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Skeletal Muscle Response to High-Intensity Interval Training (HIIT) in Older Adult Wistar Rats

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ABSTRACT

High-intensity exercise (HI), is known potentially to reduce cardiometabolic risk. However, HI is a constraint to older adults. The burdens of HI in older age can be achieved by high- intensity interval training (HIIT) which is considered to be useful. Although HIIT is considered as beneficial, its safety and feasibility in older adults should be carefully assessed. We studied the skeletal muscle response to HIIT in older adult rats. Fifteen male Wistar rats (12 months) were divided into 3 groups: HIIT group; control 1 (C1, 12 months); control 2 (C2, 14 months as sedentary control group. Parameters use are blood lactate; skeletal muscle's Troponin-T (TnT) and PGC-1α. Treatment consisted of 4 minutes of high intensity active running on a treadmill with 1 minute interval; 4X of repetition for 8 weeks. After treatment rats were sacrificed, blood and gastrocnemius muscles were collected. Results showed that blood lactate in HIIT was insignificantly higher compared to C2 and was significantly higher in C2 compared to C1 (p=0.032). PGC-1α in HIIT was significantly higher

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compared to C2 (p=0.024) and significantly lower in C2 compare to C1 (p=0.022). TnT of HIIT was significantly higher compared to C2 (p=0.002). Our results indicated that response of skeletal muscle tissue to HIIT for 8 weeks provided benefited and was feasible for older adults.

Keywords: High-intensity interval training (HIIT), lactate, older adult rats, PGC-1α, skeletal muscle, troponin T.

INTRODUCTION

Aging is a natural process in the life of a person who has passed his productive age, which is accompanied by a decrease in body function thus reducing the productivity of elderly people (Hansakul, 2010). In aging, the decrease of body functional capacity occurs gradually and is irreversible to the extent of failure and even death. Decrease of body functional capacity occurs from molecules, cells, and tissues level even at organ level (Hansakul, 2010; Masoro & Austand, 2011). One of the most commonly impaired is the musculoskeletal system. The changes that occur in the musculoskeletal system include decreased muscle mass and size, reduced contractile ability, decreased α-motor neuron nerve fibers, decreased protein synthesis, neuromuscular junction changes and decreased Ca^{2+} insarcoplasmic reticulum (Narici & Maffuli, 2010; Setiati, 2013). Reduced muscle function due to decreased muscle mass and decreased muscle strength is known as sarcopenia. Sarcopenia ultimately leads to decrease of physical activity, decreased mobility, slow walking, and low physical endurance capability (Saxon et al., 2015; Setiati, 2013).

In the elderly, the gradual decrease in skeletal muscle mass, strength and endurance is followed by ineffective response to tissue damage. Moreover age-dependent muscle wasting is also known to associate with the mechanism of uncoupling between excitation and muscle contraction, impaired muscle protein synthesis and impaired in metabolic pathways. Proteomic analysis showed that there are some changes in senescent muscle fibers including the transformation of fast-to-slow types of muscle fibers, increase in the amount and phosphorylation levels of slow myosin light chain, and a switch to slower isoforms of contractile protein including actin, myosin, tropomyosin and troponin complex (Ohlendieck, 2011). Troponin is one of the filament regulatory protein that is crucial for contraction of skeletal muscle and regulated by calcium intracellular level.

The decrease in muscle mass that causes the change of muscle contraction strength is thought to be related to changes in energy metabolism within the skeletal muscle. In this condition, the decrease in mitochondrial biogenesis contributes to the decline in skeletal muscle function (Marzetti et al., 2013). It has been reported that moderate-intensity exercise can lead to increased mitochondrial biogenesis and play an important role in preventing aging (Kang & Ji, 2013).

High-intensity interval training (HIIT) is a type of exercise with high intensity in a

short time, accompanied by recovery time or interval. HIIT duration varies from 60 seconds to 20 minutes with a heart rate of 90-100% maximum heart rate. Research on HIIT is still very limited. Shorter duration of HIIT is expected to be a great advantage for busy individuals with less time for physical exercise. However, high-intensity exercise of HIIT causes the availability of oxygen in the muscle tissue is reduced so that the generated energy comes from the anaerobic process. Anaerobic energy systems will produce lactic acid, which can cause muscle fatigue if lactic acid accumulates (Perry et al., 2008; Adeva-Andany et al., 2014). The presence of intervals after high-intensity exercise results in a combination of anaerobic and aerobic energy metabolism (MacLaren & Morton, 2012; Siahkouhian et al., 2013). High-intensity exercise of HIIT causing HIIT may allegedly improve fitness conditions, increase glucose metabolism and fat burning (MacLaren & Morton, 2012; Perry et al., 2008).

From the existing studies, it is still not known exactly about the role of HIIT associated with changes in muscle contraction and energy metabolism. Therefore this study was conducted to see the effect of HIIT on skeletal muscle response in older adult of Wistar rats as the animal model.

METHODS

All experiments in this study were performed in accordance with the guidelines animal research from the National Institute of Health and were approved by the Ethical Committe Faculty of Medicine Universitas Indonesia (Ethical approval No.676/UN2. F1/ETIK/2016). This study was performed at The Integrated Laboratory of Medical Faculty Universitas Indonesia for ELISA measurements of Troponin T and PGC 1-α, while blood lactate measurement was conducted at Laboratory of Biochemistry and Molecular Biology of Medical Faculty Universitas Indonesia.

Animal

This study used fifteen 12 months old male Wistar rats which were randomly divided into control 1 group (C1) and 14 months age as a control 2 (C2). Both control groups were not given HIIT treatment and 12 months age which were given HIIT for 8 weeks (14 months at the end of the treatment), and defined as HIIT group.

Prior to the treatment, the rats were acclimatized to be familiar with the research environment or days. The rats were fed with standard chow and had free access to drinking water ad libitum.

The rats were housed in cages that were kept clean and was set to 12 hours of light and dark cycle. The ambient temperature was maintained at 23°C.

In this research, the use of different age group was to see: the process of aging in the old adult age (12 until 14 months) sedentary rats, and the process of aging with intervention of HIIT. C1 was used as baseline data for the aged 12 months rats which was the same as 18-20 years of human age. C2 was used as a control group to compare with the treatment group that

was 12 months age with 8 weeks of exercise to become 14 months age. HIIT group was used as a Treatment group from 12 months age until 14 months age. As we know, 14 months age for a rat is the same as 30-35 years old of human age. (Andreollo et al., 2012; Sengupta, 2011 & 2013).

HIIT Treatment

This experimental work used an animal treadmill to perform exercise training. The treadmill consisted of 6 single lines and each rat could run individually in a single line. The protocol was examined from previous study (Arabmomeni et al., 2015; Hafstad et al., 2011; Manchado et al., 2005). Prior to the treatment, the rats were acclimatized using the treadmill for 5 days and the speed received was 15m/min for 10 minutes/day. HIIT was performed 5 days/week for 8 weeks. It consisted of 4 repetitions of 4 min running at high intensity interspersed by 1 min of active rest. At the first week, the rats ran on a treadmill with a speed of 16 m/min and increased gradually up to 25 m/min at week 8. In each training session, rats performed 5 min warming- up and 5 min cooling-down.

Data Collection

At the end of the 8th week exercise (after treatment), blood was taken from the tail vein for lactate measurements; then the rats were decapitated and gastrocnemius muscle was taken for measurement PGC-1α and troponin-T concentration in muscle. Skeletal muscle homogenate was prepared by weighing up skeletal muscle as much as 100mg and homogenized in 1 mL PBS solution. The homogenates were performed for 2 times storage cycle for 24 hours in -20ºC, to disrupt the cell wall. The tissue homogenates were then centrifuged at 5000 x *g* for 5 minutes, and the supernatant was taken. Aliquots of supernatant were and stored at -20ºC before measurement. The remaining tissue samples were then stored at -80ºC for further usage. Total protein concentration of the skeletal muscle extracts was measured by the Bradford method (Cusabio, 2016; Elabscience, 2016). Concentration of rat troponin T (TnT) and PGC-α were reported as mg/g total protein.

Blood Lactate Concentration

Measurement of blood lactate concentration was done according to the procedure of commercially available kit (Lactate kit, LC 2389 Randox) based on spectrophotometric colorimetric. The blood was taken from the tail vein as much as 1mL and centrifuged at 1000x*g* for 10 minutes and the plasma were taken as sample. The absorbance results were read at wavelength of 550 nm. The standard curve was made from the standard solution according to the kit's protocol. Lactate concentration was calculated by comparing absorbance of lactate in the sample with a known concentration from the standard curve.

Troponin T Quatification in Skeletal Muscle

Troponin T (Tn-T) concentration was measured with commercially available enzyme immunoassay Rat Tn-T Elisa kit (Rat TnT, ELISA E-EL-R0054, Elabscience)

based on Sandwich ELISA method. The homogenate solution was used as much as 100µL for each well. The micro ELISA plate had been pre-coated with an antibody specific to Rat TnT. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat TnT and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and then incubated. Only those wells that contained Rat TnT, biotinylated detection antibody and Avidin-HRP conjugate would appear blue in color. Concentration of Troponin T in muscle was calculated by comparing the absorbance of the sample with the absorbance of Troponin T standard curve at 450 nm wavelength.

PGC-1α Quantification in Skeletal Muscle

PGC-1**α** concentration was measured with commercially available enzyme immunoassay Rat peroxisomeproliferator-activated receptor gamma co-activator 1-alpha (PGC-1**α** ELISA CSB-ELO18425RA, CusaBio), based on sandwich ELISA technique. Antibody that was specific for PGC-1**α** had been pre-coated onto a microplate. Standards and samples were pipetted into the wells (@ 100 µL) and any PGC-1**α** present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for PGC-1**α** was added to the wells. After washing, avid in conjugated Horseradish Peroxidase (HRP) was added to the wells. The PGC-1**α** concentration was calculated from the absorbance of PGC-1**α** in sample with the absorbance of known concentration of the standardPGC-1**α** at 450 nm wavelength.

Statistical Analyses

Data obtained from each parameter is expressed in mean ± SD and analyzed using SPSS version 19. Statistical analysis was performed using student t-test. Statistical differences for each parameter between groups were considered as significant if $p < 0.05$.

RESULTS

Blood Lactate Concentration

Plasma concentration of lactate was measured right after the last exercise at the end of week 8. Our results demonstrated that control group 2 (C2) had significantly higher in plasma lactate (4.12±1.20 mmol/l) compared to control group 1 (C1) (2.50±0.71, $p = 0.032$ mmol/l). The HIIT treatment in HIIT group showed to have tendency of lower plasma lactate concentration compared to control without exercise in same age (C2), although it was insignificant ($p = 0.381$) (Figure 1).

Troponin T Content in Muscle

Our results had demonstrated that there was tendency to decrease the Troponin T Concentration (1.53 ±1.57pg/mg) from the older rats to younger age or control 1 group (2.41±1.63pg/mg, $p = 0.415$). HIIT exercise group had significantly higher troponin T

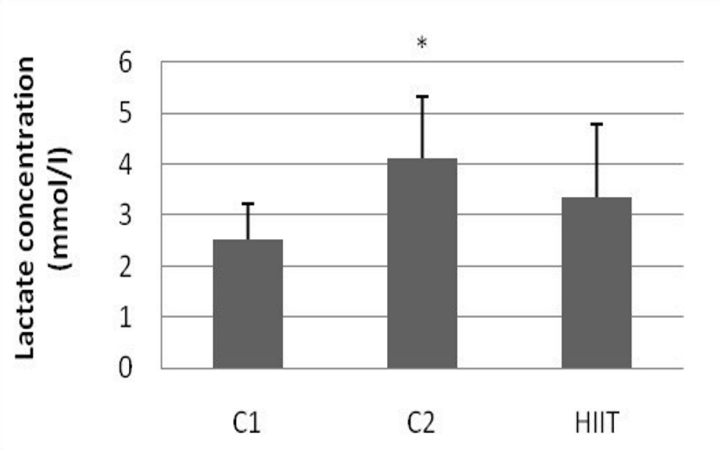

Figure 1. Lactate concentration in the blood. C1 is 12 months old without exercise, C2 is 14 months old without exercise and HIIT is 14 months old rats with HIIT treadmill exercise (HIIT group). C2 group shown to have higher blood lactate compared to C1 group ($p = 0.032$). Data are expressed in mean ± SD, analyzed with student t test, significantly difference $^{*}p<0.05$

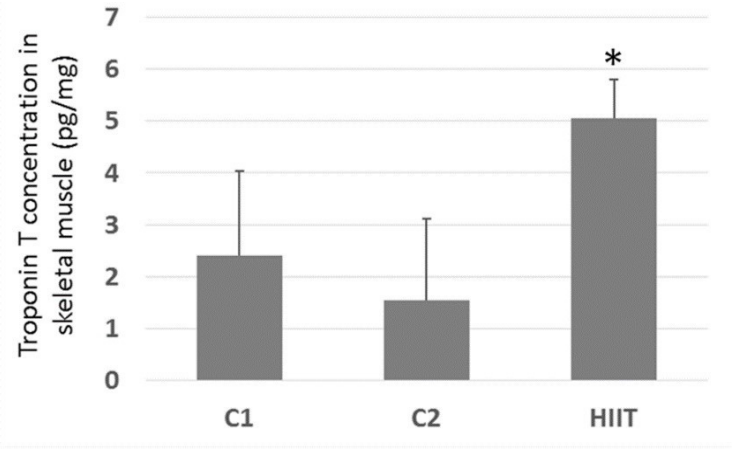

Figure 2. Troponin T concentration in skeletal muscle. HIIT exercise in HIIT group demonstrated higher concentration of troponin T in skeletal muscle compared to without exercise (C2 group) ($p = 0.002$). Data are expressed in mean ± SD, analyzed with student t test, significantly difference $^{*}p<0.05$

concentration (5.05±0.74 pg/mg) compared to C2 group (1.53±1.57 pg/mg, p = 0. 002) (Figure 2).

PGC- 1α Content in Muscle Tissue

PGC-1α in muscle tissue could represent the biogenesis of mitochondria and also play important rolein muscle metabolism during exercise. Our results showed that there was significant lower PGC-1αconcentration in skeletal muscle (148.260±102.41 pg/mg) of the older group compared to younger age or C1 group (386.33±156.43 pg/mg, p = 0.022). While exercise treatment with HIIT (HIIT group) showed higher PGC-1α concentration in skeletal muscle

(304.540±0.74 pg/mg) compared to the same age without exercise or C2 group (148.260±102.41 pg/mg, p = 0.024). (Figure 3.)

DISCUSSIONS

Our results showed that there was an insignificant difference between blood lactate levels in HIIT group and C2 group but there was a significant difference of blood lactate level between both control groups, i.e. C2 vs C1 groups. Blood lactate was identified as an indicator of an increase on glycolytic metabolism during exercise (Arabmomeni et al., 2015). The normal level of blood lactate is 0.5 - 2.2 mmol/L. This parameter is considered as a good indicator of the high-intensity exercise capacity. The peak of blood lactate concentration occurs approximately 5 min after the cessation of intense exercise (Arabmomeni et al., 2015; Manchado et al., 2005). Brito Vieira et al. (2014) defined that lactate threshold within the range of submaximal exercise intensities, normally between 50 and 80% of maximum load and blood lactate concentration approximately up to 4 mmol/L. This lactate threshold is important in examining high-intensity exercise with the interval (Arabmomeni et al., 2015). Lactate in the age of old adult rats (HIIT group), HIIT does not cause errors or disruption (performance does not go down) and does not cause injury.

Aging causes modification of body compositions that alter muscle structure and reduce the ability to perform the exercise requiring strength and power. Muscle fiber composition changes with a reduction in the number of type I (slow twitch) and type II (fast twitch) fibres with selective atrophy of type II fibres (Arabmomeni et al., 2015). This is one of the skeletal muscle response to aging. Blood lactate

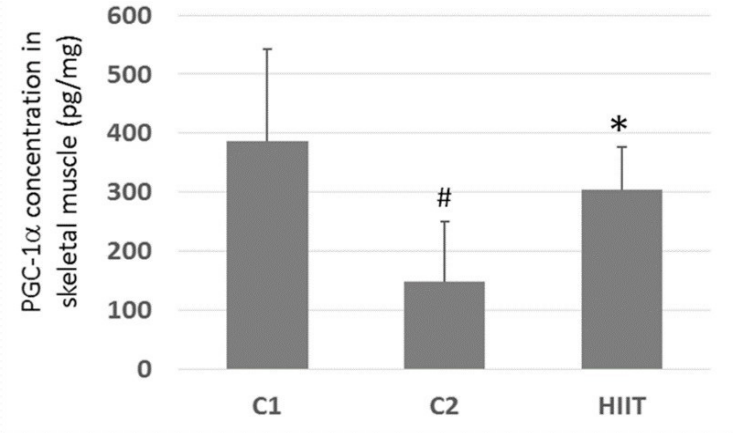

Figure 3. PGC-1α concentration in skeletal muscle. C2 group showed significantly lower in PGC-1α concentration in skeletal muscle compared to younger age or control 1 group (# p = 0.022). HIIT exercise in HIIT group demonstrate higher the concentration of PGC-1α in skeletal muscle compared to without exercise or C2 group (*p = 0.024). Data are expressed in mean ± SD, analyzed with student t test, significantly difference *p<0.05 (p=0.024). #p<0.05 (p=0.022)

level in older rats decreased during HIIT treatment compared to the same age (C2). Blood lactate level in older rat without HIIT treatment (14 months) was higher compared to the younger age (12 months). As we know, aging is associated with a minimum efficiency of glycolytic enzymes, lactate dehydrogenase and hexokinase enzymes. It is also stated that citrate and citrate synthase are reduced in muscle (Arabmomeni et al., 2015). Arabmomeni et al. (2015) stated that increased of muscle lactate would decrease muscle pH during high-intensity exercise. Following release of lactate from skeletal muscle, lactate, and hydrogen ions from red blood cells into the plasma are transported to the liver. In the liver, lactate underwent gluconeogenesis process to form glucose. It looks like exercise with enough intensity leads to lowering the blood lactate level because the interval has the greater effect on the lactate clearance.

Troponin T (TnT), as we know, as a function of contraction muscle and the sarcomeric Ca^{2+} is the regulator of striated (skeletal and cardiac) muscle contraction.

On binding Ca^{2t} TnT transmits information via structural changes throughout the actin- tropomyosin filaments, activating myosin ATPase activity and muscle contraction (Gomes et al., 2002). Our result showed that TnT protein control in the muscle of older rat with HIIT treatment was higher compared to the same age without HIIT treatment. That means that HIIT treatment alters the fibre proteome following the stimulation of AMP-activated protein kinase (AMPK) and elevation of sarcoplasmic Ca^{2t} levels during muscle contraction (Ohlendieck, 2011). In aging, there is a loss of skeletal muscle mass accompanied by the considerable decline in contractile strength (Faulkner et al., 2007). HIIT treatment proved to increase the protein content of TnT in the muscle of the old adult group. Norheim study found that there was secretion of signalling protein from human skeletal muscle cell in response to strength training (Ohlendieck, 2011).

Aging has been associated with the decline in mitochondria content and function (Menshikova et al., 2006). It is known that PGC-1α is a transcriptional coactivator that controls expressions of genes related to energy metabolism. PGC-1α control biogenesis and function of mitochondria (Jung & Kim, 2014). Our results found that PGC-1α in muscle tissue was increased in older rats with HIIT treatment compared to the same age without HIIT treatment (C2 group). Peroxisome-proliferator-activated receptor γ coactivator (PGC)-1α, is regarded as the 'master regulator' of mitochondrial biogenesis in muscle. Evidence suggests that exercise intensity is the key factor influencing PGC-1α activation in human skeletal muscle (Gibala, et al., 2012). In skeletal muscle, PGC-1α expression is activated during muscle contraction through Ca^{2+}/calmodulin dependent protein kinase IV (CamKIV) and calcineurin A, which are activated through calcium ion dynamics within the muscle in response to exercise. The increased calcium signaling during muscle contraction activities stimulates several important transcription factors such

as cAMP-response element binding protein (CREB), p38 mitogen-activated protein kinase (MAPK). Exercise will activate MAPK, increase ratio AMP: ATP and also Ca^{2t} flux, during muscle contraction (Kang & Ji, 2013). This study has limitations because in this research, soleus muscle was not used as slow twitch fiber to compare the measurements with fast twitch fiber, did not measure complete protein contractile like actin, myosin heavy chain, tropomyosin, Troponin I and Troponin C to see the difference between different age in control group and treatment group.

CONCLUSIONS

We conclude that HIIT is a powerful stimulus to muscle activity, maintaining the strength of skeletal muscle which is shown by increase Troponin T content in the muscle which is important in muscle contraction and increase PGC-1α that plays a vital role in the regulation of substrate metabolism and energy production. HIIT treatment for 8 weeks proved to be safe which was shown by the blood lactate concentration. This research uses HIIT by measuring muscle mass and strength, so we get the best HIIT formula to be applied in older adult so they can maintain healthy life. Further research is needed to determine the effect of HIIT on protein contractile level, and modifying HIIT training protocol especially when it is applied to humans especially among the elderly adults.

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REFERENCES

Adeva-Andany, M., López-Ojén, M., Funcasta-Calderón, R., Ameneiros-Rodríguez, E., Donapetry-García, C., Vila-Altesor, M., & Rodríguez-Seijas, J. (2014). Comprehensive review on lactate metabolism in human health. *Mitochondrion, 17*, 76-100.

Andreollo, N. A., Santos, E. F. D., Araújo, M. R., & Lopes, L. R. (2012). Rat's age versus human's age: What is the relationship?. ABCD. *Arquivos Brasileiros de Cirurgia Digestiva (São Paulo), 25*(1), 49-51.

Arabmomeni, A., Iravani, M. R., Sharifdoust, M., & Momeni, K. (2015) The effects of intermittent training on lactate level and lactate dehydrogenase (LDH) enzyme activity in blood of old rat. *MAGNT Research Report, 3*(2), 89-97.

Brito Vieira, W. H., Halsberghe, M. J. E., Schwantes, M. L. B., Perez, S. E. A., Baldissera. V., Prestes, J., Farias, D. L., & Parizotto, N. A. (2014). Increased lactate threshold after five weeks of treadmill aerobic training in rats. *Brazilian Journal of Biology, 74*(2), 444-449.

Cusabio. (2016). *Rat peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) ELISA kit CSB-EL018425RA*. Retrieved August 15, 2016, from http://www.cusabio.com.

Elabscience. (2016). *Rat TnT (Troponin T) ELISA kit E-EL-R0054*. Retrieved August 15, 2016, from http://www.elabsience.com.

Faulkner, J. A., Larkin, L. M., Claflin, D. R., & Brooks, S. V. (2007). Age-related changes in the structure and function of skeletal muscles. *Clinical and Experimental Pharmacology and Physiology, 34*(11), 1091-1096.

Gomes, A. V., Potter, J. D., & Szczesna-Cordary, D. (2002). The role of troponins in muscle contraction. *IUBMB Life, 54*(6), 323-333.

Gibala, M. J., Little, J. P., MacDonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of Physiology, 590*(5), 1077-1084.

Hafstad, A. D., Boardman, N. T., Lund, J., Hagve, M., Khalid, A. M., Wisløff, U., Larsen, T.S. & Aasum, E. (2011). High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. *Journal of Applied Physiology, 111*(5), 1235-1241.

Hansakul, P. (2010). Cellular aging. *Thammasat Medical Journal, 10*(3), 311-319.

Jung, S., & Kim, K. (2014). Exercise-induced PGC-1α transcriptional factors in skeletal muscle. *Integrative Medicine Research, 3*(4), 155-160.

Kang, C., & Ji, L. L. (2013) Role of PGC-1α in muscle function and aging. *Journal of Sport and Health Science, 2*(2), 81-86.

MacLaren, D., & Morton, J. (2012). *Biochemistry for sport and exercise metabolism*. Oxford, United Kingdom: Wiley-Blackwell.

Manchado, F. B., Gobatto, C. A., Contarteze, R. V., Papoti, M., & De Mello, M. A. R. (2005). Maximal lactate steady state in running rats. *Journal of Exercise Physiologyonline (JEPonline), 8*(4), 29-35.

Masoro, E. J., & Austand, S. N. (2011). *Handbook of the biology of aging* (7th Ed.). Cambridge, Massachusetts: Academic Press.

Marzetti, E., Calvani, R., Cesari, M., Buford, T. W., Lorenzi, M., Behnke, B. J., & Leeuwenburgh, C. (2013). Mitochondrial dysfunction and sarcopenia of aging: From signaling pathways to clinical trials. *The International Journal of Biochemistry & Cell Biology, 45*(10), 2288-2301.

Menshikova, E. V., Vladimir, B. R., Fairfull, L., Ferrell, R. E., Kelley, D. E., & Goodpaster, B. H. (2006). Effects of exercise on mitochondrial content and function in aging human skeletal muscle. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 61*(6), 534-540.

Narici, M. V., & Maffulli, N. (2010). Sarcopenia: Characteristics, mechanism and functional significance. *British Medical Bulettin, 95*(1), 139-159.

Ohlendieck, K. (2011). Proteomic profiling of fast-to-slow muscle transitions during aging. *Frontiers in Physiology, 2*(105), 1-5.

Perry, C. G., Heigenhauser, G. J., Bonen, A., Spriet, L. L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Applied Physiology Nutrition Metabolism, 33*(6), 1112–23.

Randox. (2016). *Lactate kit, LC 2389 based on spectrophotometric colorimetric*. Retrieved April 26, 2016, from http://www.randox.com.

Saxon, S. V., Etten, M. J., & Perkins, E. A. (2015). *Physical change and aging* (6th Ed.). New York: Springer Publishing Company.

Setiati, S. (2013). Geriatric medicine, sarkopenia, frailty dan kualitas hidup pasien usia lanjut: Tantangan masa depan pendidikan, penelitian dan pelayanan kedokteran di Indonesia (Geriatric medicine, sarcopenia, frailty and quality of life for elderly patients: The future challenges of education, research and medical services in Indonesia). *Geriatric Medicine, Sarkopenia, Frailty, 1*(3), 234-242.

Sengupta, P. (2011). Scientific review of age determination for a Laboratory Rat: How old is it in comparison with human age. *Biomedicine International, 2*(2), 81-89.

Sengupta, P. (2013). The Laboratory Rat: Relating its age with human. *International Journal of Preventive Medicine, 4*(6), 624-630.

Siahkouhian, M., Khodadadi, D., & Shahmoradi, K. (2013). Effects of high-intensity interval training on aerobic and anaerobic indices: Comparison of physically active and inactive men. *Science and Sport, 28*(5), e119-e125.