Folliculin Interacting Protein 1 (FNIP-1) expression and capillary density in gastrocnemius muscle tissue of mice after biological maturation period

La expresión de la Folliculin Interacting Protein 1 (FNIP-1) y la densidad capilar en el tejido muscular gastrocnemio de ratones después del período de maduración biológica

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Abstract

Introduction: The biological maturity period is the time or tempo of progress toward adulthood or maturity. The hormonal fluctuations play an important role in changing the characteristics of human body tissues during the biological maturity period. However, changes in tissue characteristics during biological maturity period have not been revealed. Skeletal muscle tissue is one of those believed to experience changes. Muscle fibers are thought to experience switching types from type 2 muscle fibers to type 1 muscle fibers. The transition of muscle fibers from type 2 to type 1 requires miR-499 activity from the expression of the Myh7b gene. MiR-499 directly inhibits FNIP-1. However, the biological maturity period of changing FNIP-1 expression has not been confirmed.
Objective: This study aimed to analyse the effect of the biological maturity period on FNIP-1 expression and capillary density in the gastrocnemius muscle.
Methodology: Thirty-six male mice were divided into mature groups aged eight weeks and immature groups aged four weeks. This study analyzed FNIP-1 and capillary density gastrocnemius muscle of mice using immunohistochemistry.
Results: FNIP-1 expression test showed higher in immature mice than mature mice. In comparison, the capillary density test and endurance showed higher expression in mature mice than in immature mice.
Conclusion: This study concludes maturation was characterized by a low distribution of FNIP-1 expression in the gastrocnemius muscle and a longer duration ability to run on the treadmill. Unfortunately, the capillary density was not a specific mark to determine maturation in mice.

Keywords

Maturity; FNIP-1; endurance; capillary; muscle; healthy lifestyle.

Resumen

Introduction: El período de madurez biológica es el tiempo o ritmo de progreso hacia la adultez o madurez. Las fluctuaciones hormonales desempeñan un papel importante en el cambio de las características de los tejidos del cuerpo humano durante el período de madurez biológica. Sin embargo, no se han revelado cambios en las características de los tejidos durante el período de madurez biológica. El tejido del músculo esquelético es uno de los que se cree que experimenta cambios. Se cree que las fibras musculares experimentan un cambio de tipos de fibras musculares de tipo 2 a fibras musculares de tipo 1. La transición de las fibras musculares de tipo 2 a tipo 1 requiere la actividad de miR-499 a partir de la expresión del gen Myh7b. MiR-499 inhibe directamente FNIP-1. Sin embargo, no se ha confirmado el período de madurez biológica del cambio de expresión de FNIP-1.
Objective: Este estudio tiene como objetivo analizar el efecto del período de madurez biológica en la expresión de FNIP-1 y la densidad capilar en el músculo gastrocnemio.
Methodology: Treinta y seis ratones machos se dividieron en dos grupos: grupos maduros de ocho semanas de edad y grupos inmaduros de cuatro semanas de edad. Este estudio analizó la FNIP-1 y la densidad capilar del músculo gastrocnemio de ratones mediante inmunohistoquímica.
Results: La prueba de expresión de FNIP-1 mostró una mayor en ratones inmaduros en comparación con los ratones maduros.
Conclusion: En comparación, la prueba de densidad capilar y la resistencia mostraron una mayor expresión en ratones maduros que en ratones inmaduros Este estudio concluye que la maduración se caracterizó por una baja distribución de la expresión de FNIP-1 en el músculo gastrocnemio y una capacidad de mayor duración para correr en la cinta. Lamentablemente la densidad capilar no fue una marca específica para determinar la maduración en ratones.

Palabras clave

Madurez; FNIP-1; resistencia; capilar; músculo; estilo de vida saludable.

Introduction

The biological maturity period is the time or tempo of progress toward adulthood or maturity. In skeletal muscle, biological maturity can affect performance when given physical loads (Towlson et al., 2021). The transition from adolescence to adulthood is marked by increased skeletal muscle resistance to physical loads (Costa et al., 2021; Neufer et al., 2015). Physical loads of the same volume in mature and immature individuals will put more significant stress on the skeletal muscles of immature individuals, potentially causing early injury and overuse injuries. Excessive physical loads in immature individuals may not result in optimal training adaptations and may prevent future participation in sports (Costa et al., 2021; Egger et al., 2019; Sniffen et al., 2022).

The second rapid growth, maturity of sexual function, and hormonal fluctuations, characterizes the biological maturity period. The biological maturity period in humans varies between the ages of 10-15 years. The biological maturation of women occurs earlier than men. The shock of reproductive hormones, testosterone, and estrogen play an essential role in changing the characteristics of human tissues and organs during the biological maturity period (Cameron, 2002). Unfortunately, until now, the changes in tissue characteristics during the human biological maturity period have not been revealed.

Skeletal muscle tissue is believed to change during the period of biological maturity. Skeletal muscle experiences a significant increase in muscle fiber heterogeneity during the maturation process. However, adult muscle still has substantial plasticity (Glenn et al., 2014). During development, adaptation to physiological stimuli causes extensive metabolic changes and structural remodeling of the muscle. Adaptation includes efforts to increase the muscle's ability to carry out Adenosin Triphosfat (ATP) energy metabolism and increase muscle tissue oxygenation (Neufer et al., 2015).

During biological maturity, muscle fibers are assumed to have switching type from type 2 to type 1 muscle fibers. Type I muscle fibers are characterized by being rich in mitochondria, dependent on oxidative metabolism, producing large amounts of ATP, and having high resistance to physical load. In contrast, type II muscle fibers are characterized by containing fewer mitochondria, relying on glycolytic metabolism for energy production, and having lower endurance (Liu et al., 2016). Type I muscle fibers support long-duration contraction, such as endurance sports. While muscle fibers or type II support short-duration contraction activities (Jacob et al., 2012).

The transition of muscle fibers from type II to type I requires miR-499 activity from myosin heavy chain 7b (Myh7b) gene expression. MiR-499 directly inhibits folliculin interacting protein 1 (FNIP-1). FNIP-1 protein plays a role in inhibiting the AMP-activated protein kinase (AMPK)-peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) signaling pathway in mitochondrial biogenesis, oxidative enzyme production, and myoglobin production (Liu et al., 2016; Xiao et al., 2021). Muscles expressing FNIP-1 tend to show type 2 characteristics, while type 1 characteristics are characterized by weak FNIP-1 expression (Reyes et al., 2015).

Chronic exercise can increase the number of type 1 muscle fibers, increase oxidative metabolism, and increase capillary density. Chronically trained muscles experience increased AMPK protein expression (Zhou et al., 2017). FNIP-1 expression is associated with AMPK activity in skeletal muscle that changes (Reyes et al., 2015). In addition to exercise, the period of biological maturity is thought to play a role in evolving FNIP-1 expression and AMPK activity. Unfortunately, this assumption has not been confirmed.

In skeletal muscle, AMPK induces the expression of the transcription regulator PGC-1α through Sirtuin 1 mediation towards characteristic changes leading to the specification of slow-twitch muscle fibers, oxidative metabolism, and mitochondrial biogenesis. Mitochondrial biogenesis increases the production of ATP and myoglobin in muscle (Lesmana, 2019; Liu et al., 2016). This increase in myoglobin levels and type I muscle fibers contributes to muscle specialization in long-term contractile activity and increased muscle endurance to physical stress (Jacob et al., 2012).

Biological maturity prepares skeletal muscles to support the physical activities of adults, such as standing, walking, and running for a more extended period of time (Muliani, 2016). Gastrocnemius muscles also experience changes during the biological maturity process in the secondary growth spurt phase (Kurihara et al., 2007). After the biological maturity period, gastrocnemius muscle tissue is thought to experience decreased FNIP-1 expression, increased capillary density, and resistance to physical stress. This study compares FNIP-1 expression, increased capillary density, and resistance to

physical stress between individuals who have not experienced biological maturation and those who have experienced biological maturation.

Examination of gastrocnemius muscle tissue in humans requires tissue samples taken through a biopsy method that can potentially raise ethical issues. Mice can replace humans as subjects because they have similar muscle tissue characteristics and biological maturity periods as humans (Hu et al., 2017). Research has never been conducted examining the effect of biological maturation on FNIP-1 expression, capillary density, and gastrocnemius muscle endurance in mice to physical stress. Endurance to physical stress can be evaluated through the duration of mice running activity on a treadmill. Longer running duration indicates better ability to withstand physical stress (Hu et al., 2017; Jacob et al., 2012).

Method

Animals

The experimental unit was white mice (Mus Musculus) or BALB/C male mice divided into two groups based on biological maturation age, namely groups of mice aged four weeks (immature) and eight weeks (mature) from Surabaya veterinary center. The results of calculating the sample size using the Lemeshow formula (2000) showed the minimum sample size obtained was 18, so the total sample size was at least 36 samples for two groups. Mice were divided into two groups, namely mature and immature groups, and were caged per group with a maximum of 5 mice per cage. Mice were kept in cages measuring 30cm x 16cm x 16cm. They were made of plastic with a rice husk base and a woven wire roof. Every morning, the cage was cleaned. Mice were given food and drank 2x1 day ad-lib. Mice were acclimatized for seven days with a reversed light-dark pattern. On the day of the study, mice were not fasted because there were no variables that affected the food intake of mice. All procedures of this study were approved by the Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia, with the number (49/EC/KEPK/FKUA/2024).

Materials and Location

The tools and materials used in this study included a modified Columbus treadmill, a digital stopwatch, paraffin blocks containing gastrocnemius muscle tissue, and anti-FNIP1 antibodies from Lsbio (catalog no: LS-C773237, host: rabbit). In addition, an Olympus CX21 microscope and ImageJ application were used for data analysis. This study was conducted at the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Airlangga University, and FNIP-1 expression readings were performed at the Department of Anatomy, Histology, and Pharmacology, Faculty of Medicine, Airlangga University. The study lasted for five weeks, through several stages: research preparation, research implementation, immunohistochemistry application on gastrocnemius tissue, taking pictures using a microscope, image analysis to calculate capillary density and FNIP-1 using ImageJ, and research data analysis.

Protocol

The subjects of the study were grouped based on biological maturation age. The mature group was eight weeks old, while the immature group was four weeks old. This study used an observational analytical method with a comparative study design. The subjects were given physical load of running on the flat treadmill. The physical load was determined based on the maximum running test performance by Dougherty et al. (2016). Mice were run on the treadmill with 0 inclination in to warming up and test. The first five minutes were warming up, consist of one minute at a speed of 0 cm /s, two minutes at a speed of 14 cm /s and two minutes at a speed of 21 cm /s. Mice continuously run at a speed of 30 cm/s until exhausted, which signed as unable to move forward, stay on the backgrid for five consecutive seconds, and refuse to move forward even though stimulated. The duration of running on the treadmill was determined in minutes using a digital stopwatch.

After 1x24 hours of recovery, the mice were anesthetized using ketamine injection. Then the gastrocnemius muscles were taken and stored in a container containing 1% formalin buffer to be preserved and embedded in a paraffin block. After that, the preserved tissue was sliced cross-sectionally for further FNIP1 immunohistochemistry processing. Then, the muscle tissue was photographed using a microscope for histological analysis for FNIP- 1 expression dan capillary density determination.

Histological Analysis

FNIP-1 expression was analyzed using an anti-FNIP1 antibody (Lsbio, catalog no.: LS-C773237) in mice gastrocnemius muscle. Positive cells expressing FNIP-1 were identified through brown staining of myocytes using chromogen 3,3' Diaminobenzidine (DAB), observed under a microscope at 100x magnification. FNIP-1 positive myocytes were counted using the ImageJ application (Hariharan, 2018). Figure 1 showed a comparison between mature and immature FNIP-1 expression of gastrocnemius muscle tissue in Figure 1a FNIP-1 in immature mice and Figure 1b FNIP-1 in mature mice. The arrows showed negative FNIP-1 in myocytes.

Capillary density was analyzed using the Nederveen et al. (2021) method from the histological photograph. Capillary density is the total number of capillaries divided by the total cross-sectional area of muscle fibers (Nederveen et al., 2021) carried out by experts. The images from the microscope are seen in Figure 2a, mature mice muscle, and Figure 2b, immature mice muscle. The arrows indicated the capillary density.

Data analysis

The data analysis utilised Software Statistical Packet for Social Science (SPSS) software version 22. The Shapiro-Wilk test resulted p value > 0.05 for FNIP-1 expression and capillary density, but not duration of running. Data of FNIP-1 expression and capillary density were distributed similar with the normal distribution standard, unfortunately the duration of running was not. Independent t test was performed to analyze FNIP-1 expression and capillary density between groups. Mann Whitney test was used to analyze duration of running between groups.

Results

FNIP-1 expression

FNIP-1 expression is found to be lower in percentage in the muscle of mature mice compared with the immature ones. The comparison of FNIP-1 expression between groups can be seen in Table 1. It is illustrated as brownish cytoplasm for positive FNIP-1 cells and pale cytoplasm for negative FNIP-1 cells (arrowed). Figure 1 shows the distribution of FNIP-1 cells in the gastrocnemius muscle.

Figure 1. FNIP1 Expression

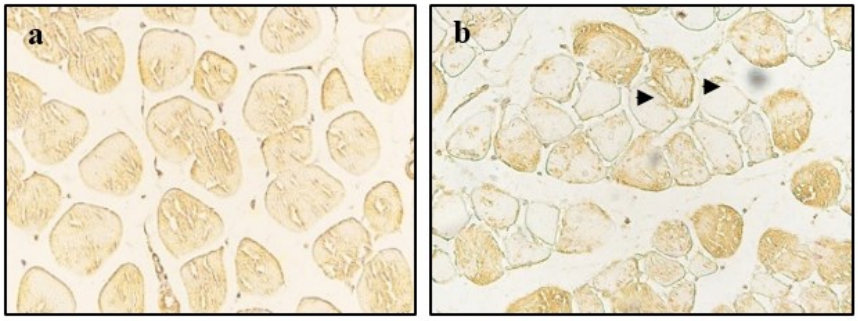

Table 1. The comparison of FNIP-1 expression between groups

Group	N	FNIP-1 Expression (%) Mean ± SD	P-value
Mature	18	26.97 ± 6.50	0.045*
Immature	18	32.80 ± 9.84	

*Significance at p-value <0.05, resulted from independent t-test

Capillary Density

The capillary density is not different between groups. The comparison of capillary density between groups can be seen in Table 2. It is illustrated as a small lumen with a single layer of endothelia filled

with blood cells (arrowed). Figure 2 shows the distribution of capillary vessels in the gastrocnemius muscle.

Figure 2. Capillary Density

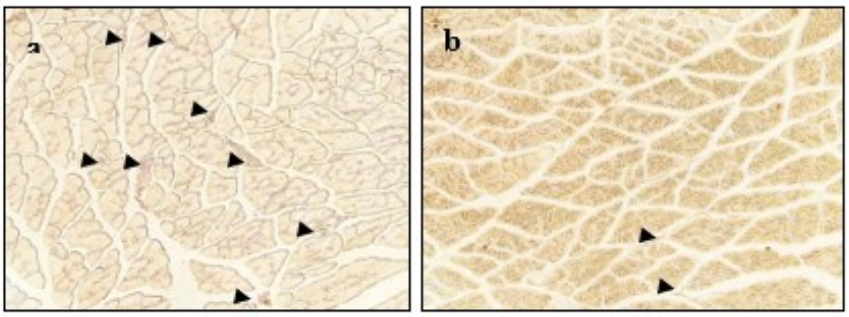

Table 2. The comparison of capillary density between groups

Group	N	Capillary Density (mm^2) Mean ± SD	P-Value
Mature	18	7,91±6,04	0,127
Immature	18	5,31±3,57	

*Significance at p-value <0.05, resulted from independent t-test

Endurance

Running duration (in minutes) is found to be longer in mature mice compared with immature ones. Mature mice has a better endurance to run on the treadmill without any inclination until exhausted. Longer running duration shows better endurance. The comparison of running duration between groups is seen in Table 3.

Table 3. The comparison of running duration between groups

Group	N	Running Duration (minutes) Mean ± SD	P-Value
Mature	18	31,99±5,47	<0,001*
Immature	18	23,58±6,29	

*Significance at p value <0.05, resulted from mann whitney test

Discussion

The results shows that the expression of FNIP-1 in the gastrocnemius muscle of mature mice was lower than that of immature mice. FNIP-1 has an inhibitory effect on mitochondrial function and type I muscle fibres in skeletal muscle. A study conducted by Reyes et al. (2015) in transgenic mice showed that loss of FNIP-1 expression in muscle fibres increased the representation of type I muscle fibres as indicated by increased myoglobin, MyH7, succinate dehydrogenase, troponin I, troponin C, troponin T, capillary density, and a number of mitochondria. Muscle fibers with little FNIP-1 expression are more resistant to post-contraction fatigue, while muscle fibers with dominant FNIP-1 expression has a higher oxidative capacity.

During development, there is adaptation to systemic physiological stimuli, and skeletal muscle fibers will undergo extensive metabolism and structural remodeling (Neufer et al., 2015). In a study using 4-week-old mice (immature), there were many II muscle fibers and few I muscle fibers (Gokhin et al., 2008). Type II muscle fibers are muscle fibers with dominant FNIP-1 expression. This is in line with the results of this study, where immature mice have higher FNIP-1 expression in the gastrocnemius muscle than mature mice. Type 1 muscle fibers are redder in color because their myoglobin content is higher, and their capillary density is also greater than type 2 muscle fibers (Lesmana, 2018).

The study results shows that the running duration in immature mice was shorter than in mature mice. Therefore, the mature group has greater endurance than the immature group. Endurance is the body's ability to carry out training activities for a long time without severe fatigue (Abdillah et al., 2021). In

running sports, in addition to requiring the ability of the lungs, heart, and blood vessels to provide sufficient oxygen to the cells (Hoeger & Hoeger, 2014), type I muscle fibers are also needed which have myoglobin, MyH7, succinate dehydrogenase, troponin I, troponin C, troponin T, capillary density, and higher number of mitochondria (Reyes et al., 2015). So that it can carry out long-term contractile activities (Jacob et al., 2012). In addition, the Yuliastrid et al. (2024) study found a relationship between FNIP-1 and endurance and myoglobin. They are negatively related, if there is FNIP-1 in muscle tissue, it interferes with the oxygen supply to connect mitochondria and inhibits endurance performance.

Age in mice affects physical activity in mice. This study's results align with the results of Reiber et al. (2022), who conducted a study on 5 phases of mice age from pre-pubescent (25 days), puberty, sexually mature, young adults, and adults. The study results showed that the running duration and distance on the spinning wheel of immature mice (pre-pubescent) were shorter than those of mature mice. This is possible in mature mice because they have more dominant type I muscle fibers.

Muscle fiber capillarisation plays an important role in regulating the maintenance of skeletal muscle mass. Adequate muscle tissue perfusion is important because it is responsible for the distribution of oxygen, growth factors, nutrients, and the removal of metabolic waste products (Hendrickse & Degens, 2019). Diffusion and transport to muscle fibers are ultimately limited by the surface area of the capillary blood vessel layer. Hence, the structure of the capillary network plays an important role in the maintenance, function, and health of skeletal muscle (Joanisse et al., 2016). Muscle tissue has a dense capillary network, with each muscle fibre surrounded by 3–6 capillaries, depending on the type of muscle fibre exercise status and the muscle tissue sample taken.

The study of Bergkamp et al. (2023) found that they did not find a significant correlation between age and capillary density. This study aligns with this study, where capillary density was not a specific mark to determine maturation in mice. Therefore, muscle fiber capillarization is related to cardiorespiratory fitness, physical activity, and glucose metabolism (Nederveen et al., 2021; Prior et al., 2016). Capillary density will increase with exercise, both resistance and aerobic exercise. Exercise can increase cardiovascular capacity and increase capillarization in muscles. To maintain a functional balance between metabolic needs and oxygen distribution, muscles will increase capillary density or the ratio of capillaries to muscle fibers (Karimian et al., 2015).

Capillary density can increase the endurance of mice in the maximum running test on a treadmill. Adequate oxygen levels will support mitochondrial oxidative enzymes to pair ATP which will help regenerate ATP to support longer-duration activity performance. Capillary density increases the oxygen supply for mitochondrial energy integration, which also contributes to the release of lactate from muscles. Lactate as a residue of anaerobic glycolysis in the cytosol, is a quick solution for ATP recovery during inhibited mitochondrial energy integration (Glancy et al., 2021). The lactate threshold is when lactate in the blood exceeds the amount that can be recycled so that there is a rapid increase in lactic acid. The lactate threshold can be used to measure endurance capacity, adaptation to exercise, and predict performance potential. The lactate threshold value can shift higher in people who have adapted to exercise (Beneke et al., 2011; Forsyth et al., 2017).

Conclusions

Based on the results of the study on FNIP-1 expression and capillary density of gastrocnemius muscle tissue in mice after the biological maturation period, it could be concluded that maturation was characterized by a low distribution of FNIP-1 expression in the gastrocnemius muscle and a longer duration ability to run on the treadmill. Unfortunately, the capillary density was not a specific mark to determine maturation in mice. Further research is needed to further evaluate myoglobin, oxygen saturation (SaO2), oxygen fraction (FiO2), and maximum oxygen volume (VO2 max) as variables. This study also did not evaluate the effects of physical activity in mice and the effects of nutrition.

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References

Abdillah, R., Risma, R., & Rofi, U. A. (2021). Kontribusi Daya Tahan Otot Fleksor Siku Dengan Ekstensor Bahu Terhadap Daya Tahan Otot Lengan Pada Siswa Ekstrakurikuler Bolabasket. *Jurnal Keolahragaan*, *7*(2), 21. https://doi.org/10.25157/jkor.v7i2.5627

Beneke, R., Leithäuser, R. M., & Ochentel, O. (2011). Blood lactate diagnostics in exercise testing and training. *International Journal of Sports Physiology and Performance*, *6*(1), 8–24. https://doi.org/10.1123/ijspp.6.1.8

Bergkamp, S. C., Smith, V., Kuijpers, T. W., Cutolo, M., van den Berg, J. M., & Schonenberg-Meinema, D. (2023). Correlations between capillary density and degree of skin pigmentation in healthy children analysed by nailfold video capillaroscopy. *Quantitative Imaging in Medicine and Surgery*, *13*(6), 3938–3947. https://doi.org/10.21037/qims-22-993

Cameron, N. (2002). *Human Growth and Development*. Elsevier Health Sciences.

Costa, T., Murara, P., Vancini, R. L., de Lira, C. A. B., & Andrade, M. S. (2021). Influence of Biological Maturity on the Muscular Strength of Young Male and Female Swimmers. *Journal of Human Kinetics*, *78*, 67–77. https://doi.org/10.2478/hukin-2021-0029

Dougherty, J. P., Springer, D. A., & Gershengorn, M. C. (2016). The treadmill fatigue test: A simple, high-throughput assay of fatigue-like behavior for the mouse. *Journal of Visualized Experiments*, *2016*(111), 1–7. https://doi.org/10.3791/54052

Egger, A. C., Oberle, L. M., & Saluan, P. (2019). The effects of endurance sports on children and youth. *Sports Medicine and Arthroscopy Review*, *27*(1), 35–39. https://doi.org/10.1097/JSA.0000000000000230

Forsyth, J., Burt, D., Ridley, F., & Mann, C. (2017). Using lactate threshold to predict 5-km treadmill running performance in veteran athletes. *Biology of Sport*, *34*(3), 233–237. https://doi.org/10.5114/biolsport.2017.65999

Glancy, B., Kane, D. A., Kavazis, A. N., Goodwin, M. L., Willis, W. T., & Gladden, L. B. (2021). Mitochondrial lactate metabolism: history and implications for exercise and disease. *Journal of Physiology*, *599*(3), 863–888. https://doi.org/10.1113/JP278930

Glenn, R., Adeel, S., & Zolt, A. (2014). Running Forward: New Frontiers in Endurance Exercise Biology. *Circulation*, *129*(7), 798–810. https://doi.org/10.1161/CIRCULATIONAHA.113.001590.Running

Gokhin, D. S., Ward, S. R., Bremner, S. N., & Lieber, R. L. (2008). Quantitative analysis of neonatal skeletal muscle functional improvement in the mouse. *Journal of Experimental Biology*, *211*(6), 837–843. https://doi.org/10.1242/jeb.014340

Hariharan, V. S. (2018). Phase fraction calculation of microstructure using ImageJ. *Research Gate*, *December*, 17. https://doi.org/10.13140/RG.2.2.22990.51521

Hendrickse, P., & Degens, H. (2019). The role of the microcirculation in muscle function and plasticity. *Journal of Muscle Research and Cell Motility*, *40*(2), 127–140. https://doi.org/10.1007/s10974-019-09520-2

Hoeger, W., & Hoeger, S. (2014). *Lifetime Physical Fitness and Wellness: A Personalized Program 13th Edition.* Paper Back Cengage Learning.

Hu, X., Charles, J. P., Akay, T., Hutchinson, J. R., & Blemker, S. S. (2017). Are mice good models for human neuromuscular disease? Comparing muscle excursions in walking between mice and humans. *Skeletal Muscle*, *7*(1), 1–14. https://doi.org/10.1186/s13395-017-0143-9

Jacob, W., Loenneke, J., Jo, E., & Wilson, G. (2012). *THE EFFECTS OF ENDURANCE,STRENGTH, AND POWER TRAINING ON MUSCLE FIBER TYPE SHIFTING*. *49*, 1724–1729.

Joanisse, S., Nederveen, J. P., Snijders, T., McKay, B. R., & Parise, G. (2016). Skeletal Muscle Regeneration, Repair and Remodelling in Aging: The Importance of Muscle Stem Cells and Vascularization. *Gerontology*, *63*(1), 91–100. https://doi.org/10.1159/000450922

Karimian, J., Khazaei, M., & Shekarchizadeh, P. (2015). Effect of resistance training on capillary density around slow and fast twitch muscle fibers in diabetic and normal rats. *Asian Journal of Sports Medicine*, *6*(4), 1–7. https://doi.org/10.5812/asjsm.24040

Kurihara, T., Kanehisa, H., Abe, T., Tsunoda, N., Fukunaga, T., & Kawakami, Y. (2007). GASTROCNEMIUS MUSCLE ARCHITECTURE AND EXTERNAL TENDON LENGTH IN YOUNG BOYS. *Journal of Biomechanics*, *40*, S690. https://doi.org/https://doi.org/10.1016/S0021-9290(07)70678-4

Lesmana, H. S. (2018). Bahan Ajar Fisiologi Olahraga. *Universitas Negeri Padang*, 79.

Lesmana, H. S. (2019). Adaptasi otot-otot skelet pada latihan. *Journal Article*, *1*, 1–10.

Liu, J., Liang, X., Zhou, D., Lai, L., Xiao, L., Liu, L., Fu, T., Kong, Y., Zhou, Q., Vega, R. B., Zhu, M., Kelly, D. P., Gao, X., & Gan, Z. (2016). Coupling of mitochondrial function and skeletal muscle fiber type by a miR-499/Fnip1/ AMPK circuit . *EMBO Molecular Medicine*, *8*(10), 1212–1228. https://doi.org/10.15252/emmm.201606372

Muliani. (2016). *BIOMEKANIKA LARI*. Fakultas Kedokteran Universtas Udayana.

Nederveen, J. P., Betz, M. W., Snijders, T., & Parise, G. (2021). The Importance of Muscle Capillarization for Optimizing Satellite Cell Plasticity. *Exercise and Sport Sciences Reviews*, *49*(4), 284–290. https://doi.org/10.1249/JES.0000000000000270

Neufer, P. D., Bamman, M. M., Muoio, D. M., Bouchard, C., Cooper, D. M., Goodpaster, B. H., Booth, F. W., Kohrt, W. M., Gerszten, R. E., Mattson, M. P., Hepple, R. T., Kraus, W. E., Reid, M. B., Bodine, S. C., Jakicic, J. M., Fleg, J. L., Williams, J. P., Joseph, L., Evans, M., … Laughlin, M. R. (2015). Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits. *Cell Metabolism*, *22*(1), 4–11. https://doi.org/10.1016/j.cmet.2015.05.011

Prior, S. J., Ryan, A. S., Blumenthal, J. B., Watson, J. M., Katzel, L. I., & Goldberg, A. P. (2016). Sarcopenia is Associated With Lower Skeletal Muscle Capillarization and Exercise Capacity in Older Adults. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *71*(8), 1096–1101. https://doi.org/10.1093/gerona/glw017

Reyes, N. L., Banks, G. B., Tsang, M., Margineantu, D., Gu, H., Djukovic, D., Chan, J., Torres, M., Liggitt, H. D., Hirenallur-S, D. K., Hockenbery, D. M., Raftery, D., & Iritani, B. M. (2015). Fnip1 regulates skeletal muscle fiber type specification, fatigue resistance, and susceptibility to muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(2), 424–429. https://doi.org/10.1073/pnas.1413021112

Sniffen, K., Noel-London, K., Schaeffer, M., & Owoeye, O. (2022). Is Cumulative Load Associated with Injuries in Youth Team Sport? A Systematic Review. *Sports Medicine - Open*, *8*(1). https://doi.org/10.1186/s40798-022-00516-w

Towlson, C., Salter, J., Ade, J. D., Enright, K., Harper, L. D., Page, R. M., & Malone, J. J. (2021). Maturity-associated considerations for training load, injury risk, and physical performance in youth soccer: One size does not fit all. *Journal of Sport and Health Science*, *10*(4), 403–412. https://doi.org/https://doi.org/10.1016/j.jshs.2020.09.003

Xiao, L., Liu, J., Sun, Z., Yin, Y., Mao, Y., Id, D. X., Liu, L., Xu, Z., Guo, Q., Ding, C., Sun, W., Yang, L., Zhou, Z., Zhou, D., Fu, T., Zhou, W., Zhu, Y., Chen, X., Id, Z. L., … Id, Z. G. (2021). *coordination of mitochondrial function and muscle fiber type by FNIP1*. 1–27. https://doi.org/10.1371/journal.pgen.1009488

Zhou, Q., Gu, Y., Lang, H., Wang, X., Chen, K., Gong, X., Zhou, M., Ran, L., Zhu, J., & Mi, M. (2017). Dihydromyricetin prevents obesity-induced slow-twitch-fiber reduction partially via FLCN/FNIP1/AMPK pathway. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1863*(6), 1282–1291. https://doi.org/https://doi.org/10.1016/j.bbadis.2017.03.019

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