Penerbit Akademia Baru

Journal of Advanced Review on Scientific Research

Journal homepage: www.akademiabaru.com/arsr.html
ISSN: 2289-7887

Fatigue onset through oxidative stress, dehydration and lactic acid accumulation and its in vivo study using experimental animals

Open Access

Hani Hafeeza Halim[1], Mohd Sabri Pak Dek[1], Azizah Abdul Hamid[1], Ahmad Haniff Jaafar[1,*]

[1] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Jalan UPM, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 30 May 2017
Received in revised form 20 July 2017
Accepted 2 August 2017
Available online 10 August 2017

ABSTRACT

Among athletes, endurance is one of the key elements to victory. In addition to training, athletes normally used supplement to prevent fatigue during the event. With prolonged and intense activity, our body started to experience decrease in muscle performance due to several factors such as oxidative stress, dehydration and accumulation of lactic acid in the body fluids. The free radicals generated during intense exercise will expose the cells to oxidative damages. In the event of dehydration, there will be significant losses of water and functional electrolytes during intense exercise which affected the body fluid balance. Fatigue will also occur during reduced oxygen in aerobic metabolism which later caused accumulation of lactic acid in the muscle. This will change the pH balance toward more acidic and caused the muscles to lose contractile efficiency. In addition, fatigue can also be studied using rats as model organism. Results from this activity can be useful to analyse cellular metabolism and physiology effects of the tested rats toward physical exercise. Therefore, this review aims to discuss the causes of fatigue through oxidative stress, dehydration and lactic acid accumulation. In addition, the effectiveness of using rats as a model system in measuring fatigue is also included in illustrating examples on fatigue assessment in vivo.

Keywords:
exercise, fatigue, oxidative stress, hydration, lactic acid

1. Introduction

Fatigue commonly occurs when a person performs intense exercise. It is induced by several attributes including the key causes such as oxidative stress, dehydration and lactic acid accumulation in muscles [1]. Essentially, oxidative stress results from increase in oxygen uptake and metabolism rate, hence triggering the release of reactive oxygen species (ROS) [2]. Performing strenuous exercise will enhance ROS formation in mitochondria and promote cellular oxidation. If fatigue is not suppressed, it may lead to DNA structural damage and further modify cell function and structure [3]. An antioxidant is a substance that can impede ROS formation. It is noteworthy that human body

---

[*] *Corresponding author.*
E-mail address: Ahmad Haniff Jaafar (a_hanif@upm.edu.my)

possess many endogenous antioxidants such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) which help to scavenge free radicals produced in exercising muscles [2]. Nevertheless, these endogenous antioxidants will not be sufficient in reducing the excessive free radicals produce during strenuous exercises. In order to impede oxidative stress-associated-fatigue during high intensity exercise, athletes are advised to increase antioxidant intake which can be done through diet or supplementations. Another key contributor to fatigue in athletes during intense exercises is dehydration. Dehydration occurs due to depletion of water in body exceeding two to three percent of body weight [4]. Body fluid balance is regulated by electrolytes, especially potassium and sodium ions which help to retain adequate water amount in extracellular and intracellular fluid, respectively [4]. Furthermore, dehydration restricts glycogen replenishment by enhancing degradation of muscular glycogen [5]. Production of water during glucose oxidation will enhance hydration status in the human body during exercise [6]. It has been reported that body fluid replacement and water intestinal absorption become more effective with the addition of sugar and sodium into water [7]. Hence, it is beneficial for the athletes to consume beverage with adequate electrolytes and sugar to replenish the electrolytes and glycogen, respectively in the body to enhance performance in subsequent exercise.

Fatigue may also result from lactic acid accumulation in the muscles. As athletes perform high intensity activities, intracellular pH of the body fluid decreases approximately by 0.5 pH units [1]. The accumulation of lactic acid in intracellular fluid occurs when glycogen is broken-down anaerobically in athletes. During glycolysis, the source of energy is obtained from glucose-6-phosphate from either glucose in blood or muscle glycogen. Lower acidity in muscle is obtained when glycogen is utilized as a source of glucose-6-phosphate instead of blood glucose during intense exercise. Therefore, the intracellular acidosis stimulates fatigue by slowing down energy metabolism in human body. While performing intense exercise, stored glycogen can readily be converted to glucose for energy production (i.e., formation of pyruvate and other reduced compounds) under complete aerobic process [8]. However, the limitation is that aerobic metabolism in muscle cannot keep up with the energy required (ie., low $O2$ level) during strenuous exercise. Under this condition, glycogen will be converted into lactate instead of pyruvate. In human body, enzyme namely lactate dehydrogenase (LDH) converts lactate back into pyruvate for energy regeneration [9].

In vivo studies on fatigue level have been widely performed using various model organisms such as humans, rats and mouse. Nevertheless, rats are the preferred model to study fatigue due to their excellence in learning many tasks, stronger survival capabilities and natural ability to swim [10]. Among all endurance exercises available to measure fatigue through in vivo study, swimming-to-exhaustion-test is commonly done due to its simplicity and low-cost equipment [11, 12]. In addition, there are also other endurance exercises that have been used such as rotarod performance test and treadmill test.

It is important that fatigue be suppressed in maximizing athletes’ performances as a preparation for subsequent exercises. As such, the occurrence of fatigue should be well understood to find alternatives to reduce fatigue and enhance performance. In fact, the comparison between models for in vivo study is vital to conduct fatigue test in an economical and effective way. Therefore, this review aimed to discuss the causes of fatigue through oxidative stress, dehydration and lactic acid accumulation and explore the best model for in vivo study of fatigue.

2. Causes of Fatigue

Exercise stimulates many types of metabolic processes in skeletal muscles, which include, increase in glucose uptake and elevation of fatty acid oxidation [13]. The usage of energy may increase up to a hundred fold when athletes perform intense exercise as compared to that of resting condition. Due to the high level of competitiveness and the desire to maximize performance, athletes will utilize their capability to the maximum and intense training e is very common among athletes. However, intense exercise will result in fatigue and if this conditions were allowed to persist, injury can be inflicted. There are many things that can lead to fatigue, nevertheless, the leading causes include oxidative stress, dehydration and accumulation of lactic acid in the muscle [1]. Therefore, understanding these causes into greater details will be beneficial in preventing fatigue and boost up exercise performance.

2.1 Oxidative stress

The major perpetrator of fatigue during strenuous exercise is oxidative stress. Athletes require oxygen during respiration, hence allowing the body to constantly react with oxygen in the effort to generate more energy. However, the process itself will generate free radicals and when this occurs excessively, the exposed cells will undergo oxidative stress [14]. Principally, the free radicals, in particular, reactive oxygen species (ROS) production is induced by some endogenous and exogenous factors such as excessive heat, radiation exposure, infection, inflammation, trauma, and intense physical exercise [3]. The ROS comprise of a single or more unpaired electron and is a highly reactive compound. Major ROS are hydroxyl radicals which could damage lipids, protein, DNA and carbohydrates hence initiating lipid peroxidation by extracting an electron from polyunsaturated fatty acids [2]. High intensity exercises enhance ROS production greatly in the working muscles hence resulting in imbalance between oxidation and antioxidant system [2, 15, 16]. Free radicals are known to be potent inflammatory agents as they can attack large molecules and organs and caused damages [15]. This occur in the presence of excessive free radicals and cellular antioxidants are in neutralizing the radicals generated [17]. Therefore, athletes are recommended to take antioxidant supplement since they have protective effects on human cells from oxidative damages by neutralizing and assisting in ROS scavenging [18].

2.1.1 Endogenous antioxidants

It is interesting to note that human body develop different mechanisms to counterbalance oxidation process via interaction between endogenous and exogenous antioxidants with the ROS [2]. Endogenous antioxidants are antioxidants which are produced by the human body which include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), serum albumins, and uric acid. They have different mechanisms in removing ROS from muscle cells and consequently protect internal organs from oxidative injuries [19]; [20]. As shown in Figure 1, oxygen (O2) is reduced to superoxide by oxidases. The SOD will dismutate O2.- to hydrogen peroxide (H2O2) which will then be converted to water (H2O) by GSH-Px or CAT. The SOD neutralizes superoxide radicals by converting them into hydrogen peroxide [2]. Both GSH-Px and CAT enzymes reduce hydrogen peroxide into water to inhibit free radical synthesis [21]. Glutathione that donates one electron to hydrogen peroxide will then reduce hydrogen peroxide to water and oxygen. The reduced glutathione then transfers a proton to membrane lipids to protect them from oxidative damage [14]. Alternatively, H2O2 can react with Fe2+ to produce hydroxyl radical [22]. Even though these endogenous antioxidants assist in mediating oxidative stress in body, however, this antioxidant defence system will not be adequate to neutralize the excess free radicals that are produced during intense exercise. The presence of endogenous antioxidants alone in the human body is thus not sufficient to prevent the damages caused by free radicals. Hence, supplementation of exogenous antioxidants will aid in scavenging the excess free radicals that can cause oxidative stress.

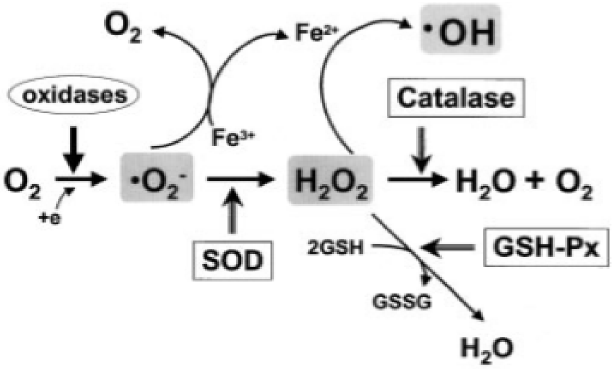

Fig. 1. Pathway of endogenous antioxidants in human body. Adapted from [21]

2.1.2 Exogenous antioxidants

The interaction between exogenous and endogenous antioxidants helps to protect cells and slows down chemical processes which stimulate oxidative damages [19]. Exogenous antioxidants are putative compounds that are able to aid in reducing oxidative stress during intense exercise as shown in Table 1 [15]. These include tocopherol, tocotrienols, ascorbic acid, phenolic compounds, carotenoids, uric acid, β-carotene, and minerals such as selenium that are needed to neutralize and destroy free radicals [14, 21, 23]. In addition, many studies have shown that phytochemicals such as phenolics, flavonoids, saponins, alkaloids and steroids help to prevent or delay oxidation process by scavenging free radicals and lowering oxidative stress [24]. Several studies reported the synergistic effects of ascorbic acid and α-tocopherol in enhancing the removal of ROS and thus reducing lipid peroxidation rate during exercise [14, 21]. Thus, it is recommended for athletes to take vitamin C, vitamin E, and vitamin A as supplements which could act as antioxidants [14, 25]. Nevertheless, these exogenous antioxidants should not be taken excessively since overconsumption of these supplements can result in adverse effects such as muscle weakness and headache [25].

2.2 Dehydration

Another key contributor to fatigue during intense exercise is dehydration. Intense physical activities elevate heat generation as a result of increase in metabolic rate which results in water and electrolyte deficits as well as glycogen depletion in both muscles and liver [4, 26]. During intense exercise, water may be lost from the body through a series of natural processes such as excretory through various systems, including renal, gastrointestinal, and respiratory systems [27]. Dehydration results from fluid losses through perspiration, urine, and respiration. Perspiration, in particular, is the main medium of fluid deficit through the excretory system under heat-stress-induced exercises [27]. The range of water losses while performing exercises are between 0.2 to 3.5 Liter/hour depending on the surrounding conditions and type of exercises performed [28]. Adequate fluid intake helps to delay the onset of fatigue and improves exercise performance during long duration exercise [29]. Athletes need to rehydrate their bodies to maximize performance for subsequent competitions or training. The most effective strategy for rehydration after exercise is to replenish body fluid balance and restore muscle glycogen that was depleted during the exercise [4]. With regards to occurrence of fatigue, glycogen exhaustion appears to be associated with dehydration during strenuous exercise. A study by Logan-Sprenger and colleagues reported that carbohydrate oxidation was greater when subjects dehydrated approximately 1.5% of their body mass after performing intense exercise [30]. Rehydration with adequate fluid containing electrolytes such as sodium and potassium is vital for physiological homeostasis and fluid balance. On the other hand, consumption of carbohydrates is vital for glycogen replenishment which helps to further hinder dehydration process. Failure to rehydrate properly the body after the exercise can be very dangerous that not only result in lower performance, but also can cause fatal injuries [31].

2.2.1 Sodium and Potassium for rehydration

During intense exercise, a significant amount of water can be lost from the body. . In addition to water, perspiration can also cause losses in important electrolytes such as sodium (Na) and potassium (K). In general, these electrolytes in their ionic form (i.e. Na+ and K+) functions to provide electrical impulses which allowed complex cell system in our body to communicate with each other via our neurons system. Depending on the cell activities; there will be specific concentration gradient (i.e., uneven distribution of ions) in the inside and the outside of cell. The concentrations of Na+ and K+ across the membrane are controlled by a specific pump known as sodium-potassium pump which activated by an enzyme known as Na+-K+ATpase.

Sodium replenishment is crucial for the hydration process since sodium is the major cation in the extracellular fluid. The extracellular fluid constitutes about two third of the total body content. Normally, sodium ions present in higher concentration within extracellular fluid (~140 mmol-1) as compared to that of intracellular fluid (10 mmol-1) [4]. There is approximately 30 to 40 mEq of sodium needed for energy recovery and to achieve euhydration [29]. Consequently, a beverage consisting of 50 mmol-1 sodium is highly effective for rehydration process [4]. Nevertheless, majority of sports drinks has sodium ranges only from 10 to 25 mmol-1 or even less. However, high sodium in beverage will not only induce excessive urine production but also produce unwanted (salty) taste. Hence, the amount of sodium in hydration beverage should be adequate to rehydrate athlete’s body as preparation for subsequent exercises without compromising the taste of the products.

Potassium is required for rehydration due to its function as a major cation in intracellular fluid and play important role in muscle contraction. Similar to sodium, potassium ions will be lost through perspiration as athletes performed intense exercises [4]. Potassium is present at a higher concentration (150 mmol-1) inside the cell compared to the concentration in the plasma (4-5 mmol-1), respectively. The higher potassium ion in intracellular fluid is due to the outward diffusion of potassium slowly through cell membranes. The potassium ions assist in retaining water effectively in the body when athletes perform intense exercise [4]. Knowing the importance of potassium in promoting substitution of intracellular water in the body after exercise, the majority of commercialized sports drinks companies formulate their products with high potassium content.

2.2.2 Carbohydrates for glycogen replenishment

Apart from electrolyte deficits, glycogen depletion will also cause dehydration and reduce performance during long duration exercises such as marathon running [1, 32]. Glycogen replenishment has been claimed as one of the effective rehydration strategy. A good trained athlete can store up to double the amount of glycogen in his or her body as compared to a typical untrained athlete [4]. Liver glycogen can be measured to indicate fatigue since high amount of liver glycogen will be converted into glucose for immediate energy during the exercise [9, 16]. Normally, active muscles absorb glucose 20 times fold greater during exercise as compared to resting condition [32]. Energy metabolism processes in our body such as glycolysis and gluconeogenesis help to regulate blood glucose concentration when athletes are experiencing dehydration [4]. The glycogen resynthesize rate depends on the amount of ingested carbohydrate. Therefore, a simple carbohydrate (i.e. sugar in the form of glucose) is required for glycogen replenishment and is the preferred fuel for energy production in exercising muscles [32]. It has been claimed that athletes should consume a beverage that contain 5 to 10% carbohydrate to achieve effective rehydration and energy recovery [29]. In spite of that, carbohydrates should be taken in adequate amount since excessive consumption of carbohydrate in sports drinks will result in adverse effects to athletes if they fail to burn equal amount of energy during their exercises routine [32]. This is because high sugar intake might predispose the athlete to other health problem such as diabetes and other chronic diseases.

2.3 Lactic acid accumulation

Lactic acid accumulation in muscle is also a key player of the occurrence of fatigue when athletes perform intense exercise. Metabolic changes such as limited energy supply stimulate lactate accumulation in the muscle by changing the metabolism’s condition from aerobic to anaerobic [13, 33]. Consequently, intracellular acidosis occurs in the athletes and stimulates fatigue by slowing down energy metabolism in the muscles [1]. Basically, the presence of lactate is vital in hindering muscular fatigue when athletes perform intense exercise. As muscles endure intense activities, lactates concentration elevates in the plasma [34]. During intense exercise, anaerobic breakdown of glycogen induces lactate accumulation and elevates plasma metabolites such as lactic acid in intracellular fluid [1, 33]. Lactic acid will then be dissociated into hydrogen ion and this can decrease the intracellular pH value by approximately 0.5 pH. It should be noted that the low pH condition inhibits enzymes involved in energy metabolism such as phosphofructokinase and phosphorylase, resulting in ATP supply reduction for body processes that requires energy [1]. Therefore, this will contribute to fatigue in athletes while performing intense exercise. Nevertheless, little is known about how to counteract fatigue through this mechanism. The causes of fatigue have been summarized in Table 1.

2.3.1 Measurement of lactate dehydrogenase activity to indicate fatigue

Another area of concern is that how fatigue can be evaluated during strenuous exercise. Fatigue level through lactic acid accumulation can be determined by measuring the activity of enzyme involved such as lactate dehydrogenase (LDH). The LDH is a moderately stable oxidoreductase enzyme and has been claimed as an accurate indicator of muscle damage. Normal range of LDH is 105 to 333 IU per liter (international unit per liter) but will be slightly different when measured in different laboratories [13]. The screening of LDH in sports medicine can indicate the prevalence of either chronic or acute damages in the muscle tissues. The LDH enzyme is released into the bloodstream when the breakdown of tissue or hemolysis of red blood cells occurs. Normally, measurement of LDH activity in blood indicates muscle damage in mammals after performing resistance exercises. Besides that, many lactate-induced-exercises have been linked to free radical damages [9]. Basically, the LDH helps to catalyze the interconversion of pyruvate and lactate [16]. In human body, lactate and glucose circulate and enter the cell through transport proteins which are located on the cell surface. Glucose will then be converted into pyruvate via glycolysis. A monocarboxylate transporter will carry pyruvate and lactate into mitochondrion of cells. The LDH enzyme converts lactate into pyruvate and the pyruvate will enters the Krebs cycle for oxidative energy production in cells [35]. As lactate accumulates in muscle during intense activities, LDH helps to convert lactate back into pyruvate for the subsequent energy production process. Hence LDH level in blood can be utilized as an indicator for fatigue level in athletes while performing intense exercise.

Table 1
Summary of fatigue pathway through muscle oxidative stress, dehydration, and lactic acid accumulation and prevention steps

Causes of fatigue	**Pathway**	**Prevention**	**References**
Oxidative stress	-Oxygen uptake increases and metabolism rate -Endogenous antioxidants (e.g. CAT, GSH-Px, SOD) not able to scavenge excessive ROS produce in muscles.	Consume dietary antioxidants (beta carotene, ascorbic acid, and α-tocopherol)	[15, 16, 21]
Dehydration	-Increased heat production and metabolic rate in athlete when performing intense exercise. -Glycogen depletes to produce energy lead to exhaustion. -Loss of fluid and electrolytes + glycogen depletion.	Consume adequate fluid, electrolytes (especially sodium and potassium) and sugars.	[4, 26-29]
Lactic acid accumulation	-Lactate accumulates in muscle when perform intense activities. -Change of aerobic to anaerobic metabolism. -Intracellular acidosis slows down energy metabolism in muscle. -Low pH inhibits energy metabolism enzymes -Reduce ATP supply to the muscle cells.	LDH enzyme converts lactates into pyruvate to regenerate energy.	[1; 9; 13; 33-34]

2.4 Selection of Model for in Vivo Fatigue Test

Many types of model organisms such as humans, mice and rats have been used to study the effectiveness of food supplement in reducing fatigue [30, 36-37]. It has been claimed that exercise performance indication is reproducible when humans are utilized as subjects for fatigue study. Despite that, the invasiveness of exercise procedures within human populations is still limited due to ethical considerations. Thus, rodents are preferred as the model organism to study cardiovascular, metabolic, and mechanism of physiological function (pulmonary regulation) throughout intense exercise [38]. It has been reported that rats are capable of learning a greater variety of tasks, have stronger memory and cognitive ability [39]. It is therefore sensible that rats are being selected as model organisms to study fatigue pathway.

2.4.1 Swimming-to-exhaustion as an endurance test using rats

The wide array of endurance exercises in measuring fatigue using experimental animals has also become another vital topic in sports industry. There are many types of exercises to indicate endurance ability of rats such as rotarod test, swimming to exhaustion, and treadmill running as shown in Figure 2.

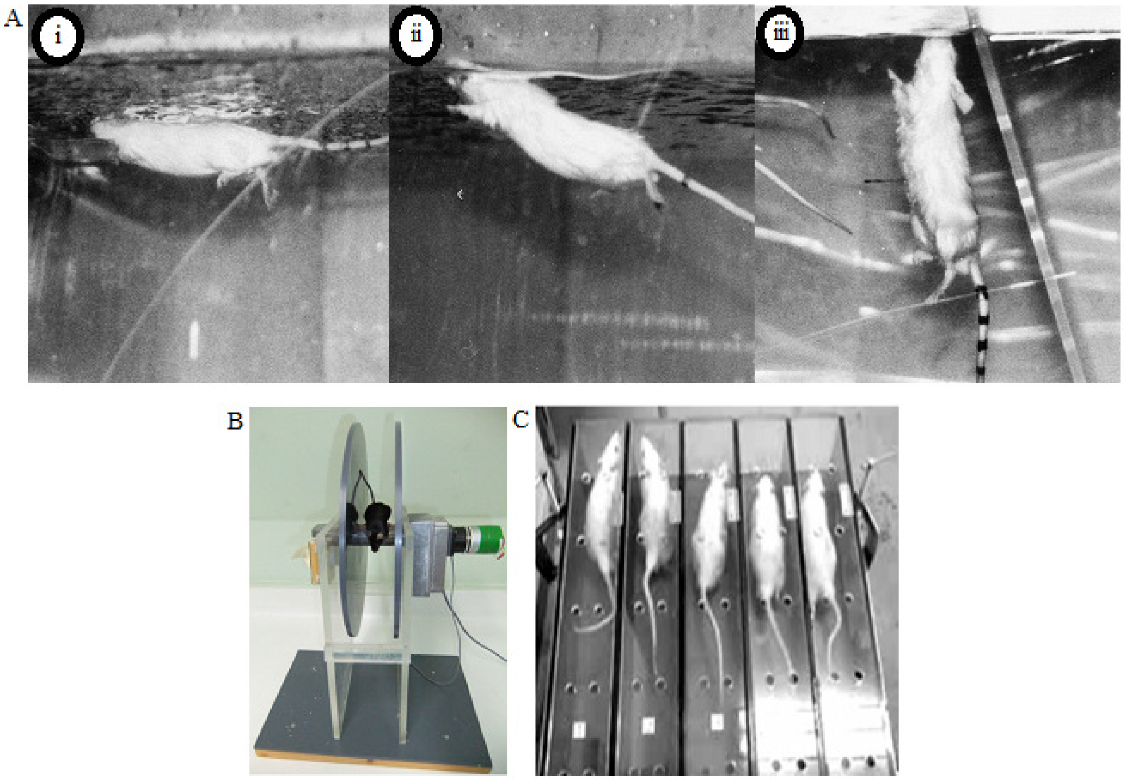

Fig. 2. A) Swimming-to-exhaustion test B) Rotarod C) Treadmill exercise (Pictures adapted from [40-42]

The swimming-to-exhaustion test has been conducted extensively using laboratory animals, in particular, rats and mice to study cellular metabolism and physiology of physical exercise. More recent reports revealed that endurance tests have been performed to analyze animal performance before and after administration of drugs, acute exercise, and long term training regimens, diets and supplements [11]. During swimming-to-exhaustion test, the animals will first be burdened with specific sinker placed on their chest or tail. The subjects will then be placed in a water filled tank. The capability of the animals to swim and float for a duration of time will determine their endurances. The weight attached to the animal helps to decrease time-to-exhaustion to a duration that is more practical since the unloaded rats will have the capability to swim for a long time (hours) in normal temperature condition. The loaded weight should be adjusted according to the body weight (5 to 10% body weight) to standardize the amount of work performed by animals from different sizes [12]. In comparison with other endurance tests, the swimming-to-exhaustion exercise is straightforward, simpler and requires inexpensive and minimum specialized equipment [11, 43]. Despite having a natural ability to swim, rodents have a strong survival instinct when exhaustion is approaching and has higher capability to work at greater intensities. These factors are very important in ensuring the rodents perform high intensity exercise in order to assess the endurance of the animals [10]. Nevertheless, some mouse strains such as FVB/NJ, Black Swiss and NIH Swiss are not suitable for swimming to-exhaustion test due to their limited immobility characteristics.

2.4.2 Other endurance tests

In addition to swimming-to-exhaustion, there are other tests that can be used to study fatigue such as voluntary wheel running, treadmill running, and rotarod climbing. In voluntary wheel running exercise, rats are allowed to run freely on the wheel while their activities will be recorded daily. However, the voluntary wheel running provides low-throughput and is expensive since the rats are required to be individually caged. Furthermore, it was reported that the rats might change their behaviour and produce different exercise performance when being placed in separate cages [44]. There is also treadmill running exercise which requires only a short time to complete and produces high throughput. Despite that, in most cases external motivators are needed in order to induce animals to run on the treadmill. The rats are motivated to run by applying shock grid, touching with brush or hand, poking, or directing short air puffs. The treadmill exercise ends when animals lay above the shock grid for five seconds and incapable to continue [45].

Table 2
Advantages and disadvantages of endurance exercises to measure fatigue

Endurance tests	Advantages	Disadvantages	References
Swimming-to-exhaustion	-Simple, straightforward - Needs inexpensive and minimum specialized equipment. -Rodents i) Natural ability to swim ii) Stronger survival instinct when exhaustion is approaching iii) Higher possibility to work at greater intensities	-Certain mouse strains are not suitable for swimming-to-exhaustion test such as FVB/NJ, Black Swiss and NIH Swiss.	[10, 11, 43]
Voluntary wheel running	Less labor use.	-Animals change behaviour when being placed in separate cages. -Low throughput. -Expensive cost since animals need an individual cage.	[44]
Treadmill running	-Short time. -High throughput	Exercise is involuntary. -Animals need stimulation to run (shock grid, touching, poking, directing short air puffs)	[45]
Rotarod	-Simple test -Easy to perform	-Expensive -Animals grip rod instead of walking forward. -Technical errors: i) Some commercial rotarods fail to accelerate at the required speed. ii) Distance between rotarod and base is not high enough making animals easy to jump off or drop.	[42]

Another type of exercise which is commonly conducted to study fatigue is rotarod test. In rotarod test, a certain indicator is needed when using accelerating rotarod such as ensuring animals to stay for 10 seconds without falling. Among the drawbacks of rotarod test is the possibility of animals with weak coordination to fall off from speed rotarod set at the beginning of the exercise. Only animals with excellent coordination will stay on the rotarod. Moreover, some commercial rotarods do not accelerate at the required speed needed and the height of the rod is not sufficient, hence making animals jump off or drops [42]. The advantages and disadvantages of the endurance tests for fatigue study are summarized in Table 2.

3. Conclusion

The occurrence of fatigue in subjects undergoing physical activities has been reviewed based on oxidative stress, dehydration and lactic acid accumulation. Oxidative stress can be overcome by consumption of antioxidant supplements such as ascorbic acid and α-tocopherol, since endogenous antioxidants are not sufficient in scavenging excessive free radicals produced during intense exercise. The loss of electrolytes and glycogen can be replenished by consuming beverages with adequate amount of minerals, especially sodium and potassium and sufficient sugars as an energy source. Lactic acid accumulation will lower body pH and consequently, inhibit enzymes involved in energy production, thus, resulting in fatigue. However, an in-depth investigation should be performed in determining fatigue attributed by lactic acid accumulation in muscle. It is also beneficial to measure LDH level in athlete due to its importance as a key indicator of fatigue during intense exercise. Even though the main focus of this review was causes of fatigue, the selection of model for fatigue test has also been taken into account. Among other model organisms, rats are preferred for the endurance test in measuring fatigue. Studies suggest that swimming-to-exhaustion test is commonly performed to measure fatigue levels through in vivo study as compared to other tests such as treadmill running and rotarod.

References

[1] Westerblad, Håkan, David G. Allen, and Jan Lännergren. "Muscle fatigue: lactic acid or inorganic phosphate the major cause?." *Physiology* 17, no. 1 (2002): 17-21.

[2] Birben, Esra, Umit Murat Sahiner, Cansin Sackesen, Serpil Erzurum, and Omer Kalayci. "Oxidative stress and antioxidant defense." *World Allergy Organization Journal* 5, no. 1 (2012): 9.

[3] Draeger, Cainara Lins, Andréia Naves, Natália Marques, Ana Beatriz Baptistella, Renata Alves Carnauba, Valéria Paschoal, and Humberto Nicastro. "Controversies of antioxidant vitamins supplementation in exercise: ergogenic or ergolytic effects in humans?." *Journal of the International Society of Sports Nutrition* 11, no. 1 (2014): 4.

[4] Saat, Mohamed, Rabindarjeet Singh, Roland Gamini Sirisinghe, and Mohd Nawawi. "Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water." *Journal of physiological anthropology and applied human science* 21, no. 2 (2002): 93-104.

[5] Evans, Gethin H., Susan M. Shirreffs, and Ronald J. Maughan. "Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum." *Applied Physiology, Nutrition, and Metabolism* 34, no. 4 (2009): 785-793.

[6] Maughan, Ronald J., Susan M. Shirreffs, and John B. Leiper. "Errors in the estimation of hydration status from changes in body mass." *Journal of sports sciences* 25, no. 7 (2007): 797-804.

[7] de Oliveira, Erick Prado, and Roberto Carlos Burini. "Food-dependent, exercise-induced gastrointestinal distress." *Journal of the International Society of Sports Nutrition* 8, no. 1 (2011): 12.

[8] Robergs, Robert A., Farzenah Ghiasvand, and Daryl Parker. "Biochemistry of exercise-induced metabolic acidosis." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 287, no. 3 (2004): R502-R516.

[9] Lindinger, M. I. "Determinants of sarcolemmal and transverse tubular excitability in skeletal muscle: implications for high intensity exercise." *Equine Comp Exerc Physiol* 2 (2006): 209-217.

[10] Kregel, K. C. "Exercise protocols using rats and mice In: American phisiology Society: Resource book for the design of animal exercise protocols." *USA, Feb* (2006): 23-57.

[11] Hohl, Rodrigo, Renato Buscariolli de Oliveira, Rodrigo Luiz Perroni Ferraresso, and Denise Vaz Macedo. "Effect of body weight variation on swimming exercise workload in rats with constant and size-adjusted loads." *Scandinavian Journal of Laboratory Animal Sciences* 38, no. 3 (2011): 145-154.

[12] Jung, Kyungah, In-Ho Kim, and Daeseok Han. "Effect of medicinal plant extracts on forced swimming capacity in mice." *Journal of ethnopharmacology* 93, no. 1 (2004): 75-81.

[13] Anugweje, K. C., and E. O. Ayalogu. "Effect of Training on the Lactate Dehydrogenase (LDH) levels of Athletes." (2014): 56-60.

[14] Aziz, A., H. Taha, N. Mohebali, Y. L. Chung, N. H. Ismail, M. Z. A. Bakar, and F. Z. M. Yusof. "Anti-Cancer Potential of Pseuduvaria Macrophylla in Human Cancer Cell Lines."

[15] Williams, Melvin H. "Dietary supplements and sports performance: introduction and vitamins." *Journal of the international society of sports nutrition* 1, no. 2 (2004): 1.

[16] You, Lijun, Mouming Zhao, Joe M. Regenstein, and Jiaoyan Ren. "In vitro antioxidant activity and in vivo anti-fatigue effect of loach (Misgurnus anguillicaudatus) peptides prepared by papain digestion." *Food Chemistry*124, no. 1 (2011): 188-194.

[17] Braakhuis, Andrea J., Will G. Hopkins, and Tim E. Lowe. "Effects of dietary antioxidants on training and performance in female runners." *European journal of sport science* 14, no. 2 (2014): 160-168.

[18] Li, Hongyan, Zeyuan Deng, Tao Wu, Ronghua Liu, Steven Loewen, and Rong Tsao. "Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes." *Food Chemistry* 130, no. 4 (2012): 928-936.

[19] Antolovich, Michael, Paul D. Prenzler, Emilios Patsalides, Suzanne McDonald, and Kevin Robards. "Methods for testing antioxidant activity." *Analyst* 127, no. 1 (2002): 183-198.

[20] Zhou, Kequan, and Liangli Yu. "Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado." *LWT-Food Science and Technology* 39, no. 10 (2006): 1155-1162.

[21] Powers, Scott K., and Edward T. Howley. "Hormonal responses to exercise." *Exercise Physiology Textbook* (1994): 69-108.

[22] Griendling, Kathy K., and Garret A. FitzGerald. "Oxidative stress and cardiovascular injury." *Circulation* 108, no. 17 (2003): 2034-2040.

[23] Ahmad, Z., R. Hasham, NF Aman Nor, and M. R. Sarmidi. "Physico-Chemical and Antioxidant Analysis of Virgin Coconut Oil Using West African Tall Variety."

[24] Mohammed, S., A. Naziru, K. Mohammed, H. Sa'idu, M. Muntari, and D. Andrawus. "Evaluation of Bacteriostatic Effect of Methanolic Extract of Guiera senegalensis on Some Clinical Bacteria."

[25] Prathapan, A., and T. Rajamohan. "Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction." *Journal of Food Biochemistry* 35, no. 5 (2011): 1501-1507.

[26] Silver, Marc D. "Use of ergogenic aids by athletes." *Journal of the American Academy of Orthopaedic Surgeons* 9, no. 1 (2001): 61-70.

[27] Moreno, Isadora Lessa, Carlos Marcelo Pastre, Celso Ferreira, Luiz Carlos de Abreu, Vitor Engrácia Valenti, and Luiz Carlos Marques Vanderlei. "Effects of an isotonic beverage on autonomic regulation during and after exercise." *Journal of the International Society of Sports Nutrition* 10, no. 1 (2013): 2.

[28] Medeiros, Aldo Cunha, and Vanessa de Fátima Lima de Paiva. "Therapeutic use of coconut water." *Journal of Surgical and Clinical Research* 3, no. 2 (2013): 83-91.

[29] Dubnov-Raz, Gal, Yair Lahav, and Naama W. Constantini. "Non-nutrients in sports nutrition: Fluids, electrolytes, and ergogenic aids." *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism* 6, no. 4 (2011): e217-e222.

[30] Kalpana, Kommi, Priti Rishi Lal, Doddipalli Lakshmi Kusuma, and Gulshan Lal Khanna. "The effects of ingestion of sugarcane juice and commercial sports drinks on cycling performance of athletes in comparison to plain water." *Asian journal of sports medicine* 4, no. 3 (2013): 181.

[31] Logan-Sprenger, Heather M., George JF Heigenhauser, Graham L. Jones, and Lawrence L. Spriet. "The effect of dehydration on muscle metabolism and time trial performance during prolonged cycling in males." *Physiological reports* 3, no. 8 (2015): e12483.

[32] DiMarco, N. M., N. P. West, L. M. Burke, S. J. Stear, and L. M. Castell. "A–Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance—Part 30." *Br J Sports Med* 46, no. 4 (2012): 299-300.

[33] Das, Sanjita, and Sunita Singh Rajput. "Toxic Level of Soft Drinks and Sports Drink on Health Status." *International Journal of Advances in Pharmacy, Biology and Chemistry [Internet]* 2, no. 4 (2013).

[34] Saey, Didier, Annie Michaud, Annabelle Couillard, Claude H. Côté, M. Jeffery Mador, Pierre LeBlanc, Jean Jobin, and François Maltais. "Contractile fatigue, muscle morphometry, and blood lactate in chronic obstructive pulmonary disease." *American journal of respiratory and critical care medicine* 171, no. 10 (2005): 1109-1115.

[35] Rodrigues, Bernardo M., Estélio Dantas, Belmiro Freitas de Salles, Humberto Miranda, Alexander J. Koch, Jeffrey M. Willardson, and Roberto Simão. "Creatine kinase and lactate dehydrogenase responses after upper-body resistance exercise with different rest intervals." *The Journal of Strength & Conditioning Research* 24, no. 6 (2010): 1657-1662.

[36] Todd, Joshua J. "Lactate: valuable for physical performance and maintenance of brain function during exercise." *Bioscience Horizons: The International Journal of Student Research* 7 (2014).

[37] Ma, De-lu, Brett J. West, Chen X. Su, Jian-hua Gao, Tin-zhong Liu, and Yu-Wen Liu. "Evaluation of the ergogenic potential of noni juice." *Phytotherapy research* 21, no. 11 (2007): 1100-1101.M

[38] Salleh, Razali Mohamed, Mizaton Hazizul Hasan, and Aishah Adam. "Prismatomeris glabra increases forced swimming time in mice." *International Journal of Pharmacognosy and Phytochemical Research* 7, no. 3 (2015): 473-479.

[39] Calil, Caroline Morini, and Fernanda Klein Marcondes. "The comparison of immobility time in experimental rat swimming models." *Life sciences* 79, no. 18 (2006): 1712-1719.

[41] Matsumoto, Keitaro, Kengo Ishihara, Kazunori Tanaka, Kazuo Inoue, and Tohru Fushiki. "An adjustable-current swimming pool for the evaluation of endurance capacity of mice." *Journal of Applied Physiology* 81, no. 4 (1996): 1843-1849.

[42] Tang, Tao, Takeshi Muneta, Young-Jin Ju, Akimoto Nimura, Kyosuke Miyazaki, Hiroyuki Masuda, Tomoyuki Mochizuki, and Ichiro Sekiya. "Serum keratan sulfate transiently increases in the early stage of osteoarthritis during strenuous running of rats: protective effect of intraarticular hyaluronan injection." *Arthritis research & therapy* 10, no. 1 (2008): R13.

[43] Deacon, Robert MJ. "Measuring motor coordination in mice." *Journal of visualized experiments: JoVE* 75 (2013).

[44] Can, Adem, David T. Dao, Michal Arad, Chantelle E. Terrillion, Sean C. Piantadosi, and Todd D. Gould. "The mouse forced swim test." *Journal of visualized experiments: JoVE* 59 (2012).

[45] Dougherty, John P., Danielle A. Springer, and Marvin C. Gershengorn. "The Treadmill Fatigue Test: A Simple, High-throughput Assay of Fatigue-like Behavior for the Mouse." *JoVE (Journal of Visualized Experiments)* 111 (2016): e54052-e54052.