 and TNF-α (Chabuk et al., 2025). CRP levels reflect overall systemic inflammation and are often used as an indicator of low-level chronic inflammation due to repetitive physical activity (Stec-martyna et al., 2025). The decrease in CRP levels in the VCO group suggests that the anti-inflammatory effects of VCO not only occur at the cytokine level but also have an impact on the systemic inflammatory response (Kasapis & Thompson, 2005).

Virgin Coconut Oil contains medium-chain fatty acids (MCFAs), especially lauric acid, which have anti-inflammatory and immunomodulatory properties (Marina et al., 2009). MCFAs are metabolized faster than long-chain fatty acids so they do not trigger the accumulation of pro-inflammatory lipids in peripheral tissues (St-Onge & Jones, 2002). Lauric acid in VCO is known to inhibit the activation of nuclear factor kappa B (NF-κB), which is the main transcription factor that regulates the expression of IL-6 and TNF-α genes (Intahphuak et al., 2010). In addition, physical activity increases the production of reactive oxygen species (ROS) which can activate inflammatory pathways and increase the production of pro-inflammatory cytokines (Powers & Jackson, 2008). Virgin Coconut Oil is reported to have antioxidant activity that is able to reduce oxidative stress due to physical exercise (Arlee et al.,

2013). This decrease in oxidative stress contributes to a reduction in inflammatory stimuli that trigger an increase in IL-6, TNF-α, and CRP (Ayala et al., 2014).

The balance between inflammatory and anti-inflammatory responses is essential to support exercise adaptation without impairing immune function. Simultaneous decreases in IL-6, TNF-α, and CRP levels in the group receiving VCO suggest that these supplementation could potentially help maintain inflammatory homeostasis during physical activity (Calder, 2011). These conditions allow the process of tissue recovery and physiological adaptation to take place more efficiently (Nieman, 2000).

Overall, the results of this study show that VCO administration provides a comprehensive inflammatory modulating effect in mice that perform activities. This effect is reflected in the decrease in proinflammatory cytokines (IL-6 and TNF-α) and acute phase proteins (CRP) as indicators of systemic inflammation. These findings reinforce the role of VCO as a functional nutrient that has the potential to be used as a support strategy in the management of inflammation due to exercise stress (Pederson, 1975).

CONCLUSION

The administration of Virgin Coconut Oil (VCO) to mice engaged in physical activity showed a tendency to lower levels of IL-6, TNF-α, and CRP compared to the control group. These findings indicate that VCO has the potential to modulate the inflammatory response due to exercise stress. Thus, VCO can be considered a supportive functional nutrient in maintaining inflammatory balance during physical activity, although further research is needed for further confirmation.

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