

# Effect of Giving Beetroot Juice During Submaximal Exercise Against Muscle Damage

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## 1 Effect of Giving Beetroot Juice During Submaximal Exercise Against Muscle Damage.

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**Abstract.** Strenuous physical activity causes muscle damage marked by an increase in C-reactive protein (CRP) concentrations. Muscle damage caused by physical activity can be prevented or reduced by giving antioxidants and anti-inflammatory. It is known that beetroot (*Beta Vulgaris* L) has antioxidant and anti-inflammatory effects. The purpose of this study was to determine the effect of giving beet juice during submaximal exercise on concentrations of C-reactive protein (CRP). This type of research is an experimental study with a randomized control group pretest-posttest design. The research was conducted at the physiology laboratory of Department of Sports Science Faculty Universitas Negeri Medan and the integrated Laboratory of the Faculty of Medicine at University of Sumatera Utara. The study used 20 futsal athletes who met the criteria. Furthermore, the sample was divided into 2 groups (experiment = 10, control = 10). The pre-test was done by checking the CRP concentrations prior to submaximal physical exercise. During one month of sub-maximal exercise program with a frequency of 3 times a week with an intensity of 80-90%, the experimental group was given 300 ml of beetroot juice one hour before submaximal exercise, while the control group was given a placebo drink. Measurement of research variables was carried out again immediately after submaximal exercise for one month. The results showed that beetroot juice supplementation at a dose of 300 ml/day in submaximal exercise significantly reduced CRP levels compared to the control group ( $18.00 \pm 5.65$  mg/L VS  $74.66 \pm 23.85$  mg/L;  $p = 0.000$ ). In conclusion, giving beetroot juice during training for one month can reduce the CRP levels that are increased in submaximal physical exercise in athletes. The decrease in CRP levels is due to the antioxidant content found in beetroot juice.

### 1. Introduction

Regular physical activity can improve health, and is protective for non-communicable diseases such as cardiovascular disease, stroke, diabetes, and some types of cancer [1], and physical activity is also associated with improved mental health [2]. However, strenuous physical activity can be dangerous, because it can cause exercise-induced muscle damage (EIMD), inflammation and also cause fatigue [3,4].

Physical activity-induced muscle damage (EIMD), especially eccentric muscle contraction with high intensity can cause inflammation by activating Nuclear Factor kappa B (NF- $\kappa$ B) [5], which triggers an increase in inflammatory markers such as C-Reactive Protein (CRP) and several inflammatory interleukins such as interleukin (IL)-6, IL-8, IL-10, interleukin 1 beta (IL-1 $\beta$ ) tumor necrosis factor (TNF- $\alpha$ ) [6,7] and the increasing quantity and activity of stress proteins, especially Heat Shock Proteins 70 (HSP70) [8].



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During strenuous exercise it can cause oxidative stress due to an imbalance between reactive oxygen species and the body's antioxidants [9,10]. As a result, excessive accumulation of ROS can also activate Nuclear Factor kappa B (NF- $\kappa$ B)[11] and also attack vital biomolecules such as plasma membrane lipids and proteins that contribute to muscle damage, inflammation and fatigue [5].

One strategy for reducing EIMD is to minimize the effects of oxidative stress and inflammatory processes with anti-inflammatory or antioxidant supplements. One of the natural ingredients that has antioxidant and anti-inflammatory effects is beets (*Beta vulgaris* L.) Red beets are known to contain betalain, which is a compound that has very high antioxidants that can neutralize free radicals [12]. Other compounds in red beet (*Beta vulgaris* L) that function as antioxidants are betaine, vitamin C, carotenoids, phenolic acids such as and flavonoids [13].

The aim of the study was to investigate the effect of beetroot juice supplementation during submaximal exercise on C-reactive protein levels. The main results of this study can be used to reduce the occurrence of muscle damage induced by strenuous physical exercise.

## 2. Methods

The research method used pre-test - post-test quasi-experimental method with control group model design.

### 2.1 Subjects

Twenty male futsal athletes who met the criteria (age  $21.25 \pm 1.48$  years, height  $170.50 \pm 0.04$  cm, weight  $64.00 \pm 4.17$  kg) participated. Subjects were instructed not to take any sports supplements, antioxidants, medical supplements or ergogenic aids for 3 months prior to the test. Exclusion criteria were smoking or cardiovascular, pulmonary, metabolic or neurological disease. Before conducting the research, the experimental protocol was first informed. Furthermore, the research subject gave written consent. This research was approved by the ethics committee of the University of North Sumatra No: 277 / KEP / USU / 2020 in accordance with the Declaration of Helsinki.

### 2.2 Exercise Program

The study used 20 futsal athletes who met the criteria. Before undergoing an exercise program, a hematological examination was carried out to measure CRP concentrations (pre test). Furthermore, the sample was divided into 2 groups, namely the experimental group who drank beetroot juice every day at 8.00 WIB after eating at a dose of 300 ml and the control group drank a placebo. Submaximal exercise is performed at a frequency of 3 times a week with an intensity of 80-90% for one month. Submaximal physical activity is done by running on a treadmill with an intensity of 80-90% of the maximum heart rate. After undergoing submaximal exercise for one month, another blood sample was taken to check the CRP concentrations again (posttest).

### 2.3 Collection Blood Samples

Blood (fasting) samples of 5 ml were obtained before and after completion of the exercise program from the forearm veins of the study subjects. Blood samples were centrifuged for 10 minutes at 3000 rotations to separate the serum, then stored at  $-80^{\circ}\text{C}$  until the research variables were analysed. Serum CRP concentrations was determined using an auto-analyzer spectrophotometer

### 2.4 Statistical analysis

All data are expressed as mean  $\pm$  standard deviation (SD) and were analysed using IBM SPSS Statistics 25 for Windows. Data normality test was done by using the Shapiro Wilk test method and data homogeneity by using the Levene's test. Independent sample t-test and paired t-test were used to evaluate changes between study variables. Data that were not normally distributed using non-parametric analysis (Wilcoxon test). The level of significance was set at  $P < 0.05$

### 3. Results

The results of measurements of age, height, body weight, BMI, age are shown in Table 1. The results of data analysis before starting training and giving beetroot juice showed no significant differences in age, height, weight and BMI between the experimental group and the control group.

**Table 1:** Participant characteristics

Variable	Group	M±SD
Age (Year)	Experiment	21.5±1.76
	Control	21.0±1.26
Height (cm)	Experiment	171±0.05
	Control	170±0.02
Weight (kg)	Experiment	64.33±5.75
	Control	63.66±2.25
BMI (kg/m <sup>2</sup> )	Experiment	21.95±0.65
	Control	22.03±0.92

Values are mean ± standard deviation. Tested by the independent sample t-test; no statistically significant differences between groups.

The results of measurements of CRP shown in Figure 1. Based on the research results obtained pre-test CRP levels in the treatment group were  $6.0 \pm 0.0$  mg/L, while the control group  $6.0 \pm 0.0$  mg/L. The results of statistical tests showed that there was no difference in CRP levels between the experimental group and the control group. The results of the measurement of the CRP post-test of the treatment group were  $18.00 \pm 5.65$  mg/L, while the control group obtained CRP levels of  $74.66 \pm 23.85$  mg/L. The results of statistical tests using the t-test showed differences in pre-test-post-test CRP levels in the experimental group or in the control group ( $p = 0.004$ ). The results of statistical tests using the independent sample t-test showed a significant difference in the levels of posttest CRP between the treatment and control groups ( $p = 0.000$ ).



**Figure 1.** Impact of supplementation with beetroot juice on the level of CRP. In each group, the data is mean±SD for n=10. Different letters suggest a major difference between paired t-test samples at  $p < 0.05$ . \* Significant, analysed by the independent sample t-test ( $p < 0.05$ ).

#### 4. Discussion

In this study, there were no statistically significant differences in body mass index, body weight and height between the experimental and control groups. With no differences in body height, weight, body mass index and age of the samples in the study, it is expected that muscle maturation between groups will not differ either. Muscle maturation is one indicator of the ability of muscle tension strength. With the similarity in height, weight, body mass index and age of the samples in this study, the study sample has met the established criteria without showing any variations that interfere with the homogeneity of the sample.

The findings of our study reveal that sub-maximal physical activity can increase CRP levels and administration of beetroot juice at a dose of 300 ml daily during an exercise program can reduce the increase in CRP levels during sub-maximal physical activity. It is known, physical activity, especially eccentric muscle contraction with high intensity, will induce muscle damage (Exercise-induced Muscle damage) [3]. Exercise-induced muscle damage will trigger an inflammatory response by activating Nuclear Factor kappa B (NF- $\kappa$ B) [5]. Activation of NF- $\kappa$ B will trigger an increase in inflammatory markers such as C-reactive protein (CRP) and several inflammatory interleukins such as interleukin (IL) -6, IL-8, IL-10, interleukin 1 beta (IL-1 $\beta$ ), tumor necrosis factor (TNF- $\alpha$ ) [6]. The increase in CRP levels in this study was supported by several studies conducted by Draganidis et al. [14] which reported the level of recovery in soccer skills performance after resistance exercise of moderate or high intensity. In his research, it was reported that an increase in exercise intensity would lead to an increase in CRP levels [14]. Our results are also supported by the study of Fatouros *et al.*, who reported chronic overuse resistance exercise, plasma CRP concentrations increased in proportion to training load, suggesting that CRP is a sensitive marker of inflammation induced by excessive exercise [15]. In the study of Draganidis et al. the increase in CRP persisted for 1-2 days before returning to baseline concentrations, while the increase in CRP in the study of Fatouros et al. happens immediately after exercise.

Our study findings suggest that daily administration of beetroot juice during exercise can reduce CRP build-up in sub-maximal physical activity. Lowered CRP levels in this study due to the effect of antioxidant content on red beetroot. It is known, beetroot (*Beta vulgaris* L) contains various types of natural antioxidants, one of them is betalain. Beetroot contains betalain pigment as much as 1,000 mg/g weight dry or 120 mg/100g wet weight. Betalain pigments are compounds that give red beets their deep red color. These pigments also function as antioxidants that can bind free radicals and prevent damage due to oxidative stress. There are two groups of betalain pigments in beetroot, namely the red pigment violet betasianin and the yellow pigment betasantin. The concentration ratio between betasianin pigment and betasantin pigment ranged from 1: 3 depending on the beet variety [12,16]. The results reported that the scavenging activity of betalaine was comparable to hydroxytoluene butylation, a widely used synthetic anti-oxidant [17]. Besides functioning as an antioxidant, betalain, especially betacyanin, plays an important role in human health because of its pharmacological properties as anti-cancer, anti-inflammatory and hepatoprotective [18]. Several studies that reported betalain to function as anti-inflammatory include betalaine significantly reduced carrageenan-induced superoxide anions, tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin (IL)-1 $\beta$  in peritoneal fluid, and increased levels of IL-6 [19].

Other compounds that act as powerful antioxidants in beetroot include betaine, [13,20], phenolic acids, vitamin C, carotenoids, triterpenes, coumarins and flavonoids. The flavonoid compounds in beet root include tiliroside, rhamnetin, kaempferol, astragaloside, rhamnocitrin [13,21], cochliophillin, betavulgarin, betagarin, and dihydroisorhamnetin [22]. Phenolic acid compounds in red beetroots include N-trans-feruloyltyramine, N-trans-feruloylhomovanillylamine [22], caffeic acid, gallic acid, ferulic acid, chlorogenic acid, quercetin, p-coumaric acid, kampferol, syringic acid, ellagic acid, pyricetin, ellagic acid and vanillic acid [23,24]. Betaine acts as an anti-inflammatory [20,25] by suppressing nuclear factor kappa-light chain enhancer of activated B cells (NF- $\kappa$ B) and Akt activation [26], and initiation of inflammasomes [20]. Ferulic acid (FA) is a potent mercurane antioxidant and is considered to be an effective free radical scavenger [27]. As an antioxidant, caffeic acid significantly

reduces lipid peroxidation and decreases DNA damage in UVB irradiated lymphocytes. In addition, caffeic acid was shown to reduce oxidative stress and inflammation caused by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in vivo in mouse skin

## 5. Conclusion

In conclusion, giving beetroot juice during training for one month can reduce the CRP levels that are increased in submaximal physical exercise in athletes. The decrease in CRP levels is due to the antioxidant content found in beetroot juice. The results of this study are very useful for reducing the occurrence of muscle damage during strenuous exercise.

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