Analysis Of Changes In Nitric Oxide (NO) And F₂-Isoprostane Levels After The 30 Km Cycling Event In The Makassar Bike Community

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ABSTRACT
This study aims to determine changes in Nitric Oxide (NO) and F₂-Isoprostane levels after a 30 km cycling event in the Makassar cycling community. This research was a pre-experimental study with a one-group pretest & post-test design with 30 cyclists as subjects in the city of Makassar, men aged 30-60 years. Blood sampling was carried out pre-event and post-event cycling as far as 30 Km. Measurement of F₂-Isoprostane & Nitric Oxide levels using the ELISA method. Data analysis used the Wilcoxon test to see changes in Nitric Oxide (NO) and F₂-Isoprostane levels after participating in a 30 Km cycling event. Data analysis using the Mann-Whitney test was used to see differences in changes in Nitric Oxide (NO) and F₂-Isoprostane levels in the age group, average speed, exercise category, BMI, and smokers. Spearman test to see the correlation of changes in Nitric Oxide (NO) and F₂-Isoprostane levels. The results of this study found that the 30 km cycling event had a significant effect on changes in Nitric Oxide (NO) levels which could improve the function of the vascular endothelium with p=0.0001. Meanwhile, the 30 km cycling event had no significant effect on changes in F₂-Isoprostane levels, which meant that it had no risk of oxidative stress and tissue damage (p=0.688). There is no positive correlation between changes in Nitric Oxide (NO) levels and changes in F₂-Isoprostane levels, which means that changes in the repair of vascular endothelial function seem to suppress free radical production which leads to oxidative stress after cycling as far as 30 Km.

Keywords: Nitric Oxide; F₂-Isoprostane; Cycling; Exercise.

INTRODUCTION
During the Covid-19 pandemic, cycling was the choice of the wider community to continue exercising while maintaining physical distancing. According to data from the Institute for Transportation and Development Policy (ITDP), bicycle users have increased
10 times in Jakarta, Indonesia (Alfirdaus & Susanto, 2021; Ambarsari, 2019). Several cases of sudden death due to cycling during the Covid-19 pandemic have been in the spotlight on several news portals, although currently reporting of cases of death due to cycling in Indonesia have not been recorded with certainty. The study by Toukola et al., (2015) showed that cycling is the 2nd most frequently reported type of sport due to sudden death. In France, cases of death in cycling have been reported to be higher, namely, 30.61% compared to running, football, hiking, swimming, and basketball (Marijon et al., 2011).

Cycling alone certainly has many benefits for health, but there are also safety risks and injuries in this case if it is done excessively beyond the capacity and ability of the body or overexercise (Bopp et al., 2018; Wahyuni, 2021). Compared to moderate-intensity exercise, vigorous-intensity exercise can increase the risk of an unexpected death by 5 to 7 times higher. 90% of Sudden Cardiac Death (SCD) occur in men and recreational sports (de Gouveia et al., 2016).

The main factors in bicycle-related injuries or even deaths apart from infrequent and irregular physical activity are also due to the extreme intensity, in this case, average speed and duration, which depend on the type of muscle and cardiovascular condition, in a person with impaired cardiovascular function, even without symptoms. which is responsible for 80% of sudden cardiac death in old age compared to young age (de Gouveia et al., 2016). Adolescents to adults over 40 years of age have a higher risk of death due to cycling, while those aged 5-24 years have the highest risk of injury (Bopp et al., 2018).

During acute exercise, oxygen consumption increases and there is a significant change in blood flow to various organs, it is these physiological changes that will lead to ROS-mediated oxidative stress which causes the accumulation of lipid peroxidation products including free radicals (Kawamura & Muraoka, 2018; Parshukova et al., 2020). F2-Isoprostane is a clinical marker to see free radical production which is stable and accurate (Lidapraja, 2013).

Oxidative stress is the main reason for changes in Nitric Oxide (NO) activity. Nitric Oxide (NO) acts as a good vasodilator, increasing blood flow to muscles, nutrient transport and gas exchange. Oxidative stress will create lesions on the endothelium with the Nitric Oxide (NO) reaction. If there is a decrease in Nitric Oxide (NO) levels in the vascular endothelium, then the body's adaptive capacity decreases and pathological changes occur in metabolism and can potentially lead to coronary heart disease and vascular damage (Astutik et al., 2014; Baraas et al., 2013; Persio et al., 2020). The purpose of this study was to determine the formation of free radicals through F2-Isoprostane levels and the function of the endothelium and blood vessels through Nitric Oxide (NO) levels when cycling 30 Km.
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METHOD

Study Design
This research is a pre-experimental study with one group pretest-posttest design. The study was conducted after obtaining ethical approval from the Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Approval No: 597/UN4.6.4.5.31/PP36/2022, Protocol No: UH22080483).

Subject Recruitment
The subjects in this study amounted to 30 people who are a cyclist community in the city of Makassar. The sampling technique was purposive sampling which met the inclusion criteria. The inclusion criteria for this study were men aged 30-60 years, actively cycling for more than one year, with normal resting EKG, and willing to have blood drawn. Exclusion criteria were having a history of cardiovascular disease, alcohol consumption and obesity. This research was conducted in October 2022.

Anthropometric measurements, VO2 Max, resting ECG examination, 24-hour food recall, and blood sampling was taken as pre-test data before the 30 Km cycling event. On a different day, the 30 Km cycling event started by activating the STRAVA application at the start line. While cycling, the researcher monitors the participants along the route. After arriving at the finish line, the participants took a short break and after that, a second blood sample was taken as post-test data and recorded data from the STRAVA application, namely, the distance traveled, average speed and duration of the cyclists.

Blood Sample Collection
3cc blood samples were taken, then stored in the HUM-RC laboratory freezer at Hasanuddin University Hospital. Serum was separated by centrifuge at 5000 rpm for 5 minutes, then blood samples were tested using the ELISA method.

Statistical analysis
Test the normality of the data with the Shapiro-Wilk test with abnormally distributed data (p <0.05). The Wilcoxon test was performed to see changes in levels of Nitric Oxide (NO) and F2-Isoprostane. Spearman correlation test was performed to determine the relationship between changes in Nitric Oxide (NO) and F2-Isoprostane. Statistical analysis using IBM SPSS Statistics version 26.
RESULTS AND DISCUSSION

Subject Characteristics

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 30-45 years</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>• 46-60 years</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td><strong>Cycling time</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• &gt; 1 year</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>• &lt; 1 year</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Cycling frequency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Trained</td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td>• Untrained</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>• No</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td><strong>Consumption of drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>• No</td>
<td>28</td>
<td>93.3</td>
</tr>
<tr>
<td><strong>Body Mass Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Underweight</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>• Ideal</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>• Overweight</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Energy intake (food recall 24 hours)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• &lt; 80% inadequate</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>• 80-110% enough</td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>• &gt; 110% over</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>Level VO\textsubscript{2}max</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Good</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>• Poor</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td><strong>Average speed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• &gt; Median</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>• &lt; Median</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td><strong>EKG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>• Abnormal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 30-45 year</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>• 46-60 year</td>
<td>15</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 1 shows that the subject characteristics for age are grouped into two, namely the age group 30-45 years (50%) and 46-60 years (50%). While the characteristics of the subjects for the average speed were grouped into 2, namely the >median speed group (50%) and the <median speed group (50%). There are 13 people trained in the category, and 17 people in the untrained category. Smokers as many as 5 people and 25 people who do not smoke. The ideal BMI is 16 people while overweight is 14 people. VO2 Max has a good category of 9 people and 21 poor people.
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Changes in NO Levels Before and After a 30 km Cycling Event

Table 2. Analysis of changes in NO levels after a 30 km cycling event

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>30 km cycling event</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-test Mean±SD (µmol/L)</td>
<td>Post-test Mean±SD (µmol/L)</td>
</tr>
<tr>
<td>Nitric Oxide (NO)</td>
<td>30</td>
<td>39.3±12.6</td>
<td>103.8±156.9</td>
</tr>
</tbody>
</table>

Wilcoxon test, meaning when p<0.05

Based on table 2, it shows that there was an increase in pre and post test with mean ± SD pre-test 39.3 ± 12.6 and post test 103.8 ± 156.9. The results of the Wilcoxon test were obtained p = 0.0001, which means that there was a very significant change in Nitric Oxide (NO) levels after cycling 30 km.

Changes in F2-Isoprostane Levels Before and After a 30 km Cycling Event

Table 3. Analysis of changes in F2-Isoprostane levels before and after cycling 30 km

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>30 km cycling event</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-test Mean±SD (pg/mL)</td>
<td>Post-test Mean±SD (pg/mL)</td>
</tr>
<tr>
<td>F2-Isoprostane</td>
<td>30</td>
<td>102.51±50.16</td>
<td>109.22±42.94</td>
</tr>
</tbody>
</table>

Wilcoxon test, meaning when p<0.05

Based on table 3, shows that there was an increase in pre and post-test with mean ± SD pre-test 102.5 ± 50.1 and post-test 109.2 ± 42.9. The results of the Wilcoxon test were obtained p = 0.688 which means that there was no significant change in F2-Isoprostane levels after cycling 30 km.

Correlation of Changes in F2-Isoprostane Levels with Changes in ET-1 before and after a 30 km cycling event

Table 4. Correlation of changes in F2-Isoprostane levels with changes in Nitric Oxide (NO) levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔF2-Isoprostane - ΔNO</td>
<td>30</td>
<td>-0.248</td>
<td>0.094</td>
</tr>
</tbody>
</table>

Spearman rank, meaning when p<0.05

The results showed that changes in F2-Isoprostane content with Nitric Oxide (NO) levels had a correlation coefficient r = -0.248 indicating a negative correlation. The
significant value shows 0.094 (p>0.05). This means that there is no significant relationship between changes in levels of F₂-Isoprostane and Nitric Oxide (NO) after the 30 km event.

![Graph showing no significant relationship between F₂-Isoprostane and NO](image)

**Figure 1.**
The scatter dot graph shows that there is no significant relationship between changes in F₂-Isoprostane and Nitric Oxide (NO) levels

**Discussion**

**Changes in Nitric Oxide (NO) Levels Before and After a 30 Km Cycling Event**

In the results of this study, the aerobic exercise by cycling 30 km can increase NO levels, which is consistent with previous studies (Otsuki et al., 2019; Pan et al., 2015; Tanaka et al., 2015; Tsukiyama et al., 2017) which showed an increase in Nitric Oxide (NO) concentration after doing aerobic exercise. Isral & Sulastri (2014) said that there is a significant relationship between physical activity and changes in Nitric Oxide (NO) levels. Exercise has been shown to increase plasma nitrite levels by increasing Nitric Oxide (NO) synthesis in endothelial cells (Arikawa et al., 2013). During aerobic exercise, local and systemic Nitric Oxide (NO) production increases in the cardiovascular system including arteries, veins and capillaries. Nitric Oxide (NO)-mediated vasodilation occurs due to the response of the endothelium which can reduce vascular resistance at rest and blood pressure response after exercise (Marino et al., 2021; Otsuki et al., 2019).

When doing physical exercise, physical and chemical responses occur that can control Nitric Oxide (NO) production. In endothelial cells, exercise can stimulate Nitric Oxide (NO) synthesis through a chemical response. This chemical mechanism involves the interaction of endogenous/exogenous agonists (acetylcholine, bradykinin, and ATP) with specific receptors on endothelial cells. The physical impact on the walls of the blood vessels stimulates the release of Nitric Oxide (NO) in the blood vessels. An increase in shear stress due to exercise can stimulate the release of vasorelaxation factor Nitric Oxide (NO) (Green et al., 2017).
Shear stress is the main mechanism for increasing Nitric Oxide (NO) production during physical exercise. Mechanical stress generated by friction between red blood cells and endothelial cells activates endothelial NOS and increases Nitric Oxide (NO) production. Nitric Oxide (NO) is produced by eNOS through the metabolism of L-arginine to L-citrulline. Nitric Oxide (NO) diffuses into the underlying vascular smooth muscle and activates the enzyme guanylate cyclase. It then induces cGMP production which activates the cGMP-dependent protein kinase G (PKG) metabolic pathway, causing vascular relaxation. Thus shear stress is considered a strong stimulus for the release of vasodilator factors produced by the vascular endothelium (Facioli et al., 2022; Marino et al., 2021).

Changes in F2-Isoprostane Levels Before and After a 30 km Cycling Event

Every cell in the body carries out a metabolic process that will produce free radicals and is characterized by the formation of reactive oxygen species (ROS). Free radicals can be formed because they are triggered by stressors, namely ultraviolet light, radiation, and physical activity (Berawi & Agverianti, 2017). The results of the analysis found that there was no significant increase in F2-isoprostane levels after exercise, which is in line with the study of Goods et al., (2016) and Maghsoudi et al., (2016) which showed that there was no significant increase in F2-isoprostane post-exercise.

Although no significant change was found, based on the results it was found that there was an increase in F2-isoprostane levels after cycling as far as 30 Km. This research is in line with the research of Syahrastani et al., (2019) and Wadsworth & Lark, (2020) there was an increase in F2-Isoprostane levels after doing physical exercise. Strenuous exercise induces oxidative stress and also results in tissue hypoxia. The high demand for muscle oxygen during exercise can lead to low oxygen availability for other tissues. It is this increase in oxygen consumption during exercise that will increase the occurrence of ROS formation (Green et al., 2017). As long as the intensity and duration are low, the body has an effective antioxidant defence response that may meet the production of ROS, however, as the intensity and duration of exercise increases, the antioxidant defence is no longer sufficient which has the potential to result in oxidative damage (Thirupathi et al., 2021).

High-intensity exercise increases total antioxidant capacity when compared to moderate to low-intensity exercise which causes no changes in lipid peroxidation which is a product of free radicals. Previously it was believed that exercise of increased intensity must be overwhelmed by antioxidant defences that induce conditions of oxidative stress. However, recent reports have shown that even low or moderate intensity can lead to oxidative stress, suggesting that training volume (exercise duration and intensity) and a failing antioxidant defence system are the main...
mediators of exercise-induced oxidative stress (Thirupathi et al., 2021).

Different intensities of resistance training can effectively reduce oxidative damage in individuals when compared to the same intensity. This could be due to exercise-induced redox-related health adaptations through enhanced antioxidant defence systems. Regarding aerobic exercise, moderate intensity improves exercise performance by reducing oxidative damage (González-Bartholin et al., 2019).

However, short-term, higher-intensity aerobic exercise can strongly induce oxidative damage, but this effect does not extend to the DNA level. The type of exercise is an important factor for inducing oxidative damage because high cycling intensity reduces oxidative damage and increases enzymatic antioxidants (Bogdanis et al., 2013).

**Correlation of Changes in F2-Isoprostane Levels with Changes in Nitric Oxide (NO) before and after a 30 km cycling event**

The results of the statistical analysis did not show a positive correlation between changes in F2-Isoprostane and Nitric Oxide (NO) levels. Nitric Oxide (NO) has increased significantly and F2-Isoprostane has not increased significantly after exercise (cycling), which means that an increase in Nitric Oxide (NO) in the body can reduce the production of ROS (Fenty-Stewart et al., 2010). Moderate aerobic exercise can improve endothelial function by increasing Nitric Oxide (NO) production, meanwhile, superoxide and hydrogen peroxide which are products of ROS can be produced in a controlled manner (Tanaka et al., 2015).

The study of Baraas et al., (2013) showed that there was no positive correlation between F2-Isoprostane and Nitric Oxide (NO). Nitric Oxide (NO) is a negative predictor variable for F2-Isoprostane levels because the higher the Nitric Oxide (NO) level, the lower the F2-Isoprostane. As a gas molecule, Nitric Oxide (NO) plays an important role in regulating all of the rapid vasoconstriction and vasodilation signals, especially catecholamine signals and sympathetic nerves. However, Nitric Oxide (NO) can control ROS production so Nitric Oxide (NO) is a negative predictor variable for F2-Isoprostane which contribution to suppressing ROS production.

Increased Nitric Oxide (NO) levels have been associated with the absence or negative effect of increasing levels of oxidative stress. During acute exercise, the potential for increased ROS will be responded to along with Nitric Oxide (NO) production which is beneficial for improving endothelial function. Hydrogen peroxide also increases with acute exercise. Hydrogen peroxide has the potential to positively regulate vasorelaxation through indirect eNOS activation (Tanaka et al., 2015).
CONCLUSIONS AND SUGGESTIONS

Conclusions

Cycling as far as 30 Km can improve the function of blood vessel endothelium, and also can not increase the occurrence of oxidative stress which leads to free radicals. Changes in the repair of the vascular endothelial function appear to suppress free radical production after cycling 30 km. The higher the Nitric Oxide (NO), the lower the ROS level because an increase in Nitric Oxide (NO) can reduce the production of ROS after cycling (exercise). Therefore, judging from the clinical results obtained, cycling can improve vascular endothelial function and can also reduce ROS activity. But it is advisable to continue to carry out clinical examinations periodically before doing sports, especially with moderate-severe intensity to determine that cycling (exercise) is in the safe category.

Suggestions

For further research, it is necessary to take a second posttest blood sample after 24 hours of cycling to see whether tissue damage is temporary or persistent after cycling. When taking baseline blood samples, respondents were advised not to do a strenuous physical activity so that it would not affect the results of the data.

REFERENCES


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