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Effect of acute submaximal physical exercise before decompression dive on tumor necrosis factor alpha concentration among male trained divers

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ABSTRACT

The increase of inflammatory biomarkers due to decompression dive is one of the factors that could cause decompression sickness (DCS), one of them is tumor necrosis alpha (TNFα). According to the preconditioning theory, exercise before dive can reduce amount of gas bubble to prevent DCS. This study aimed to prove that exercise before diving can prevent increase of TNFα. This study employed quasi-experimental design with trained male divers. The subject divided into two groups, treatment and control. The treatment group got submaximal exercise with 70% heart rate intensity, using cycle ergometer with young men's Christian association (YMCA) procedure modify by Guritno, 24 hours before decompression dive 280 kPa bottom time 80 minute with US-NAVY table, whereas the control group only do decompression dive. TNFα expression was checked three times, at beginning of study, before dive and after dive. In treatment group there was insignificant decrease TNFα, from 7.06±1.85pg./ml to 6.75±1.81pg./ml, whereas the control group showed a significant increased TNFα, from 8.22 (1.45 to 13.11)pg./ml to 8.39 (1.73 to 12.18)pg/ml, and significant difference was found between the mean difference for two groups $p<0.05$. It can be concluded that acute submaximal exercise prevents an increase of TNFα after single dive decompression to prevent possibility occurring DCS).

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1. INTRODUCTION

Decompression illness is a disease that occurs due to changes in environmental pressure. Decompression illness can affect by divers, compressed air workers, aviators and astronauts. Changes in environmental pressure cause the formation of inert gas bubbles in blood circulation and its causes decompression sickness (DCS) [1].

The Diver Alert Network released that the death rate due to diving in the last 10 years reached 1067 cases, and specifically in 2019 the number of cases reached 228 cases worldwide. By presentation 37.5% of deaths were due to decompression sickness (DCS). Decompression sickness in 2019 reached 2423 incidents worldwide. This figure is the number of incidents reported by professional divers who have insurance, so it is almost certain that the actual incidence is higher because many divers do not work formally and do not have insurance [2]. Meanwhile in Indonesia, the incidence of decompression sickness has not been reported nationally, because the majority of divers are traditional divers. It was noted that fishermen in 2006 in

Bungin Island, NTB 57.5% reported joint pain, which is a direct symptom of decompression sickness type 1, in the pramuka islands of Jakarta, the prevalence of decompression sickness was as much as 6.9% and cumulatively in karimun jawa islands between 2007-2014 there were 104 cases with 7 people died [3]-[4]. Many divers and compressed air workers have tried to prevent decompression sickness (DCS) by carrying out work according to deco-stop procedures, but decompression sickness incidents are still difficult to avoid. This is in accordance with Mayden's theory, which states: Decompression sickness due to dives not only caused by bubble gas, it can be caused by endothelial dysfunction [5]. Endothelial dysfunction due to dives is triggered by hyperoxia-induced vasoconstriction at depth that causes Oxidative Stress. In studies conducted with rats, an association was found between the incidence of endothelial dysfunction and gas bubbles [5]. This can be seen from changes in inflammatory biomarkers such as endothelin-1 (ET-1), 6-keto prostaglandin F1α (6-keto-PGF1α), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion protein-1 (VCAM-1) and malondialdehyd (MDA), while serum nitric oxide (NO) decreased following rapid decompression and also the increased of tumor necrosis factor alpha (TNFα) expression [6], [7].

Tumor necrosis alpha (TNFα) is a pleiotropic acute cytokine that initiates cytokine cascade by increasing membrane permeability of the blood vessels, this contributing to the body's defenses and stimulating cell growth. Excessive TNFα activation increases the risk of tumor and cell death. TNFα is influential in the process of fat metabolism, in the hypercholesterolemia diver TNFα levels is above the normal value. TNFα also increases with regard to the arterosclerotic process or the aging process of blood vessels, in obese patients and increased blood sugar TNFα also increased [8].

Research in professional divers in Indonesia, decompression dive induced inflammatory biomarkes changes like the decrease of endothelial-NOS (eNOS) synthesis, increased interleukin-1 synthesis and increased thrombocyte aggregation processe [9]-[11].

Precautions against the occurrence of oxidative stress due to decompression dive is by providing a pre-dive condition, known as preconditioning theory. One of them is physical exercise before diving. Physical exercise before dives with specific intensity, duration and frequency has been shown to reduce bubble gas formation [12], [13]. No studies have yet reported on pre-dive exercise on the expression of anti-inflammatory biomarkers. As we know that physical exercise with specific intensity, duration and planned frequency increases metabolism and adaptation response through increased antioxidant enzymes [13]. It increases oxygen consumption resulting in the formation of hydrogen peroxide that provides physiological responses through redox-sensitive factor transcription that suppresses inflammatory cytokine synthesis like tumor necrosis factor alpha (TNFα) and increases antiinflammatory cytokines [7], [14]. And also the absence has about the effect of decompression dive on tumor necrosis factor alpha (TNFα) expression in humans, and it has not been proven also about the effect of physical exercise on markers of TNFα as an inflammatory biomarkers. So that this study aims to prove that physical exercise before decompression diving can prevent the occurrence of oxidative stress due to decompression diving, this is seen from the prevention of an increase in inflammatory markers tumor necrosis factor alpha and hope it can be used to prevent of occurring decompression sickness induced from oxidative stress in diving. In this study, we also wanted to see the effect of age, smoking habits, body mass index (BMI) on tumor necrosis factor alpha (TNFα) expression in healthy divers.

2. RESEARCH METHOD

This research is a collaboration study with an quasi-experimental design, subjects were divided into two groups, treatment and control based on age groups, with pre and post designs, which was carried out in 2016. This quasi-experimental research implemented by looking at the effect of submaximal physical exercise with submaximal intensity of exercise 70% maximal heart rate frequency, given 24 hours before dives, physical exercise given using multistage young men's Christian association (YMCA) cycle-ergometer modif by Guritno's as shown in Figure 1 [15], [16]. Every subjek has their own exercise intensity target, intensity calculation using heart rate. Heart rate is calculated by the formula 220-age x 70% (submaxsimal intensity). Decompression dive is done by using hyperbaric chamber with pressure 280 kPa with bottom time 80 minutes, recompression with U.S.Navy diving procedure. The TNFα level expression was performed three times at the beginning of the study, before diving and after diving. The total cholesterol level and low-density lipoprotein (LDL) level perfomed only one time in the beginning of study. The entire study subjects were trained male divers, who had met the criteria and will be divide into treatment and control group. The treatment group was the group that received the acute submaximal physical exercise of pre-dive and the control group did not receive acute submaximal physical exercise. This criterion is made to control the confounding factors for TNFα expression, the criteria divide into inclusion and exclusion criteria.

Inclusion criteria used (1) Trained divers aged 21-40 years, (2) Fit do dive, (3) Did not dive in the last 48 hours before the research, (4) Did not do physical exercise in the last 48 hours before the research, (5) Did not consume painkiller, anti-inflammatory or herbal medicine, (6) Not currently suffering from chronic disease. The exclusion criteria in this study were; (1) TNFα levels are lower than 0.54pg/μ, (2) TNFα levels are higher than 15pg/μ, (3) Total cholesterol levels are more than 240mg /dl, (4) Low-density lipoprotein (LDL) levels are more than 160mg/dl. The research met the research ethic on the ethics commission of the medical faculty Universitas Indonesia. The TNFα level at the beginning of the study, cholesterol total and low-density lipoprotein (LDL) was used to see if any subjects would be excluded.

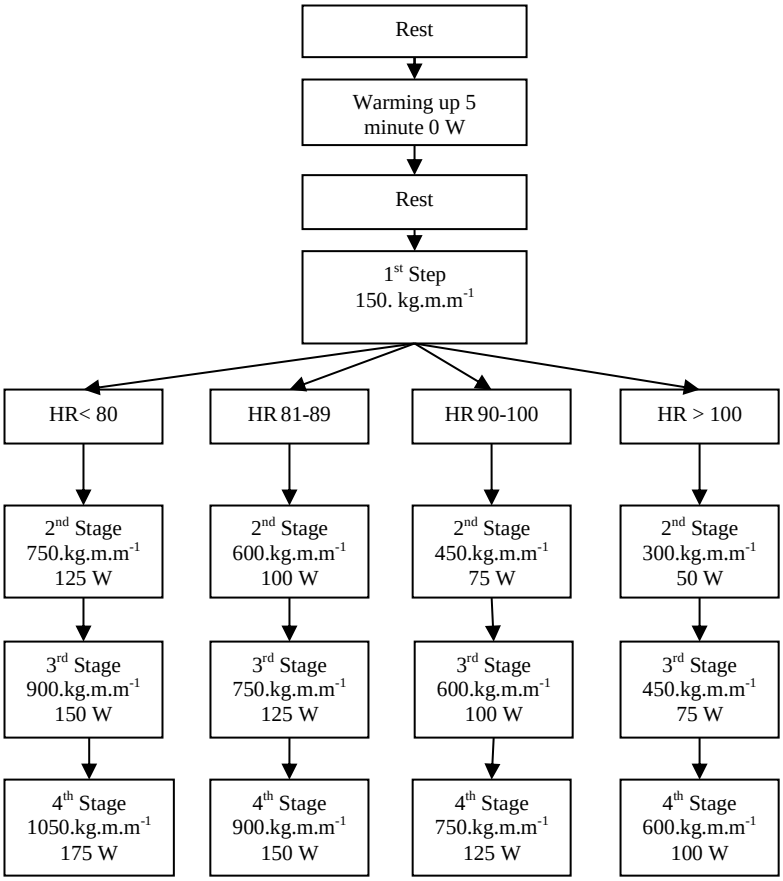

Figure 1.YCMA procedure modify by Guritno

The calculation of the number of research subjects is based on the research of Ye Chen, *et al.* [7]. This is because there are no studies in humans that have seen the provision of acclimation before diving by doing physical exercise in preventing oxidative stress by looking at TNFα expression. The sample size was calculated as a power of 90% and a tolerable error of type one was 5%. After calculating, the number of research subjects required is 34 people. However, in this study, 44 research subjects were obtained. Then all selected subjects were sorted alphabetically in order to obtain a sampling frame, the subjects divided into two age groups, namely 21-30 years and 31-40 years, then the subjects were selected by random sampling in each age group. Total 44 research subjects were obtained and divided into 22 people in the 21-30 year age group and 22 people in the 31-40 year age group, which were divided proportionally into 22 people in the treatment group and 22 people in the control group.

Statistical analysis was performed using SPSS version 22, data analysis was adjusted to the results of the distribution of data from research subjects, data distribution tests were performed using the Shaphiro Wilk test. Paired t-test is performed if data distribution is normal, and non-parametric or Wilcoxon test is performed when data distribution is abnormal. The calculation of the mean difference between the two groups was carried out using the Mann-Whitney test.

3. RESULTS AND DISCUSSION

Total subjects were 44 research subject. But only 40 research subjects who can complete the research and still meet the data processing criteria. This is because in the treatment group there were two subjects who were excluded because their total cholesterol, low-density lipoprotein (LDL) and TNFα levels exceeded the specified limits and in control group there there are two subjects who withdrew from the research due to reasons of getting a sudden assignment letter from the leadership. Total 40 subject with an age range of 30±4.03 years. The mean body mass index (BMI) of the study subjects was 24.3±2.53 kg/m2, with 45% of the subjects as smokers as shown in Table 1.

The equivalence test based on the results of the baseline tumor necrosis factor alpha (TNFα) examination between the treatment group and the control group using the parametric test showed that there was no significant difference between the treatment group and the control group (p=0.220), meaning that the TNFα level at the beginning of the study was equivalent as shown in Table 2.

Table 1. Distribution of subjects according to demographic and group characteristics

Variable	Treatment n=20		Control n=20		Total
	Frequency	%	Frequency	%	
Age :					
21-30 years	10	50	10	50	20
31-40 years	10	50	10	50	20
BMI (kg/m^2) :					
Norm weight	13	65	15	75	28
Overweight	7	35	5	25	12
Smoking habit					
Non-smoker	13	65	9	45	22
Smoker	7	38	11	62	18

Table 2. TNFα beginning of the study equivalence in both groups

Variable	Treatment	Control	p
TNFα (pg/ml)	7.06±1.85	7.69±3.08	0.220

3.1. The effectiveness of the pre-dive treatment

To assess the effectiveness treatment group, an analysis was carried out on the mean TNFα at the beginningof the study and TNFα after the dive. TNFα distribution at the beginning of the study and after diving in the treatment group had a normal distribution. While the TNFα distribution at the beginning of the study and after diving in the control group had an abnormal distribution. Bivariate analysis of TNFα levels at the beginning of the study with after diving in the treatment group with the parametric test, there was a decrease in the mean TNFα level after diving compared to the beginning of the study, but it was not statistically significant, or in other words the treatment prevented an increase in TNFα levels after the dive. Bivariate analysis of TNFα levels at the beginning of the study with after diving in the control group with non-parametric test, there was a significant increase in the mean of TNFα level after diving compared to the beginning of the study, or in other words, decompression diving caused an increase in TNFα levels as shown in Table 3.

Table 3. Differences in mean TNFα beginning of the study and after diving

Variable	Beginning of study	After diving	90%CI of difference	p
TNFα treatment (pg/ml)	7.06±1.85	6,75±1,81	(-0.68-0.59)	0.0815
TNFα control (pg/ml)	7.69±3.08	7.94±0.51	(0.13-0.46)	0.005*

***** Non parametric test

The difference delta mean in TNFα levels after diving and the beginning of the study had an abnormal distribution. The difference mean changes in TNFα levels between the treatment and control groups using the non-parametric test, found a statistically significant difference in TNFα levels between the treatment groups as shown in Table 4.

Table 4. The difference in TNFα levels at beginning and after diving in both groups

TNFα	Median(Min-Max)	p
ΔTNFα treatment group (pg/ml)	-0.24 (-2.74 – 1.67)	0.0015*
ΔTNFα control group (pg/ml)	0.45(-0.94 – 0.95)	

***** Non parametric test

In a preliminary study conducted on mice, it was found that giving acclimation to mice before decompression diving can reduce TNFα levels [6]. Physical exercise with intensity of 70% maximum heart rate 24 hours before diving in the treatment group, decreased TNFα levels after a single dive 280 kPa decompression 80 minutes bottom time compared to the initial expression of the study, or in other words physical exercise before diving can prevent the increase in TNFα after dive. This situation is reinforced by the theory that Physical exercise with intensity, durations and planned frequencies, cause a good adaptation response.

In theory, an increase in the immune system can occur through the mechanism of increasing the number of leukocytes in the circulatory system, an increase in body temperature which causes a hormonal response trough epinephrine and norepinephrine activation [17]. In another mechanism, aerobic exercise will increase oxygen consumption 15 times greater than normal in cellular metabolism. This increase emerges from the increased demand for adenosine triphosphate (ATP) during exercise, while the intracellular supply of adenosine triphosphate (ATP) is very limited. As a result from a continuous demand for adenosine triphosphate (ATP) production through oxidative processes, Krebs-cycle and electron transfer the consumption of oxygen in the respiratory chain increased, and its produce the formation of free radicals. Free radical like reactive oxygen species (ROS) is increase; it caused by an imperfect electron transfer process causes the reduction of oxygen molecules to produce superoxide ions. Reactive oxygen species (ROS) is also influenced by the auto-oxidation of myoglobin and hemoglobin during exercise. Superoxide ion is formed from oxyhemoglobin and oxymyoglobin based on metal catalysis reactions. The superoxide ion produced in large quantities, cell make an adaptive response by increasing the amount of the enzyme superoxide dismutase (SOD) so that the superoxide ion is converted into hydrogen peroxide [18]. Hydrogen peroxide acts as a 2nd messenger who can provide a positive response as well as a negative response from the body. In normal levels less than 0.4μmol hydrogen peroxide acts as a 2^{nd} messenger to NF-κβ and its regulates the formation and activation of anti-oxidant enzymes such as superoxide dismutase (SOD), zinc superoxide dismutase (Zn-SOD), ferritin heavy chain (FHC), Catalase, thioredoxin-1 (Trx-1), thioredoxin-2 (Trx-2), glutathione peroxidase (GPX) which provide a protective effect against oxidative stress through the mechanism of increasing enzymatic reactions in the metabolism of reactive oxygen species (ROS). Hydrogen peroxide acts as a regulator of the autophagy mechanism, improves the immune system, suppresses the increase in inflammatory factors, one of which is TNF-α by increasing the activity of tumor necrosis factor receptor antigen (TNFRA) [14], [19], [20]. The activation tumor necrosis factor receptor antigen (TNFRA) provide by tumor necrosis factor receptor-2 (TNFR2) pathway through the mitogen-activated protein kinase (MAPK) pathway to stimulate gene formation, increased cell proliferation, increased cell differentiation thereby increasing the response of cell resistance to decompression dive [12], [21].

In the study result, the reduction in TNFα levels after dive based on the literature is due to increased tumor necrosis factor receptor antigen (TNFRA) activity [14]. Physical exercise also results in increased plasma of soluble tumor necrosis factor-α receptor (sTNF-αR) levels which occurs since three hours after physical exercise, reaches a peak within 24 hours and can gradually disappear up to four days after exercise, this human soluble tumor necrosis factor-α receptor (sTNF-aR) which maintains and suppresses the amount of TNFα in plasma to remain in a normal range state and also gives the effect of growth and increased cell strength [14], [21], [22]. Another mechanism, the response after exercise, the pressure in the aorta increases due to an increase in heart rate and systolic pressure. Increased pressure in the aorta results in an extraordinary strain on endothelial cells, biomarkers of endothelial cell vascular, including increased endothelial nitric oxide synthase (eNOS), endothelial-derived hyperpolarization factor synthase (EDHF) and cytochromes P450 (CYP450), as well as increased release of reactive oxygen species (ROS) forms such hydrogen peroxide. Hydrogen peroxide (H_2O_2) is a signal to activate expression of adhesion molecules, such as intercellular adhesion molecules (ICAM), selectin, and chemotactic protein monocytes-1 (MCP-1), this indirectly prevents vasoconstriction at depth due to decompression dive [23].

In the control group, there was an increase in the mean levels of TNFα after decompression dive compared to the beginning of the dive which caused a significant difference. This happened because in diving activity, the diver breathes by using the pressurized air and with an increased environmental pressure, this causing an increase in partial pressure of gas from the breath media in the body. It is called as hyperoxia-induced vasoconstriction in depth. In this state the increase of reactive oxygen (ROS) such as superoxide ions and hydrogen peroxides trigger the occurrence of oxidative stress [5]. Superoxide ion and hydrogen

peroxide ions act as second messengers via redox-sensitive pathways. These redox-sensitive pathways include receptor and non-receptor tyrosine kinase, tyrosine phosphatase, serine threonine kinase, and transcription factors such as activator protein (AP-1), nuclear factor kappa-β (NF-κB), tumor protein P53 (p53), nuclear factor of activated T-cells (NFAT), hypoxia-inducible factors (HIF-1) [23]. Tumor necrosis factor alpha (TNFα) also plays a role in causing endothelial dysfunction, through the mechanism of inhibition of nitric oxide (NO) synthesis through the activation barrier of the argininosuccinate syntetase enzyme that inhibits citruline and L-argine metabolism thereby reducing the production of endothelial nitric oxide synthase (eNOS) [8], [25]. This study result also consistent with preliminary studies with the object of research in rats, which found an increase in TNFα levels due to decompression diving and also another inflammatory cytokine [7], [26]. In this control group, also in accordance with Spisni *et al.* who stated that the deco-stop strategy provides an advantage in terms of inhibiting gas expansion, but other things show that the deco-stop procedure cannot guarantee that the diver is free from decompression sickness (DCS), this is because there is still an increase in inflammation biomarker secretion, that causes vascular damage, and it seen from biomarkers of C-C motif chemokine ligand-2 (CCL2) and C-C motif chemokine ligand-5 (CCL5) [27].

3.2. Effect of submaximal intensity exercise on TNFα expression

In this section, the discussion concerns TNFα expression 24 hours post-exercise in both study groups. Bivariate analysis of TNFα expression at the beginning of the study with TNFα before diving in the treatment group analyse using parametric test. Meanwhile, in the control group, the distribution of TNFα data was not normal, so a non-parametric bivariate analysis was carried out as shown in Table 5.

Table 5. Effect of submaximal exercise on TNFα levels before diving

Variable	Beginning of study	Before diving	90%CI of difference	p
TNFα treatment (pg/ml)	7.06±1.85	7.46±2.07	(-0.68-0.59)	0.066
TNFα control (pg/ml)	7.69±3.08	7.63±3.01	(-1.12-0.24)	0.261*

***** Non parametric test

The difference delta mean in TNFα levels before diving and the beginning of the study had an abnormal distribution. The difference mean changes in TNFα levels between the treatment and control groups using the non-parametric test, found a statistically significant difference in TNFα levels between the treatment and control groups as shown in Table 6.

Table 6. The difference in mean changes in TNFα levels before diving treatment and control group

TNFα	Median(Min-Max)	p
ΔTNFα treatment group (pg/ml)	-0.24 (-2.74–1.67)	0.0015*
ΔTNFα control group (pg/ml)	0.45(-0.94–0.95)	

***** Non parametric test

Acute submaximal physical exercise affects TNFα levels 24 hours after exercise, this is illustrated by an increase in mean TNFα levels 24 hours after physical exercise but it is not statistically significant. This situation is in accordance with the literature which says, physical exercise will increase TNFα plasma along with an increase in sTNF-α receptor activity starting three hours after exercise, induced by a process of increasing tissue lymphocyte mobilization in response to increased keratin kinase due to physical exercise [28], [29]. According to the theory; physical exercise with good dose, which mean in this case the appropriate intensity, duration and frequency give a positive adaptation response to the body. The highest superoxide dismutase (SOD) enzyme activation is 60 minutes post-exercise which reaches 10000±2828 and returns to normal levels 72 hours after training [14]. Also in theory, this happen due to an increase in soluble tumor necrosis factor-α receptor (sTNF-αR) in plasma, which in theory increases since three hours post-exercise and reaches a peak of 24 hours post-exercise, and persists for up to four days post-exercise [14], [21]. This is also in accordance with the research of Hamada *et al.* who explained that the acute exercise can stimulate the mobilization of hematopoietic stem cells from bone marrow and old immune cells from peripheral tissues to the circulation. In addition, exercise increases the action of noradrenaline which is responsible for the effect of acute exercise on lymphocyte changes, including natural killer (NK)-cell and T-cell activity and increased

catecholamine growth-mediated hormones to carry out neutrophil changes and increase in TNF-α mRNA [30]. In this study, we did not examine soluble tumor necrosis factor-α receptor (sTNF-αR) and tumor necrosis factor alpha-messenger RNA (TNF-α mRNA) biomarkers, so we cannot conclude the cause. Meanwhile in the control group there was no change in TNFα expression.

3.3. Effect of subject characteristics on TNFα expression at the beginning of study

This study also wanted to see, the effect of TNFα expression at the beginning of the study on the characteristics of the research subjects consisting of age, body mass index (BMI) and smoking habits. Characteristics of research subjects were made based on dichotomous categories. Body mass index (BMI) grouped into norm weight and overweight, smoking habits are made into smokers and non-smokers as shown in Table 7.

Table 7. Effect of subject characteristics on TNFα expression at the beginning of study

Variable	TNFα (n= 40)	90% CI of difference	p
Age:			
1. 21-30 years	7.08±2.83pg/ml	(-1.95-0.76)	0.23
2. 31-40 years	7.68±2.22pg/ml		
Smoking habits:			
1. Non-smokers	7.14±3.03pg/ml	(-1.90-0.83)	0.257
2. Smokers	7.67±1.78pg/ml		
BMI:			
1. Norm weight	6.94±2.64pg/ml	(-3.06-0.13)	0.037
2. Overweight	8.54±1.84pg/ml		

TNFα at the beginning of the study with smoking status, there was no significant difference between non-smokers and smokers, which meant TNFα levels were not affected by smoking habits. As for the classification of Body Mass Index, the initial TNFα expression of the study with norm weight and overweight body mass index (BMI) there was a significant difference, or in other words TNFα levels may influenced by body mass index (BMI). It appears that subjects who are overweight have higher levels of TNFα than norm weight. But overweight here is not related to the amount of cholesterol in the blood, but related to the anthropometry of the subject's body with muscular or fat mass. This is in line with research which states that TNFα will increase with the occurrence of body mass index (BMI), this is due to changes in cellular metabolic processes due to being overweight, and it can be caused by muscular mass or body composition and leptin [31]. Other studies suggest that, TNFα biomarker concentration is not only related to body composition but may depend on individual variation in adipocytokines and myosin secretion as a result of various genetic factors [32]. However, in this study, body composition, leptin, adipocytokines and myokines were not examined, so we cannot conclude the cause.

3.4. Limitations

This study only used one research biomarker tumor necrosis factor alpha. For the future research, it is necessary to examine other supporting biomarkers such as endothelial nitric oxide synthase, Interleukin-6, Interleukin-1, soluble tumor necrosis factor-α receptor and tumor necrosis factor alpha-messenger RNA as markers of endothelial dysfunction. And also in this study using trained diver as a subject, which do not reflect the same level of fitness as the community.

4. CONCLUSION

Pre-dive physical exercise with a target exercise intensity of 70% of heart rate (submaximal) performed once (acute) 24 hours before single dive 280 kPa bottom time 80 minutes can prevent the increase in TNFα levels after diving. There was a significant difference between the levels of TNFα after diving and the beginning of the study between the treatment group who received acute submaximal physical exercise 24 hours before diving and the control group who did not receive physical exercise. In this study, there was a significant increase in TNFα levels due to single dive 280 kPa decompression 80 minutes bottom time in the group that did not get physical exercise before diving. Acute submaximal physical exercise increased TNFα levels after 24 hours after exercise but it was not significant. So, exercise before diving can be used as one of the prevention of oxidative stress that can induce DCS on divers. Further studies are needed regarding the prevention of decompression sickness due to diving-induced metabolic changes, through administration of antioxidants or hyperbaric oxygen therapy (HBOT) before immersion as seen from TNFα biomarkers as a comparison of effectiveness.

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