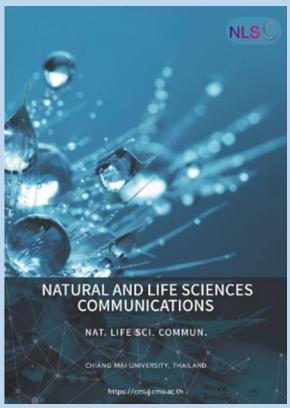
Research article



Higher Activity of Glutathione Peroxidase in Mature Mice Following a Single Bout of Exercise-Induced Muscle Damage

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ABSTRACT

Oxidative stress is the underlying mechanism of muscle damage following a single bout of exercise. Glutathione peroxidase (GPx) is an endogenous antioxidant that reduces oxidative stress and prevents cell damage in many cases. Following maturation, the GPx protein in the muscle is present at higher levels. The effect of increased GPx protein on oxidative stress and muscle damage is still unknown. Serum skeletal muscle troponin I (sTnI) levels are widely used as a marker for acute muscle damage. In this study, we compared GPx activity and sTnI levels in mature and impremature mice following a single bout of exercise-induced muscle damage. Forty healthy Bbalb/c mice were randomly divided into two groups. Twenty mice were at a mature age of 8 weeks, whereas the other 20 were at an impremature age of 4 weeks. All of the animals performed a single bout of maximum running exercise. Blood serum and calf muscles were collected 24 hours following the end of exercise. GPx activity in the muscle was higher in mature mice compared with that in impremature mice. Serum sTnI levels were lower in mature mice compared with those in impremature mice. Maturation prepares muscles more for defense against oxidative stress and muscle damage.

Keywords: Antioxidant, Endurance, Exercise, Glutathione peroxidase, Healthy lifestyle, Maturation, Muscle damage, sTnI

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INTRODUCTION

Nurturing nature is believed to be a fundamental concept for achieving optimal human performance within physiological limits (Klissouras, 2001). There are periods during growth and development to be trained, in which organs adapt and respond better to a physical load. Each organ has a specific window of optimal trainability, and structured load administered outside of these periods may induce stress or, even worse, injury (Malina, 2006).

It is essential to train skeletal muscle during exercise physiology. Trained muscles improve speed, strength, power, and endurance (Radnor et al., 2018); however, in practice, our daily exercise is not built under a window of trainability consideration. Many believe that the earlier the physical load is trained, the faster it will improve speed, strength, power, and endurance. Some protocols do not differentiate the physical load between young children and adults (Aubert et al., 2021; Malina, 2006).

Skeletal muscles develop during myogenesis from embryonic to differentiated steps. At the end of myogenesis, myotubes merge to form a strong, fast, single myocyte characteristic (Nesvadbova and Borilova, 2018). Fast and strong are the two basic characteristics of skeletal muscle. Over time, along with new capillarization, muscles receive more oxygenation to support aerobic metabolism in the mitochondria (Samora et al., 2008). This results in more energy in the form of ATP, which supports muscles to maintain longer performance. This is known as the endurance characteristic.

Exercise and an active, healthy lifestyle improve health, increase blood flow, and muscle vascularization. Muscles receive more oxygen during exercise. Consequently, they must manage a higher risk of reactive oxygen species (ROS) formation and activity during exercise. Higher oxidative activity increases the risk of more free radicals, oxidative stress, and damage (Phillips et al., 2003). Muscles depend on endogenous antioxidants to protect against oxidative stress and damage. Glutathione peroxidase (GPx) is an antioxidant that protects muscles from oxidative damage during exercise (Kozakowska et al., 2015).

Skeletal muscles require more GPx for better oxygenation (Kozakowska et al., 2015; Leelarungrayub et al., 2005). GPx activity differs between slow and fast skeletal muscle fibers. Slow fiber has a higher number and activity of endogenous antioxidants compared with fast fiber muscles (Lawler et al., 1993; Proctor et al., 1995). Because slow fibers develop continuously following maturation (Papenkort et al., 2021), we hypothesize that GPx activity is higher in mature, exercised mice compared with that in immature mice.

Maturation is characterized by a hormonal surge, puberty, and growth spurts (Cole et al., 2015). New capillarization and slow fiber formation peak during maturation (Samora et al., 2008). Maturation improves protection against exercise-induced oxidative damage (Meylan et al., 2014); however, whether mature mice exhibit a better response to exercise compared with immature mice has not been determined. This study aimed to examine the effect of maturation on the antioxidant activity of glutathione peroxidase (GPx) following a single exercise-induced episode of muscle damage in mice.

MATERIAL AND METHODS

Experimental animals

Forty healthy male Balb/c mice were used as subjects. The sample size in this study (each experimental group) was calculated using the Federer (1966) formula ($n \geq 16$) with a correction factor of 20%, so the total sample size was 20 for each experimental group. Therefore, this study used 40 mice, which were divided into two groups, namely mature (n= 20, aged 8 weeks as mice attain sexual maturity at

8–12 weeks of age (Dutta and Sengupta, 2016)) and immature (n= 20, aged 4 weeks). The mice were obtained from the Pharmaceutical Veterinary Center, Surabaya, Indonesia, and housed individually in a cage (10 cm x 10 cm x 10 cm) with an inverted daily light-to-dark cycle. Before the exercise-induced damage protocol, the mice were fed standard pellets and water ad libitum for a week. All procedures were approved by the Research Ethics Commission, Faculty of Medicine, Universitas Airlangga, as stated in ethical clearance no. 31/EC/KEPK/FKUA/2023.

Exercise-induced muscle damage (EIMD) protocol

The mice were randomly divided into two equal groups. Twenty mice of eight weeks of age comprised the mature group, whereas the second twenty mice were 4 weeks of age and designated the immature group. At the end of acclimatization, the mice were tested for maximum running ability on a Columbus treadmill with a speed of 34 cm/s without any inclination. Maximum running ability was determined as the longest duration to maintain running. This was considered steady running for the mouse for five consecutive seconds on the back grid and refusal to continue running after stimulation (Davies et al., 2008; Peake et al., 2017).

The shortest running time was used as a reference of physical load for all mice to perform in a single bout of exercise. All of the mice received an equal load of physical stress to induce muscle damage. Three days of recovery were permitted before the mice performed the exercise. The mice performed a single bout of exercise in pairs; two mice per group per running session. The mice recovered within 24 hours after an episode of exercise (Purwanto and Sudiana, 2016).

Measurement of glutathione peroxidase (GPx) activity and serum skeletal muscle troponin I levels

Mice were sacrificed for intracardiac blood collection and tissue collection using an established anesthetic procedure. Serum was prepared to measure skeletal muscle troponin I levels as a specific exercise-induced muscle damage marker. The right calf muscles were ground in 10 ml of 10% cold polybuffer saline (PBS) and centrifuged to obtain a supernatant to measure GPx activity. Serum skeletal muscle troponin I levels were measured using an ELISA kit for mouse troponin I (catalog no. E1167Mo, BT Lab of China). GPx activity was measured using an ELISA kit containing the glutathione peroxidase assay reagent (catalog no. E-BC-K096-S, Elabscience, China).

RESULTS

Table 1 shows the results of the maximum running test. The longest duration of running was 34 minutes and the shortest was 11.35 minutes. Mature mice ran longer compared with immature mice. The mature mice also had superior endurance compared with the immature mice. A duration of 11.35 minutes was determined as a reference task of running for all mice in a single bout of exercise.

Table 1. Duration of the maximum running test on a treadmill between groups.

	Group	n	Mean ± SD	Min-max	*P*-value
Duration (minutes)	Mature	20	31.04 ± 8.04	14.20–34.00	0.001
	Immature	20	20.18 ± 6.33	11.35–28.00	

Note: Differences were assessed using the independent sample t-test with significance at $P < 0.05$.

A single bout of exercise induced GPx activity in mouse calf muscles. A comparison of GPx activity between the groups is shown in Table 2. GPx activity in mature mice was significantly higher compared with that of immature mice. Mature

mice exhibited superior antioxidant protection compared with that in immature mice. The data were not distributed normally (*P*-value Saphiro-Wilk test < 0.05); therefore, the Mann–Whitney test was used to analyze differences.

Table 2. Comparison of glutathione peroxidase (GPx) activity between groups.

	Group	n	Median	Min-max	*P*-value
GPx activity (U/ml)	Mature	20	33.54	13.92–77.22	0.008
	Immature	20	25.31	12.66–54.43	

Note: Differences were determined using the Mann–Whitney test with significance at $P < 0.05$.

The mice experienced a single bout of exercise-induced muscle damage. The calf muscle released troponin I into the blood, which peaked 24 hours after exercise. Table 3 shows a comparison between groups of skeletal muscle troponin I (sTnI) levels in mouse serum. sTnI levels were higher in immature mouse serum compared with that in mature mouse serum. The data were distributed normally; therefore, an independent t-test was used to analyze the differences.

Table 3. Comparison of skeletal muscle troponin I (sTnI) levels between groups.

	Group	n	Mean ± SD	Mean difference	*P*-value
Troponin I (µg/ml)	Mature	20	332.97 ± 31.15	−63.78	0.008
	Immature	20	396.74 ± 36.23		

Note: Differences were determined using an independent sample t-test with significance at $P < 0.05$.

DISCUSSION

The maximum running test distinguished the endurance level between immature and mature mice. Mature mice could run for longer durations compared with immature mice. New capillarization developed more during maturation, particularly in the muscles (Samora et al., 2008). Muscles receive increased blood flow and oxygenation during exercise. Oxygen is needed for fuel to metabolize glucose and free fatty acids to produce ATP. Oxygen also increases mitochondrial activity to perform aerobic metabolism (Korthuis, 2011). Aerobic metabolism produces more ATP compared with anaerobic metabolism (Van Loon et al., 2001). Increased oxygenation induces greater aerobic metabolism, resulting in a higher ATP pool inside the muscle and superior endurance performance (Baker et al., 2010). Unfortunately, we did not assess muscle capillarization, oxygenation, or ATP levels in the present study to confirm this hypothesis.

Maturation increases hormone levels in the blood, particularly reproductive hormones. Testosterone levels surge during maturation (Reynolds et al., 2007). Testosterone induces skeletal muscle fiber switching from fast to slow muscle fibers (Cardinale et al., 2020). Testosterone also inhibits FNIP-1 on AMPK/PGC-1α signals to mitochondria biogenesis (Usui et al., 2014). Mature muscle develops more aerobic properties, such as myoglobin, mitochondria, and oxidative enzymes. Better oxygenation results in superior endurance; however, it also leads to a higher risk of oxidative stress and damage (Srivastava and Kumar, 2015).

GPx is an important enzyme that reduces oxidative chain reactions in myocytes (Semin et al., 2000). It converts reactive hydrogen peroxide (H2O2) to stable water molecules (Ji et al., 1992). GPx activity determines the level of oxidative stress (Powers and Jackson, 2008). Higher GPx activity reduces the risk of muscle damage during exercise (Di Meo et al., 2019). Our results confirmed that mature mice with

higher GPx activity exhibited lower serum levels of sTnI, which is a marker of exercise-induced muscle damage.

GPx activity is dependent upon the nuclear factor E2-related factor 2 antioxidant responsive element (Nrf2-ARE) signal pathway (Banning et al., 2005). Testosterone activates the Nrf2-ARE signaling pathway to synthesize endogenous enzymatic antioxidants, such as GPx. During maturation, testosterone surges in the blood serum and can stimulate the Nrf2-ARE signaling pathway in skeletal muscle (Zhang et al., 2019). Mature skeletal muscle contains more GPx protein compared with immature muscle. An episode of exercise induces more GPx activity in mature muscles compared with that in immature muscles. Our results confirmed that GPx activity in mature mice was higher compared with that in immature mice.

Mature mice were characterized by higher endurance levels and greater protection against radical oxidant exposure during physical load tests (Paltoglou et al., 2015). This extra protection results from higher oxygenation, oxidative reactions, and aerobic metabolism following maturation (Vázquez-Medina et al., 2011). Testosterone plays an important role in both AMPK/PGC-1α and Nrf2-ARE signaling to produce durable mice in "safe mode." Our results confirmed that mature mice run longer with extra protection from GPx activity compared with immature mice.

GPx is essential to protect muscles against radical oxidant exposure during the physical load stress of exercise (Lian et al., 2022). Radical oxidants, such as reactive oxygen species (ROS), induce protein carbonylation of myofibrils. Skeletal muscle troponin I (sTnI) is a part of the myofibril and is commonly carbonylated during oxidative stress (Mollica et al., 2012). Carbonylated sTnI is expelled into the bloodstream from myocytes (Akagawa, 2021). During exercise, sTnI levels in the blood serum increase until they reach a peak after 24 hours of recovery (Liu et al., 2019). High sTnI serum levels 24 h postexercise is a specific marker for exercise-induced muscle damage (Purwanto and Sudiana, 2016; Song et al., 2020).

Troponin I inhibits the sliding attachment of myosin heads to actin to promote muscle contraction. Oxidative stress induces the release of sTnI from the muscle so that there is no negative regulator of contraction (Sorichter et al., 1997). The muscle contracts intensively, resulting in a considerable recoil force known as a spasm. A spasm is followed by unpleasant, intense soreness for a period of time. Muscle spasms protect muscles against tears even if they cause pain (Proske and Morgan, 2001). Soreness is an important signal to stop exercising and limit endurance performance.

Mature mice showed better performance in endurance runs during single strenuous exercise. Our findings also confirmed that mature mice had lower serum levels of sTnI compared with those in immature mice. Maturation prevented exercise-induced damage, which enabled mice to run longer on high-speed treadmills (Meylan et al., 2014). Mature mice exhibited higher GPx activity to resolve oxidative stress following an episode of exercise-induced muscle damage.

In the future, the physical load of exercise should be customized to match the age of body maturation. An excessive load on immature individuals promotes stress and oxidative damage. Immature damage induces early fibrotic formation in the muscles. Fibrosis decreases flexibility, agility, strength, power, and endurance performance (Gardner et al., 2020). These conditions limit an individual's ability to be more productive.

CONCLUSIONS

Exercise is essential to nurture the muscle nature and improve physical performance. An unsuitable physical load of exercise with the nature of muscles induces stress and damage. Maturation is part of muscle nature, which is an essential guide to prescribing suitable loads for physical exercise. Immature individuals should not be overloaded because of less protection from oxidative stress and a higher risk

of inducing damage. It is worth waiting for maturation for better protection and physical performance in daily life.

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AUTHOR CONTRIBUTIONS

Muhammad Ilham Mauluddin performed the experiments and drafted the manuscript. Bambang Purwanto, Irfiansyah Irwadi, and Sundari Indah Wiyasihati designed the study and analyzed the data. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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