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Analysis of Changes in Malondialdehyde (MDA) and Lactate Dehydrogenase (LDH) Levels After a 30 km Cycling Event in The Makassar Cycling Community

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ABSTRACT
This study looks at the changes in MDA and LDH levels after a 30 km cycling event in the Makassar cycling community. This research is a pre-experimental with a one-group pretest-posttest design on 30 male cyclists aged 30-60 years. The pre-test was carried out the day before the cycling event, and then the post-test was carried out 2 hours after the 30 km cycling event. Blood samples were tested using the ELISA Kit. Data were analyzed using the Wilcoxon test to see the effect of a 30 km cycling event on changes in MDA and LDH levels. Spearman correlation test to see the correlation between changes in MDA levels and LDH. The results of the study found that the 30 km cycling event had a significant effect on changes in MDA levels due to lipid peroxidation (p<0.05) and a significant effect on changes in LDH levels due to increased lactate secretion (p<0.05). There was no significant correlation between changes in MDA and LDH levels (p=0.60) with a negative correlation, which means that the more significant the change in MDA levels, the lower the LDH level.

Keywords: *Cyclists; Malondialdehyde; Lactate Dehydrogenase; Oxidative Stress.*

INTRODUCTION

Cycling is an activity that is now loved by many people. Referring to data collected by The Institute for Transportation and Development Policy (ITDP), during the COVID-19 pandemic, cycling increased by up to 1,000 percent and production increased by 30 percent (Rupaka et al., 2021). Cycling provides an opportunity for individuals to incorporate physical activity into daily life (Oja et al., 2011).

According to Fonterra in Prasetyo, Indonesia is a country in Southeast Asia where

68% of respondents do sports with intensity, type, and time only according to their leisure time, without adjusting the dose for exercise. All sports that are carried out need to fulfill the frequency, intensity, type and time formulas to make it easier to calculate the level of physical activity during sports (Prasetyo, 2015). This data is supported by Wahyuni's research (2021) which reports that they only do cycling activities and do not set targets so that cycling can benefit physical and muscle fitness (Wahyuni, 2021).

It's still rare for people to think about the negative impact of excessive exercise on muscle conditions. The detrimental effect of changing the condition of the muscles has not received much attention, so research is still needed. Fatigue muscle condition is a change in muscle cells that occurs as a result of oxidative stress due to free radical attacks during and after exercise in the form of muscle damage (Brancaccio et al., 2010).

MDA is a substance that is formed from oxidative processes and is used as an indicator of changes in muscle condition as a result of changes in the muscle cell walls. Fatigue muscle condition due to radical attack (Gea et al., 2015). The increase in free radicals begins after exercise, Michailidis et al. reported a study on changes in the levels of oxidative stress markers after aerobic exercise, showing that levels of TBARS (thiobarbituric acid reactive substance) will increase when doing physical exercise, reach a peak within 1 hour afterwards, and a drastic decrease occurs within the next 4 hours (Michailidis et al., 2007).

Then the production of free radicals in the form of MDA will cause macromolecular oxidative damage, immune dysfunction, muscle damage, and fatigue which will trigger the process of lactic acid formation and LDH levels (Vasudevan et al., 2016). In some sports, an increase in MDA will be followed by an increase in serum creatine kinase (CK) and serum lactate dehydrogenase (LDH) (Bouchez, 2015).

This increase occurs due to an increase in the permeability of the muscle cell membrane due to a decrease in energy metabolism. Also due to damage to muscle cells due to the physical activity carried out for a long time LDH which is in the tissues is released into circulation (Rubio-Arias et al., 2019). Under normal circumstances, LDH levels are low in plasma. Increased LDH in plasma is an indication of muscle damage (Lieberman, Marks, Allan D., Peet, Alisa., 2013).

Some examples of negative impacts in the form of changes in muscle condition or muscle damage due to exercise in society are recreational sports such as cycling, the aim is recreational or benefits from physical and socio-psychological aspects, but it is not uncommon for people to experience poor muscle condition, with complaints of pain and soreness in the muscles. especially on long distances (Torres et al., 2009).

Increased muscle breakdown biomarkers. In 200 km ultra-marathon runners it was shown to increase biomarkers related to muscle and cartilage damage, as well as anti-inflammatory cytokine markers IL-6 (Kim et al., 2007).

Changes in MDA and LDH levels can be related to several factors, namely: in old age, free radicals are responsible for damage at the cellular and tissue level (Tyagita et al., 2021). Research on changes in MDA and LDH levels after cycling events is still very limited. Researchers are interested in conducting this research so that the basic mechanism for increasing MDA and LDH levels can be identified to be used as a monitoring process and material for consideration of effective training interventions in the cycling community in the future. Determining the criteria for the training zone, namely Frequency, Intensity, Time, and Type, is very important to be known by the cyclist community so that they can identify safe groups and at risk in cycling.

METHOD

This research is a pre-experimental study with one group pretest-posttest design. This research was conducted in September-October 2022 in the Makassar bicycle community. The researcher gave informed consent, demographic data questionnaires, and an explanation of the research procedures to the subjects regarding the actions to be taken. The sample in this study was a blood sample from members of the Makassar bicycle community. Calculation of the number of samples in this study used the formula (Saryono, 2011) with a 95% confidence level and the sample was determined to be 30 people.

The sampling technique used a purposive sampling technique that met the inclusion and exclusion criteria. The sample inclusion criteria to be studied were men aged 30-60 years, active cyclists (scheduled), cycle time (more than one year), healthy (normal resting ECG), willing to fill out informed consent and willing to have blood drawn. Exclusion criteria were active cycling for less than 1 year, BMI obesity, history of cardiovascular disease, history of hypertension, alcohol consumption, and unwillingness to have blood drawn.

Before the cycling event begins, BMI measurements are taken, resting ECG examinations, VO2 max measurements, initial nutritional status examinations, and initial blood sampling as pre-test values. Then activate the STRAVA application starting from the starting line at Makassar State University. After finishing at FK Hasanuddin University, cyclists are encouraged to drink, then rest for 1 hour and then take a second blood as a post-test value and record the final STRAVA data results (mileage, average speed, and duration). Blood sample testing using the ELISA Kit from Elabscience.

Statistical analysis using Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corporation, Armonk, NY, USA). The data normality test used the Shapiro-Wilk test and obtained a p-value <0.05 indicating that the data was not normally distributed. To see changes in MDA and LDH levels before and after a 30 km cycling event, the Wilcoxon test was used. Spearman's correlation test was used to determine the correlation between changes in MDA levels and LDH.

RESULTS AND DISCUSSION

Results

Data processing was carried out with the IBM SPSS 24 program using the *Wilcoxon* test (abnormal distribution) to find out how the influence before and after the 30 km cycling event had on MDA and LDH levels. The *Spearman* test was used to see the correlation between changes in MDA levels and LDH levels.

Table 1.
Subject Characteristics, Measurement Results and Baseline Examination Data

Characteristics	N	%
Age		
• 30-45 year	15	50
• 46-60 year	15	50
Cycling Time		
• >1 year	30	100
• < 1 year	0	0
Cycling Frequency (Training category)		
• Trained	13	43.3
• Untrained	17	56.7
Smoking History		
• Yes	5	16.7
• No	25	83.3
Consumption of Drugs		
• Yes	2	6.7
• No	28	93.3
Body Mass Index		
• Underweight	0	0
• Ideal	16	53.3
• Overweight	14	46.7
Energy intake (Food Recall 24 hours)		
• < 80 % inadequate	9	30
• 80-110 % adequate	14	46.7
• > 110 % over	7	23.3
Category VO^2max Level		
• > Median (Poor)	21	70
• < Median (Good)	9	30

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Characteristics	N	%
VO^2max Level		
• Excellence	0	0
• Good	0	0
• Above average	1	3.3
• Average	1	3.3
• Below average	7	23.3
• Poor	8	26.7
• Very poor	13	43.3
Average Speed		
• > Median	15	50
• < Median	15	50
EKG		
• Normal	30	100
• Abnormal	0	0

Table 1 shows that the age subject cyclist is divided into 2 groups ie 30-45 (50%) and 46-60 (50%) years each of 15 people. Speed subject divided above 2 ie group speed >median (50%) and <median (50%) with the same number of 15 people each. There are 13 trained people in the category and 17 untrained people. The ideal BMI is 16 people, overweight is 14 people. The good VO2 max group is 9 people, the bad is 21 people, the group smokers 5 people, non -smokers 25 people.

Changes in MDA Levels Before and After Cycling 30 km

Table 2.
Analysis of changes in MDA levels before and after a 30 km cycling event

Variable	n	30 km Cycling Event		p
		Pre-test Mean ± SD	Post-test Mean ± SD	
MDA (ng/ml)	30	408.89±187.18	581.01±172.83	0.000

Wilcoxon test, $p<0.05$, is considered significant

The MDA level before the cycling event was 408.89 ± 187.18 ng/ml and after the 30 km cycling event, it became 581.01 ± 172.83 ng/ml. From the Wilcoxon test, a p-value = 0.000 was obtained, so it can be concluded that there was a very significant change in MDA levels after a 30 km cycling event.

Changes in LDH Levels Before and After Cycling 30 km

Table 3.
Analysis of changes in LDH levels before and after a 30 km cycling

Variable	n	30 km Cycling Event		p
		Pre-test Mean ± SD	Post-test Mean ± SD	
LDH (µkat/L)	30	0.887±0.330	1.743±0.674	0.000

Wilcoxon test, $p<0.05$, is considered significant

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The LDH level before the cycling event was 0.887 ± 0.330 µkat/L and after the 30 km cycling event, it became 1.743 ± 0.674 µkat/L. From the Wilcoxon test, a p = 0.000 value was obtained, so it can be concluded that there was a very significant change in LDH levels after a 30 km cycling event.

Correlation of Changes in MDA Levels with LDH Levels After a 30 km Cycling Event

Table 4.
Correlation of changes in MDA levels with changes in LDH levels

Variable	N	r	p-value
ΔMDA <--> ΔLDH	30	-0.09	0.60

Spearman rank, p<0,05 is considered significant

The results of the Spearman correlation test, the value of the correlation coefficient is negative r = 0.09 (very weak correlation) indicating a non-unidirectional relationship between variables. That is, the greater the change in MDA levels, the lower the LDH levels, and vice versa. It is known that a significance value of 0.60 (p> 0.05) means that there is no significant relationship between changes in MDA levels and LDH levels (Figure 1).

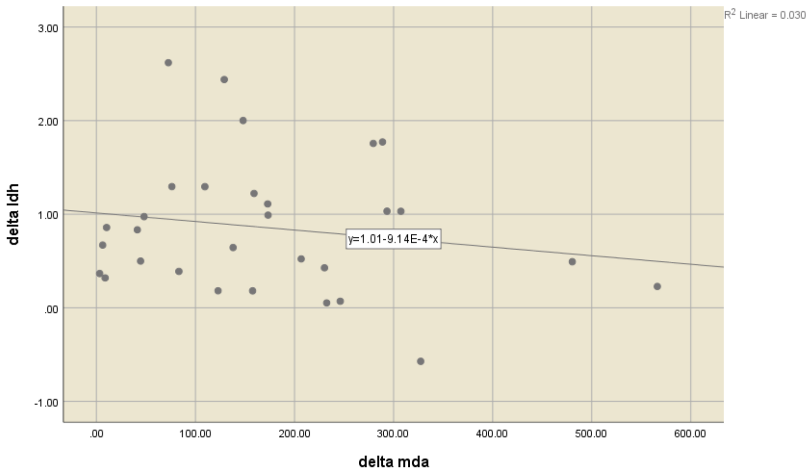

Figure 1.
The scatter plot graph shows no significant relationship between changes in MDA levels and LDH levels

DISCUSSION

Changes in MDA Levels After a 30 km Cycling Event

The results of the Wilcoxon statistical test showed that cycling with a distance of 30 km had a significant effect on changes in MDA levels ($p < 0.05$). The results of this study are in line with research conducted by Bloomer et al. (2009) which stated that there was an increase in MDA levels as an indicator of oxidative stress in the aerobic and

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anaerobic physical activity carried out for 30 minutes (Fisher-Wellman & Bloomer, 2009). Likewise, research conducted by Yunus (2016) stated that there was a significant effect of badminton exercise on oxidative stress as indicated by high levels of MDA in blood plasma in the untrained group (Yunus, 2016).

Research conducted by Chevion et al. (2003) that an increase in MDA in the body occurs due to strenuous physical activity causing an increase in metabolism and oxygen consumption which can increase the production of ROS (Chevion et al., 2003).

When doing strenuous physical activity can increase oxygen consumption 100-200 times compared to resting conditions. Increased use of oxygen, especially by contracting muscles, causes electron leakage from mitochondria which will become ROS (Reactive Oxygen Species) (Talarowska et al., 2012). Increased energy requirements in muscles that are excessively contracted, meaning that the need for processing oxygen into the tissues also increases and turns off electrons into the respiratory chain in the mitochondria also increases (Brancaccio et al., 2010).

Increased metabolism and oxygen consumption are required to meet the increased energy requirements during strenuous physical activity. Part of the oxygen consumed will be converted into ROS so an increase in oxygen consumption will cause an increase in ROS production. Energy requirements increase sharply during strenuous physical activity, spurring the metabolism of energy supply in the body (Sinaga, 2016).

During strenuous physical activity, another important source of energy is anaerobic glycolysis, especially during hypoxaemia. Anaerobic glycolysis produces lactic acid. The higher the exercise intensity, the higher the lactic acid produced, thus causing the accumulation of lactic acid. Lactic acid can make ROS more reactive (Helianti & Hairrudin, 2009). The higher the production of ROS, the more it fails to be neutralized by the antioxidant system in the body. ROS that is not neutralized will react with body components including PUFA, causing lipid peroxidation. Lipid peroxidation produces various products including MDA so increased ROS production in strenuous physical activity will result in MDA production (Ayala et al., 2014).

This increase in oxygen volume will result in increased free radical formation. A state of balance will occur between the production of free radicals with antioxidant defences. This balance can be lost due to the excessive production of free radicals after excessive or irregular exercise. As a result of the disappointment between free radicals and antioxidants, oxidative stress will arise which can damage cell membranes, DNA or proteins, causing pain. Poor muscle condition as indicated by increased MDA levels. The

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released MDA is then released into the blood (Kawamura & Muraoka, 2018).

Effect of a 30 km Cycling Event on Changes in LDH Levels

The results of the Wilcoxon statistical test showed that cycling at a distance of 30 km had a significant effect on changes in LDH levels ($p<0.05$). This is because the physical activity does not purely use only one of the aerobic and anaerobic metabolic systems, but uses a combination of the two systems. Energy formed from aerobic and anaerobic metabolism in cells is a continuous process of energy formation (Flora, 2015). The transition of metabolism from aerobic and anaerobic types is an adaptation response so that energy is still available even in the absence of oxygen. Anaerobic metabolism has an impact on the formation of lactic acid. LDH is needed to convert lactic acid into pyruvate (Khonsary, 2017).

This research is in line with the research of Li et al. (2012) who reported that plasma LDH activity increased immediately after exercise/physical activity was performed (Li et al., 2012). Research by Kobayashi et al. (2005) demonstrated that aerobic exercise, such as running, can promote an increase in LDH activity for 12 to 24 hours (Kobayashi et al., 2005).

According to Flora (2015), conditions that are more hypoxic in anaerobic physical activity have an impact on increasing the use of LDH in catalyzing lactate to pyruvate (Flora, 2015). LDH plays a role in the glycolysis process in anaerobic conditions which produces lactate. The LDH enzyme also plays a role in glycogenolysis in the muscles which always ends in lactate. When in aerobic conditions the end product of glycolysis is pyruvic acid which will enter the citric acid cycle. LDH can be detected because of its ability to catalyze pyruvate reduction in the presence of NADH or catalyze lactate oxidation in the presence of NAD+. LDH can be used as an indicator that supports tissue damage (Goodwin et al., 2007).

The results of this study are also supported by research conducted by Sari Octarina Piko et al. a comparison of aerobic and anaerobic physical activity to lactate and lactate dehydrogenase (LDH) levels in FKIP students of the Department of Sports Education, Bina Darma University in Palembang City, showed that there was an increase in LDH levels in aerobic and anaerobic physical activity (Admin et al., 2019).

The enzyme lactate dehydrogenase (LDH) in the blood can convert pyruvate to lactate or vice versa. This interconversion can occur quickly and at the same rate in both directions, even under severely hypoxic conditions (Khonsary, 2017). Research by Rumley

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& Rafla (2015) states that physical activity can result in an increase in LDH in plasma. This increase occurs due to an increase in the permeability of muscle cell membranes due to a decrease in energy metabolism and due to damage to muscle cells due to physical activity carried out for a long time so that LDH in the tissues is released into circulation (Flora, 2015). Under normal circumstances, LDH levels are very low in plasma. Increased LDH in plasma is an indication of muscle damage (Torres et al., 2009).

According to the researchers in this study, the increase in plasma LDH levels in cyclists was not due to tissue damage. The increase in LDH is due to an increase in lactate secretion as the result of anaerobic metabolism so LDH is needed to convert lactate into pyruvate so that it can be used again as an energy source.

Correlation of Changes in MDA Levels with LDH Levels After Cycling Events

There was no significant relationship between changes in MDA levels and LDH levels (p=0.60). The value of the correlation coefficient is negative, r=-0.09, indicating that the variable relationship is not unidirectional. That is, the greater the change in MDA levels, the lower the LDH levels, and vice versa.

According to Liguori et al (2020), the factor that can affect levels of oxidative stress in the body is age. At an older age, physiological functions decrease, including the process of forming antioxidants (Völter et al., 2020). Meanwhile, the increase in LDH levels depends on the intensity and duration of exercise (Brancaccio et al., 2010).

CONCLUSIONS AND SUGGESTIONS

Conclusions

The 30 km cycling event affects changes in MDA (free radical) levels due to lipid peroxidation during cycling and affects changes in LDH levels due to increased lactate secretion. There was no significant correlation between changes in MDA and LDH levels with a negative correlation, which means that the greater the change in MDA levels, the lower the LDH level.

Suggestions

It is recommended by the cycling community to cycle according to the frequency, intensity, type and time determined by professional health. It is necessary to carry out comprehensive health checks regularly including heart health so that the health status of cyclists can be properly monitored.

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