



The Effect of Moderate-Intensity Resistance Training and Astaxanthin Supplementation on Lactic Acid Levels in Adult Men at Fitness Centers in Tangerang City

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KEYWORDS

astaxanthin; resistance training; blood lactate; trained and untrained subjects

ABSTRACT

Moderate-intensity resistance training induces lactate production through the activation of anaerobic glycolysis and represents a strong metabolic stimulus. Astaxanthin, a carotenoid antioxidant, has been proposed to support mitochondrial function and metabolic recovery during exercise. This study aimed to compare blood lactate responses to moderate-intensity resistance training between trained and untrained adult men who received astaxanthin or a placebo. A randomized controlled experimental study with a mixed factorial design was performed. Adult men aged 18–25 years were classified as trained or untrained and randomly assigned to astaxanthin supplementation (6 mg/day) or placebo for 14 days while performing a standardized moderate-intensity resistance training program. Blood lactate levels were measured at baseline, one week, and two weeks post-intervention and analyzed using repeated-measures ANOVA. Blood lactate levels increased significantly from baseline to week one and decreased toward baseline at week two in all groups ($p < 0.001$; partial $\eta^2 = 0.886$). Comparison between supplementation groups (astaxanthin vs. placebo) showed no significant differences in lactate changes over time (time \times group interaction, $p = 0.595$). Similarly, no significant differences were observed between trained and untrained participants in the overall lactate response patterns. Descriptively, untrained participants demonstrated a steeper acute increase in lactate, whereas participants receiving astaxanthin, particularly trained subjects, exhibited a greater reduction from peak lactate levels during the recovery phase. In conclusion, the lactate responses to moderate-intensity resistance training were primarily driven by the exercise stimulus itself.

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INTRODUCTION

Astaxanthin is a carotenoid pigment that does not have provitamin A activity in humans. The compound was first isolated from lobsters in 1938. (Stachowiak & Szulc, 2021) Commercially, astaxanthin is used as a supplement in fish feed and also in the supplementation and nutraceutical industries. In aquatic environments, astaxanthin gives pink to red pigmentation in some species of fish. In humans, astaxanthin supplementation shows antioxidant effects. Although its use in humans is still limited, several studies have reported the powerful antioxidant effects of astaxanthin as well as its potential to help modulate the inflammatory response, immune system, and improve other health conditions (Hussein et al., 2006).

Physical activity can lead to the accumulation of lactic acid as a metabolic waste substance. This accumulation, especially in muscle fibers and blood circulation, results in an increase in hydrogen ions from lactic acid that triggers lactic acidosis. (Cairns, 2006; Cairns & Lindinger, 2025) Such accumulation can interfere with the process of muscle contraction and cause fatigue characterized by a progressive decrease in muscle contraction strength, especially

when performed repeatedly. Lactate production comes from various organs such as the skin (25%), erythrocytes (20%), central nervous system (20%), muscles (25%), and digestive tract (10%). However, lactate production will increase significantly under conditions of strenuous physical activity (Seheult et al., 2017).

The administration of astaxanthin has the potential to improve physical fitness through interactions with various macronutrient metabolic pathways in humans. A meta-analysis by Hasan, which included nine randomized clinical trials, found a significant improvement in cycling ability as well as an increase in total antioxidant capacity (TAC). (Hasani et al., 2024) Talbott et al reported that astaxanthin supplementation of 12 mg/day for eight weeks in amateur runners (28 subjects) lowered average pulse rate by about 10% ($p < 0.05$) at aerobic and anaerobic intensity compared to the placebo group. (Talbott et al., 2016) In overweight individuals with a BMI of >25 , also reported a significant decrease ($\sim 7\%$) in pulse and carbohydrate oxidation. A non-significant decrease ($p > 0.05$) was found in the oxidation of fat, lactic acid, glucose, and rating of perceived exertion (RPE). (Wika et al., 2023) Liu et al showed that supplementation of astaxanthin (12 mg), tocotrienol (10 mg), and zinc (6 mg) twice daily in the elderly (65–82 years) resulted in better metabolic adaptation (Liu et al., 2021).

Regular consumption of astaxanthin has the potential to provide benefits that are felt by both individuals who regularly practice and those who do not practice regularly. In individuals who are accustomed to exercise, the body has better metabolic capacity, including the ability to utilize lactate as an energy source and the ability to recover faster due to physiological adaptations such as increased lactate transporters (MCT-1 and MCT-4) as well as higher oxidative capacity. (Brooks, 2000) With astaxanthin supplementation, this adaptation can be further supported through a decrease in free radical accumulation, stabilization of mitochondrial membranes, and increased efficiency of energy metabolism, resulting in faster recovery and reduced muscle fatigue (Barker et al., 2023; Wu et al., 2019).

In individuals who do not exercise regularly, lactate utilization capacity and resistance to oxidative stress are relatively lower, so physical activity easily triggers lactic acid accumulation and muscle fatigue. Astaxanthin supplementation in this group has the potential to have a more pronounced impact because it can reduce lactic acid accumulation, reduce oxidative stress, and help speed recovery even though basic physiological adaptations are not optimal. This suggests that in both individuals who already have exercise adaptations and those who do not, regular consumption of astaxanthin plays a role in supporting metabolic recovery and increasing comfort in physical activity (Aoi et al., 2008; Earnest et al., 2011; Fasset & Coombes, 2011).

Physiologically, the relationship between lactic acid and astaxanthin lies in the role of astaxanthin as a lipophilic antioxidant that can stabilize mitochondrial membranes and improve the efficiency of the electron transport chain. Thus, the body is better able to maintain aerobic metabolism, reduce lactic acid formation from the anaerobic glycolysis pathway, and accelerate the lactic clearance produced during exercise. This is particularly relevant for both individuals who have adapted to exercise and those who have not, as both can experience reduced muscle fatigue and increased post-workout comfort with regular consumption of astaxanthin (Brooks, 2009; Packer et al., 2005; Powers & Jackson, 2008).

A number of literature states that high doses of antioxidant supplementation can potentially interfere with physiological adaptations resulting from physical exercise, especially

in endurance-type training (Pingitore et al., 2015; Powers et al., 2016). This is because the reactive oxygen species (ROS) formed during exercise not only induce oxidative stress, but also serve as signaling molecules that trigger various adaptation processes, including the activation of mitochondrial biogenesis pathways mediated by peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α). Administration of high-dose antioxidants such as vitamin C ($\geq 1,000$ mg/day) and vitamin E (≥ 400 IU/day) has been reported to reduce availability ROS as an adaptation signal, thereby decreasing the adaptive response to resistance training emphasizing long-term oxidative capacity enhancement.

However, such inhibitory effects are mainly present in resistance-based exercises that require a progressive increase in mitochondrial capacity. In resistance-based training or weight training, the dominant adaptation pathways differ, namely increased muscle mass (hypertrophy), strength, and metabolic buffering capacity against hydrogen ions (H⁺). Therefore, the risk of adaptation disorders due to moderate doses of antioxidant supplementation in the context of weight training is relatively low.

Astaxanthin, the antioxidant used in the study, has different characteristics than vitamins C and E. Astaxanthin is lipophilic, can integrate into cell membranes and mitochondria, and in clinical studies is generally given in intermediate doses (6–12 mg/day). Human studies report that astaxanthin does not interfere with chronic adaptation parameters, but instead helps accelerate metabolic recovery and lower post-exercise lactic acid levels. Thus, the use of astaxanthin in this study is considered safe and does not hinder the exercise adaptation process, especially since the purpose of the study is focused on the acute response of lactic acid levels and metabolic recovery, rather than on long-term physiological adaptation changes.

Antioxidant supplementation may improve physical performance, particularly in individuals who have a previous history of exercise. With respect to the antioxidant potential and its effects on the body's metabolism, further research is needed to evaluate the effectiveness of astaxanthin as a supplement in improving physical fitness.

The selection of a fitness center in the city of Tangerang as the location of the study is based on the availability of adequate facilities and the relevance of the user population to the research objectives. The gym is widely accessible to young adult individuals who have a high interest in fitness and health, providing an ideal opportunity to evaluate the impact of weight training on physiological parameters, including lactic acid levels. In addition, the presence of certified professional instructors ensures the implementation of a structured weight training program that complies with safety standards and the effectiveness of the exercises.

The combination of interventions in the form of physical activity and antioxidants is seen as having the potential to optimize metabolic recovery, reduce the impact of oxidative stress, and improve comfort and exercise performance. By utilizing astaxanthin, it is hoped that a more comprehensive understanding can be achieved of the mechanism of reducing lactic acid accumulation and its relation to post-exercise recovery.

The central theme of this study is the evaluation of the effect of astaxanthin supplementation on blood lactic acid levels in moderate-intensity weight training, with the aim of finding out the extent to which astaxanthin can improve energy metabolism efficiency, decrease lactate accumulation, and accelerate muscle recovery.

Astaxanthin, as a powerful natural carotenoid antioxidant, is known to be able to protect cell membranes and mitochondria from damage caused by oxidative stress generated during physical activity. This mechanism has the potential to increase muscle oxidative capacity, slow down the use of anaerobic glycolysis pathways, and ultimately decrease lactic acid production.

Lactic acid levels were measured at three time points before the intervention, one week after supplementation, and two weeks after supplementation with weight training to obtain a longitudinal picture of changes in lactic metabolism. This approach not only measures acute effects, but also monitors the physiological adaptations that occur during the intervention period.

Thus, through an approach that combines moderate-intensity weight training and astaxanthin supplementation, this study is expected to provide a new perspective on safe and effective oxidative stress management strategies. The results are expected to be the basis for the preparation of evidence-based health recommendations, which are applicable to support fitness programs, health maintenance, and anti-aging efforts that are growing in Indonesia.

This study aims to determine the effect of moderate-intensity weight training on increasing lactic acid levels in adult men who are classified as trained and untrained. In addition, this study also aims to analyze the effect of astaxanthin supplementation for 14 days in reducing lactic acid levels after weight training in both groups of subjects. Furthermore, this study was directed to evaluate the effect of the combination of weight training and astaxanthin supplementation in reducing lactic acid levels compared to the administration of a single intervention, either in the form of weight training or astaxanthin supplementation alone.

This research is expected to provide benefits both theoretically and applied. Theoretically, the results of this study are expected to make a scientific contribution in expanding understanding of the mechanism of action of astaxanthin in modulating lactic acid metabolism, as well as enriching the study of nutritional strategies in the field of exercise. Appliedly, the findings of this study are expected to be used as a basis for further research related to the use of astaxanthin in the context of physical fitness and anti-aging efforts, so that it can provide practical implications for the development of nutritional interventions based on scientific evidence.

METHOD

Place and time of the research

This research was conducted at a fitness center in Tangerang City. This research was conducted for 3 months from August to October 2025

Research Variables

Independent variables:

1. Age (in years)
2. Gender (adult male)
3. Training status (trained vs untrained)
4. Astaxanthin supplementation (6 mg/day)

Dependent variables :

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Table 1. Variable operational definition

o..	Variabel	Operational Definition	Measuring Instruments	Skala Ukur
1.	Age	Age in years for study participants	Interview	Numerical
2.	Gender	Gender of the study participants	Interview	Nominal
3.	Asam lactate until	The concentration of lactic acid in the blood is measured in mmol/L.	Lactic acid assay Assessed before astaxanthin supplementation, after 1 week and 2 weeks, then blood is taken after a physical test	Numerical
4.	Weight training	Trained group: Individuals who consistently performed weight training at least 3 times per week for the last ≥ 12 weeks of moderate to high intensity. Untrained group: Individuals who have not had a history of regular weight training for at least the past 3 months, either in the form of light, moderate, or heavy weight training.	Direct observation of the training program carried out by the participants and recording of weights and reps Weights that can be lifted with $>80\%$ intensity in 5 types of exercises (<i>Push Incline Bench Press, Pull Lat Pull Down, Squat Dumbbell Squat, Hinge Romanian Deadlift (RDL), Core Plank (1-2 minutes)</i>)	Numerical
5.	Astaxanthin supplementation	Giving the antioxidant astaxanthin in capsule form to study participants for 14 days before the test	Provision of astaxanthin capsules 6mg/day and recording of consumption compliance through self-report and direct supervision	Numerical

Source: Researchers' compilation, 2025

Research Sample Population

The study sample population was adults aged 18 – 25 years who agreed to participate in the study. The population is divided into 4 groups (8 people each):

Subjects in this group were individuals who had been trained regularly with a frequency of weight training 2-4 times a week. Exercise intensity ranges from 60-70% of a maximum rep (1RM), with workout durations ranging from 30-60 minutes per session. With a minimum of 6 months of exercise experience, this group is expected to have a better physiological adaptation to oxidative stress, so as to provide a clear picture of the effects of astaxanthin supplementation in the established fitness context.

This group also consisted of trained individuals with the same characteristics as the first group, but they received placebo as a control. It is important to compare the real effects of astaxanthin with the psychological or placebo effects that may arise from taking the supplement

Subjects in this group were individuals who exercised irregularly, with a frequency of less than once per week. They received astaxanthin supplementation, so the study could explore whether astaxanthin can provide antioxidant benefits in individuals who do not have exercise habits. It is also important to understand how the untrained body responds to antioxidant supplementation.

As the final control group, subjects in this group were also untrained and received a placebo. This allowed researchers to evaluate the difference in response between untrained individuals who received astaxanthin and those who only got a placebo.

The inclusion criteria for the trained group include adult men aged 18-25 years who are active members of fitness centers in Tangerang City and routinely do weightlifting exercises 2-3 times a week in the last 6 months. For the untrained group, the inclusion criteria are adult men aged 18-25 years who do not routinely train weights for less than once a week.

As for the exclusion criteria, among others, inconsistent consumption of the research supplement for 2 weeks, and the absence of an injury or medical condition or chronic illness that prevents participation in weight training during the study period

Research instruments

A research instrument is a tool or method used by researchers to collect data and information necessary to achieve research objectives. The Strength Training Test in this study is designed to comprehensively evaluate muscle strength and trigger acute oxidative stress. The training protocol consists of five main types of movements that represent the basic movement patterns of the body, namely: push, pull, squat, hinge, and core. Each exercise is carried out with a load of 60-70% of 1 Repetition Maximum (1RM) of 10 reps in 3 sets, with a recovery time of 2 minutes between sets.

Types of Exercises

Table 2. Strength training test

Movement Patterns	Exercise Name	Major Muscle Groups
<i>Push</i>	<i>Incline Bench Press</i>	Upper chest, front shoulders, triceps
<i>Sweater</i>	<i>Lat Pull Down</i>	Upper back (<i>Latissimus dorsi</i>), biceps
<i>Squat</i>	<i>Dumbell Squat</i>	Squirrel front, hamstrings, <i>Gluteus</i>
<i>Hinge</i>	<i>Romanian Deadlift (RDL)</i>	<i>Hamstrings, gluteus, lower back</i>
<i>Core</i>	<i>Plank (1-2 minutes)</i>	<i>Core: rectus abdominis, obliques, transverse abdominis</i>

Source: Researchers' compilation, 2025

The exercises were conducted in one structured session and supervised by a Certified Personal Trainer at the A fitness center. Before the training session begins, each subject undergoes a 1 Repetition Maximum (1RM) measurement test for each major movement. Since direct measurement of 1RM is at risk of injury, an estimation approach is used using the Brzycki formula based on submaximal reps:

$$1RM = \frac{B}{1.0278 - 0.0278R}$$

Description:

1RM = estimated maximum load (kg)

B = load successfully lifted (kg)

R = number of reps performed with the load (maximum 10)

Example: if a person can lift 60 kg 8 reps, then the estimated 1RM is:

$$1RM = \frac{60}{1.0278 - (0.0278 \times 8)} = \frac{60}{0.8062} = 74.4 \text{ kg}$$

The results of this estimate are used as a basis to determine 60-70% of 1RM in the Strength Training Test session.

Sampling Techniques

The sample size calculation was calculated by statistical power analysis using G*Power 3.1 software. The study design involved repeated measurements (Repeated Measures ANOVA) with 4 treatment groups and 3 measurement times (week 0, week 1, and week 3) Compute required sample size - given α , power, and effect size. The results of the analysis show that the minimum number of subjects required is 28 subjects, assuming a balanced distribution of subjects in each group (7 subjects per group). To anticipate the possibility of drop-out or loss of data during the study, increase the number of subjects by 10–15%, so that the ideal total of subjects is around 32 people.

Astaxanthin and Placebo Supplementation

The intervention group will receive 6 mg of astaxanthin supplementation and the placebo group will receive a placebo pill. Capsules for astaxanthin supplementation use the Astria Force 6 mg supplement product, the supplement is produced by PT. Pertiwi Agung, the distribution permit of the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI) SD 191355041 with the active ingredient astaxanthin extract synthesized from *Haematococcus pluvialis* as much as 6 mg. The supplement pill was given to the group that followed the exercise program and did not participate in the exercise program. To ensure the double blinding process, in the study participants, both types of capsules (astaxanthin and placebo) will be inserted into empty gel capsules of the same color.

The research assistants in the field and the research participants in their respective groups did not know the type of capsules to be received (double-blinding). For 2 weeks, patients will be given placebo capsules and 6 mg astaxanthin capsules. Study participants were instructed to take the capsules before the exercise program.

Physical Test

Study participants who were included in the intervention and placebo groups would have a physical test during the 2 weeks of the intervention. Physical tests were conducted 3 times a week with an interval of 1 day for each physical exercise session during the study period. On the day of the study, 5 types of physical tests were push (incline bench press), pull (lat pulldown), squat (dumbbell squat), hinge (romanian deadlift or RDL), and core (plank 1 – 2 minutes). All exercises except planks are performed with a heavy load intensity of 60–70% of 1 RM, 10 reps, 3 sets, duration of 5–10 minutes, recovery time of 3 minutes between sets. Heavy load is a load of 60-70% of the maximum load that can be lifted in 1 rep (1RM).

Blood Lactic Acid Examination

Blood collection was carried out three times, before the supplementation intervention, 1 week after the administration of astaxanthin supplementation and 2 weeks after the administration of astaxanthin supplementation after physical exercise, using The Edge Blood Lactate Monitoring System tool working on the principle of enzymatic electrochemistry. The test strips contain the enzyme lactate oxidase that reacts specifically with lactic acid in the blood sample to produce an electric current. The amount of electric current produced is

proportional to the concentration of lactic acid in the blood, so results can be obtained in less than 15 seconds. The measurement range of the device ranges from 0.0 to 25.0 mmol/L, with moderate accuracy and good compatibility with conventional laboratory methods.²⁸

The Edge appliance is switched on and ensured is in a ready state. Sensor strips (lactate test strips) are inserted into the appliance according to the instructions. Each strip sensor has a specific calibration code that will be automatically read by the tool. Officers wear disposable gloves to maintain sterility and prevent cross-contamination.

The subject's fingertips (usually ring or index fingers) are cleaned with 70% alcohol cotton and dried. A sterile lancet is used to pierce the subject's fingertips to obtain drops of blood. The first droplets are usually removed to avoid contamination of the interstitial tissue fluid. The second drop of blood is left to stand out and ready to be applied to the sensor strip. The strip sensor is brought closer to the blood droplets until the device automatically absorbs blood through a capillary mechanism. Once the blood is absorbed, the device begins to calculate lactic acid levels digitally. The measurement results are displayed on the instrument screen in mmol/L units in approximately 10–15 seconds.

The entire process of checking lactic acid levels is carried out by professional laboratory officers from Prodia's Clinical Laboratory, to ensure the accuracy of sampling, the validity of the results, and maintaining the standard operating procedures according to medical protocols.

Kriteria Inklusi

1. Adult men aged 18 – 25 years with regular weight training 2-3 times a week in the last 6 months
2. Adult men aged 18-25 years with weight training < once every 1 week
3. Be willing to follow the entire research procedure

Exclusion Criteria

1. History of consumption of antioxidant supplements (carotenoids or astaxanthin)
2. Medical conditions that limit the ability to be physically active
3. Have an intolerance to moderate-intensity exercise
4. Allergy to supplement ingredients
5. Taking chronic immunosuppressive or anti-inflammatory medications

Tools and Materials

1. The Edge Blood Lactate Monitoring System
2. lactate test strips
3. Kapsul Astaxanthin Astria Force 6 mg
4. Smooth capsules (containing astaxanthin 6 mg for the intervention group and placebo for the control group)

Data Analysis

This study used an experimental quantitative approach with a pre-test post-test control group design and repeated measurements in 4 treatment groups, with the main variable being blood lactic acid levels. Measurements were taken three times, at baseline at week 0, after 1 week of intervention, and after 2 weeks of intervention. Before inferential analysis is performed, the data will be tested against several statistical assumptions of the Shapiro-Wilk Test for each time and group. Levene's Test Variance Homogeneity Test to compare variants

between groups. Sphericity Test: Mauchly's Test for Repeated Measures ANOVA. If the assumption is not met, a Greenhouse-Geisser correction is made.

The inferential analysis in this study will use the Repeated Measures ANOVA method (Re-Measured Variance Analysis) to evaluate changes in blood lactic acid levels between time and between groups. This technique is suitable because the study design involves repeated measurements of the same variable (lactic acid levels) in the same subjects over multiple periods of time (week 0, week 1, and week 3) and multiple treatment groups (4 different groups) based on a combination of exercise and astaxanthin supplementation. An interaction between time and group will be evaluated, to assess whether the trend of changes in lactic acid levels differs between treatment groups.

If significant differences are found, Bonferroni Correction is used for comparison between time and between groups. Focus on comparisons between astaxanthin and placebo at the same time, comparisons between trained and untrained, evaluation of combination interactions (exercise status \times supplementation). The significance level was set at $p < 0.05$, the confidence interval was set at 95%.

Data analysis will be carried out using, the latest version of SPSS, data visualization using line plots with error bars to illustrate the trend of changes in lactic acid levels between time and groups.

RESULT AND DISCUSSION

Asam Lactate Up

This experimental study was conducted to test the effects of astaxanthin supplementation on blood lactic acid levels in adult men undergoing a moderate-intensity weight training program. The research design used a mixed factorial design approach with two inter-subject factors (exercise status and type of supplementation) and one in-subject factor (measurement time). A total of 32 participants met the inclusion criteria and were randomly allocated into four groups of moderate-intensity weight training interventions with similar demographic characteristics. However, only 29 participants were included in the data analysis, as 3 participants had incomplete data.

Lactic acid levels were measured using the calibrated The Edge Blood Lactate instrument. Measurements were carried out three times, namely during pre-intervention (Week 0), 1 week post-intervention (Week 1), and 2 weeks post-intervention (Week 2). The results of blood lactic acid level measurements at week 0 (pre-intervention), week 1, and week 2 are presented descriptively which can be seen in table3 below.

Table 3. Average value of lactic acid levels.

Groups	N	Average Lactic Acid Levels (mg/dL)		
		Week 0	Week 1	Week 2
1	8	80.50 \pm 43.48	184.13 \pm 27.90	88.50 \pm 24.86
2	7	71.00 \pm 21.27	189.29 \pm 14.65	98.00 \pm 38.64
3	7	65.71 \pm 60.99	145.57 \pm 52.05	80.71 \pm 55.53
4	7	37.43 \pm 15.1	152.71 \pm 41.03	73.29 \pm 14.33

Source: Processed research data, 2025

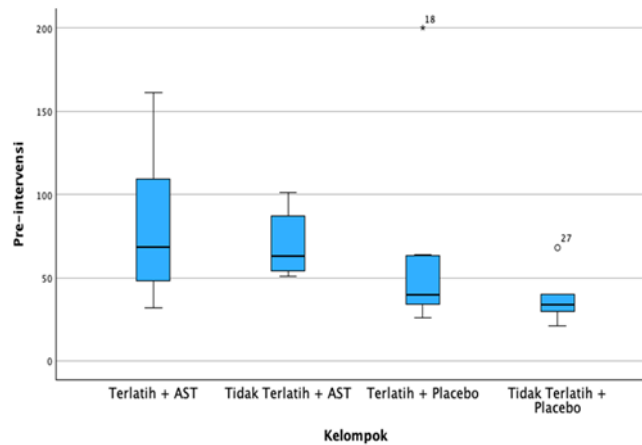


Figure 1. Visualization of lactic acid levels in each study group at the pre-intervention period
Source: Processed research data, 2025

Based on table 1, all groups experienced a relatively sharp increase in lactic acid levels between baseline (Week 0) and post-intervention conditions at 1 week (Week 1). The largest absolute improvement was observed in the untrained group supplemented with Astaxanthin, with an average value of lactic acid levels of 118.29 mg/dL. Meanwhile, the lowest increase was observed in the untrained group who received Placebo supplementation with a value of 79.86 mg/dL. A graph of the increase in lactic acid levels for each group can be seen in figure 4.1. The following.

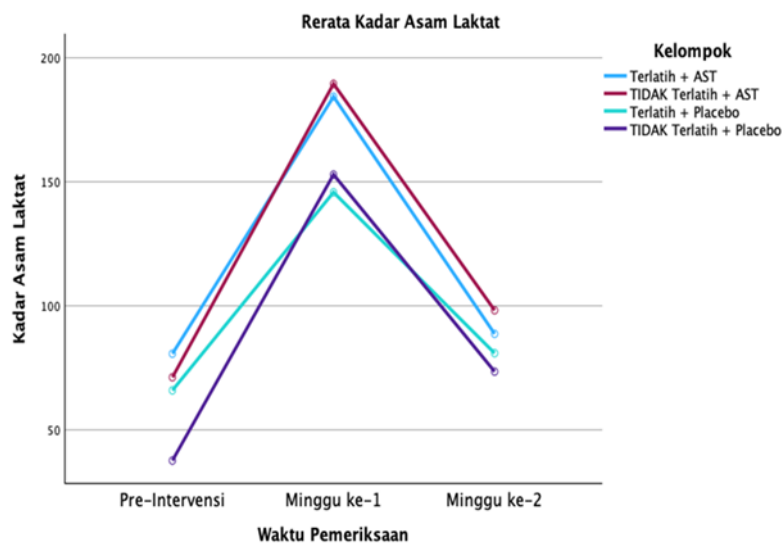


Figure 2. Graph of lactic acid levels for each study group
Source: Processed research data, 2025

At the 2nd week post-intervention measurement, lactic acid levels in all groups decreased close to or were assessed to be relatively higher than baseline, with a decrease range of approximately 64.86–95.63 mg/dl. The largest absolute decrease from peak (Week 1 to Week 2) was observed in the trained group receiving Astaxanthin (-95.63 mg/dL), while the smallest decrease was in the trained group receiving placebo (-64.86 mg/dL).

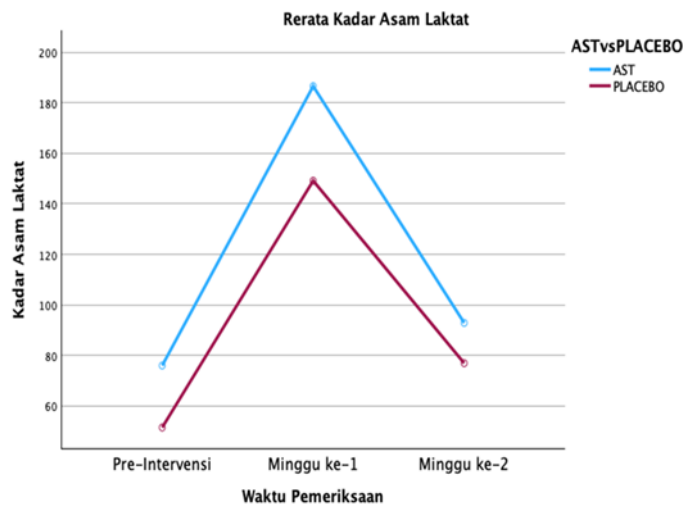


Figure 3. Comparison graph of the increase between the AST and Placebo groups
 Source: Processed research data, 2025

The graph of improvement between the AST and placebo groups can be seen more closely in Figure 3 above, without looking at the training status, where based on the visualization of the data on the graph it was observed that the pattern of improvement from the pre-intervention condition to week 1 did not show a marked difference between the groups receiving Astaxanthin (AST) and placebo. Visually, the trend lines of the two groups increased with a more or less parallel slope, indicating that the magnitude of the acute increase in lactic acid after moderate-intensity weight training was relatively similar, regardless of the status of AST supplementation. Therefore, the acute physiological response of increased lactic acid levels appears to be more influenced by the stimulus of such exercise than by supplementation interventions during this period.

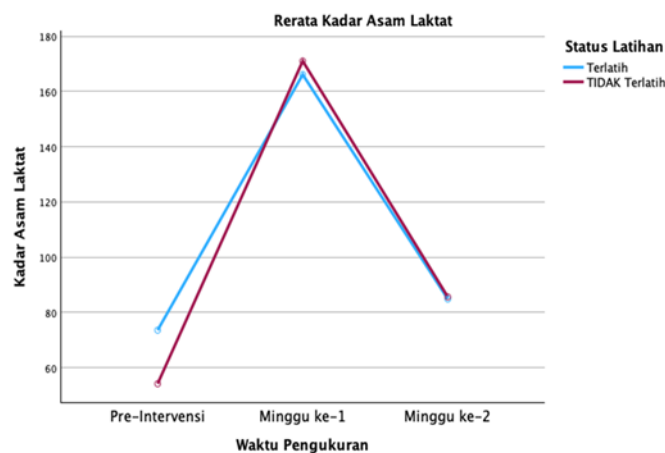


Figure 4. Comparison graph of the increase between the trained and untrained groups
 Source: Processed research data, 2025

Furthermore, further analysis was carried out by separating based on exercise status showing interesting patterns. As visualized in the graph, the untrained group showed a relatively higher slope response from before the intervention to 1 week post-intervention,

compared to the trained group even though the untrained group had a relatively lower baseline at the pre-intervention time. An interesting pattern can be seen at the peak of the average lactic acid levels of the two groups which are relatively the same in week 1 and week 2. This pattern indicates that although the untrained group showed a more sensitive anaerobic metabolic response to the given exercise stimulus (as seen from the sharper increase), the adaptive capacity or workload of the given exercise resulted in a similar final metabolic load (peak lactate) and recovery profile between trained and untrained subjects after one week of intervention.

Statistical Analysis

After the lactic acid level values were known at the three measurement times, a normality test was then carried out using Shapiro-Wilk because the number of samples in each group was relatively small ($n < 50$). The normality test will be assessed with the help of the SPSS 25 program. The data is declared to be normally distributed if the significance value (p) obtained from the calculation results is greater than the significance level (α)=0.05 (significance level 5%). The results of the Shapiro-Wilk normality test can be seen in Table 2 below.

Table 4. Shapiro-Wilk normality test results.

Groups	Time	<i>p-value</i>
Trained, Getting AST	Pre-intervention	0,534
	Week 1	0,001*
	Week 2	0,181
Untrained, Got AST	Pre-intervention	0,127
	Week 1	0,021
	Week 2	0,456
Trained, Getting a Placebo	Pre-intervention	0,002
	Week 1	0,108
	Week 2	0,015*
Untrained, Getting a Placebo	Pre-intervention	0,161
	Week 1	0,663
	Week 2	0,709

$p > 0.05$ indicates normal data distribution

* $p < 0.05$ indicates deviation from the normal distribution

Source: SPSS 25 output, 2025

Based on the data from the normality test results listed in Table 4, the evaluation of the data distribution showed a pattern that varied between groups and the measurement time. In the baseline (pre-intervention) condition, three groups met the assumption of normality with a significance value of (p) > 0.05 , while one group (the regular group and received placebo) showed a significant deviation ($p=0.002$). At the 1-week post-intervention measurement, two groups were normally distributed, while both groups receiving AST showed deviations from the normal distribution ($p=0.001$; $p=0.021$). At the 2-week post-intervention measurement, three groups were normally distributed and one group (regular and placebo) again showed abnormalities ($p=0.015$). Quantitatively, 8 of the 12 measurement conditions (66.7%) had met the normality assumption, indicating that overall the majority of the study data were normally distributed.

Significant deviations from normality were identified consistently in the regular and placebo groups, at two of the three measurement times, and in both groups receiving AST supplementation in the 1-week post-intervention acute phase. This pattern is interesting because it suggests that interventions, either in the form of exercise or a combination of exercise and AST supplementation, can affect the distribution of physiological response data, which can be attributed to the higher variability of individual responses within the group. Although there are violations of normality assumptions in some data, the variance analysis method for repeated measurements (Repeated Measures ANOVA) has been shown to have a fairly high robustness to mild violations of normality assumptions, especially when the sample sizes between groups are relatively balanced and the study design is factorial. Therefore, after considering the proportion of the dominant normal data, the balance of the design, and the robustness of the planned statistical method, it was decided that parametric analysis can and should still be performed to validly test the research hypothesis.

Before performing hypothesis tests with ANOVA Repeated Measures, a series of prerequisite tests were carried out to ensure the feasibility and validity of parametric statistical analysis. The tests include the homogeneity test of the homogeneity of the variance of error (Lavene's test) and the sphericity test (Mauchly's test).

The homogeneity of variance error test (Levene's test) was performed to identify the similarity of lactic acid content data variance between groups at each measurement time separately. The homogeneity criterion will be met if the significance value (p) >0.05 is at the significance level (5%). The full homogeneity test results can be seen in Table 5 below.

Table 5. Levene's Test Homogeneity Test Results.

Measurement Time	Levene Statistic	p -value	Interpretasi
Pre-Intervention	1,894	0,157	Homogeneous
Week 1	4,377	0,013	Not Homogeneous
Week 2	1,629	0,208	Homogeneous

$p > 0.05$ indicates homogeneous data variants

Source: SPSS 25 output, 2025

Based on Table 5, it can be concluded that lactic acid content data at baseline (pre-intervention; $p=0.157$) and at the end of the intervention (2nd week; $p=0.208$) have met the assumption of variance homogeneity. However, data at the peak of acute response (Week 1) showed significant variance heterogeneity ($p=0.013$). This heterogeneity reflects greater variability of physiological responses between individuals in the acute phase after receiving exercise loads and supplementation interventions. The regular exercise and placebo groups showed the highest variability at Week 1 ($SD = 52.05$ mg/dL), which may reflect an inconsistent response due to the absence of external antioxidant support in the trained subjects. In contrast, the exercise irregular group receiving AST showed the lowest variability ($SD = 14.65$ mg/dL), indicating a more uniform stabilizing or response effect of AST supplementation in previously untrained populations.

Data analysis was followed by a sphericity test to test the assumption of sphericity or homogeneity of variance between measurement conditions between groups. The sphericity test

is performed with the Mauchly test. If the Mauchly test is not statistically significant ($p > 0.05$), then it can be concluded that the sphericity assumption is met. The test results showed a value of Mauchly's $W = 0.919$ with a significance value (p) of 0.365. So it can be concluded that the assumption of sphericity is not violated. Therefore, there is no need to correct the degree of freedom, and interpretation can refer to the test results assuming that the sphericity is met. In addition, the Epsilon Greenhouse-Geisser value (0.925) which is close to 1 also corroborates the conclusion that the sphericity violations, if any, are minimal.

Inferential analysis to test the research hypothesis was carried out using Repeated Measure ANOVA with a mixed factorial design. This design allows simultaneous evaluation of the influence of time factors (pre-intervention, week 1, week 2) and intervention factors (exercise status and supplements). This analysis procedure is designed to test: (1) the main effect of time as an indicator of the overall influence of exercise on lactic acid dynamics; (2) the effects of time and group interactions (evaluation of lactic acid change patterns at each time of measurement differed significantly between intervention protocols); (3) the main effect between the groups as a whole, regardless of the time factor.

The results of ANOVA's Repeated Measures analysis comparing the four groups are presented comprehensively in Table 6 below.

Table 6. Results of ANOVA Repeated Measures Analysis Test.

Interaction	<i>Partial Eta Squared</i>	<i>Observed Power</i>	<i>p-value</i>
Time	0,761	1,000	<0.001
Time x Group	0,078	0,253	0,648
Groups	0,236	0,561	0,077

Source: SPSS 25 output, 2025

The analysis revealed that there was a statistically significant difference in pre-intervention, 1 week post-intervention, and 2 weeks post-intervention lactic acid levels ($p < 0.001$) with a large effect size ($\eta^2 = 0.761$) indicating that as much as 76.1% of the variance in total blood lactic acid levels could be explained by the measurement time variable. The findings strongly confirm that the stimulus of moderate-intensity weight training has induced substantial physiological changes in all participants. Thus, it can be concluded that moderate intensity weight training increases lactic acid levels in adult men, both trained and untrained.

This study also showed that the effect of interaction between time and group was found to be statistically meaningless (observed power = 0.253; $p = 0.648$). Similarly, the group's main effect as a whole was not statistically significant, although it showed a moderate effect size (observed power = 0.561; $p = 0.077$). This indicates that the pattern of changes in lactic acid levels over time, characterized by a sharp increase in week 1 followed by a decrease in week 2, was parallel and homogeneous in all four intervention groups. It can be concluded that there is insufficient statistical evidence to state that one of the intervention protocols results in changes in lactic acid levels that differ from the others.

Considering that the results of the analysis were not entirely in accordance with the research hypothesis, additional statistical analysis was carried out using the Chi-Square test and included data from the examination of high lactic acid levels to see the distribution and

differences in the proportion of lactic acid content categories at each measurement time. The results of the analysis are presented in Table 7.

Table 7. Chi-Square Test Results.

Measurement Time	χ^2 (Pearson)	df	p-value	Remarks
Pre-intervention	3,097	3	0,377	Insignifikan
Week 1	9,911	3	0,019	Signifikan
Week 2	2,971	3	0.396	Insignifikan

Source: SPSS 25 output, 2025

Based on Table 7, the results of the Chi-Square test showed that in the pre-intervention there was no significant relationship between the intervention group and the status of lactic acid levels ($\chi^2 = 3.097$; $df = 3$; $p = 0.377$), indicating that the distribution of lactic acid levels between the groups was relatively homogeneous before the intervention.

At week 1, a statistically significant association was found between the intervention group and lactic acid level status ($\chi^2 = 9.911$; $df = 3$; $p = 0.019$), indicating a difference in the proportion of lactic acid levels between groups in the early phase of the intervention.

However, in week 2, the Chi-Square test results again showed no meaningful association ($\chi^2 = 2.971$; $df = 3$; $p = 0.396$), so the difference in the proportion of lactic acid levels between groups did not continue in this phase.

Overall, these results suggest that the difference in the proportion of lactic acid levels between groups is only temporary and is mainly seen in the first week of the intervention.

CONCLUSION

Moderate-intensity weight training was shown to be a major determinant in changes in blood lactic acid levels during the intervention period. The time effect showed a very significant influence, confirming that exercise stimuli play a dominant role in the increase in acute lactic acid levels as well as its decrease in the early recovery and adaptation phases. Differences in exercise status only affected response patterns descriptively and did not show statistically significant differences, indicating that acute metabolic responses to standardized exercise load were relatively similar between groups.

Astaxanthin supplementation did not show a significant synergistic effect with time or exercise status on changes in lactic acid levels. However, based on descriptive analysis and additional statistical analysis using the Chi-Square test, astaxanthin supplementation makes a supportive contribution in the form of a tendency to decrease the average lactic acid level, especially in the recovery phase. This reflects an improvement in the metabolic environment without replacing the primary role of exercise adaptation. Thus, physical exercise remains a dominant factor in metabolic adaptation, while astaxanthin plays a supporting role in supporting metabolic recovery .

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