Physical Exercise in Clinical Stage IIhuman Immunodeficiency Virus Infection Patients' Increasesskeletal Muscle MAss Through the Increasing of Myogenic Regulatory Factors Expression

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Abstract

Background: Human Immunodeficiency Virus infection is a chronic diseasethat threatensthe health ofmillions of people inthe worldand causing disability. One of the factors that causedisabilityis HIVmusclewasting, causing a decrease inthe quality of lifethat interferethe daily activitiesand evenincreased mortality. Proper physical exercise is needed to prevent and treat muscle wasting.

Methods: This studyusinghuman subjectswithclinicalstageII HIVinfection. Subjectswere groupedinto two: Intervention (n =9) that subjects whogetmoderate-intensity physicalexercise (*HIVA*) for 8weeks andControl (n =9) as subjects wereobservedfor 8 weeks. Muscles samples were taken fromthe vastuslateralismusclebiopsies that performed24 hoursafterthe lastphysical exercise,immunohistochemicalexaminationwithmonoclonal antibodyanti-Pax7, anti-MyoD1 andanti-myogenin and measurement of thigh circumference.

Results: Therewere significant increase in regeneration of skeletal muscle in the intervention group (increasing of Pax7, MyoD1 and myogenin) than the control groupand significant increase in quadriceps muscles mass as measured by thigh circumference ($p < 0.001$).

Conclusion: *HIVA* physical exerciseof moderate intensityincreasesskeletal muscleregenerationinclinical stageII HIVinfectionthroughthe increasingPax7, MyoD1, myogeninexpression and followed by an increase of quadriceps muscles mass.

***Keyword**: HIV, Rehabilitation Exercise, Myogenic Regularly Factors, Indonesia.*

Introduction

HumanImmunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/AIDS) is a health risk for millions of people who are currently a pandemic. The percentage of HIV infection in Indonesia, the highest reported in the age group 25-49 years (69.1%)[1], leads to reduced life quality due to muscle wasting. Hence, people with HIV / AIDS cannot perform daily activities and participate in society's social members. People with HIV / AIDS will experience impairment and disability due to their progression [2].The impact of HIV infection in the form of muscle-wasting disease progression, increased mortality, loss of muscle protein mass, weakness, impaired function [3,4], and decreased aerobic capacity [5] were also a predictor of mortality.

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The quadriceps muscle is one muscle that is essential to maintaining the function of ambulation, so it needs physical exercise to prevent the progression of muscle wasting and stimulate the regeneration process[3]. Vastuslateralis musclerepresents quadriceps musclebecause the strength is essential for weight-bearing and ambulation. It can show the response to physical exercise intervention and the biopsy's safest[6].

Skeletal muscle massrepresented a person's physical performance[7]. Physical exercise is known to increase muscle mass, improve protein balance through a mechanical signal, chemically maintain or increase muscle mass[8], and increase muscle strength.Skeletal muscle strength is a determinant of a person's functional capacity, providing a higher level of independence and quality of life[9].In this study, patients with HIV infection clinical stage IIperformed *HIVA* aerobic physical exercise (The name of tailored physical therapy is *HIVA*). This study aimed to compare the effect of *HIVA* exercise in skeletal muscle regeneration and muscle massin HIV infection clinical stage II patients through increased expression of Pax7, MyoD, and Myogenin.

Materials and Methods

Participants

The subjects in this study were male of HIV infection clinical stage II, aged 21-50 years, who came to the outpatient clinic of infectious unit Dr. Soetomo General Hospital. Human Immunodeficiency Virus infection clinical stage II is HIV infection defined by WHO criteria [10]. All study subjects get antiretroviral therapy.The ethics committee of Dr. Soetomo General Hospital Surabaya approved the study protocol of this research.(No: 258/Panke.KKE/IV/2015).

Allparticipants in this study provided written informed consent.

Physical Exercise Protocol.

The subjects in the intervention group were given a physical exercise as aerobics with a frequency of 2 times per week, intensity 60-70% of maximum heart rate, duration of 23 minutes (warm-up, and stretching 6 minutes, the core exercise 13 minutes and cooling-down 4 minutes) for eight weeks. The time adjusted with an intensity of 60-70% maximal heart rate.

Vastuslateralis muscle biopsy procedure.

Materials needed in this study are: muscle tissue biopsy results of vastuslateralis, anti-TNF- αmonoclonal antibody clone 52B83, Novus Biological product (NB600-1422), anti-calcineurin monoclonal antibody clone 2G8 Novus Biological product (H0005530-M03), anti-Pax7 monoclonal antibody clone 1E12 Novus Biological product (H00005081-M05), anti-NF-κB p65 monoclonal antibody clone 112A1021 Novus Biological product (NB100-56712), anti-MyoD1 monoclonal antibody clone 5.8A Dako product (M3512),

At the point of the bottom third of the line connecting the SIAS-patella performed muscle biopsy. Local anesthesia by injection of Lidocaine 2%, skin sterilized with povidone-iodine. Muscle biopsy using a reusable Medcore gun and Unicoreneedle size 16G. Specimen storage with formalin 10%.Each slide of the vastus lateralis muscle was viewed under light microscopy, 400 times magnification, counted the number of satellite cells showing Pax-7, Myo D, and Myogenin in 10 fields of view, then the averaged per field of view.

Statistic Analysis

Statistical analysis is using the SPSS program. To determine the homogeneity of the data in each group, using the one-sample Kolmogorov-Smirnov test anddetermining the data normalityusing the Shapiro-Wilk test. Thigh circumference before and after the physical exercise comparison in each group using a paired t-test. The alteration of thigh circumference after the physical exercise among the two groups used the Lavene test. The significance level was at $p \leq 0, 05$.

Results

The characteristics of the subjects showed in Table 1. Before intervention, there were no significant differences between age, Body Mass Index, and Thigh

Circumference between the intervention and control groups.

Table1. Characteristics of subjects

Variables	Intervention					Control				
	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Age (year)	9	27.00	50.00	36.33	7.29	9	25.00	388.00	32.00	4.12
BMI (kg/m2)	9	19.40	26.70	22.12	2.89	9	15.30	28.50	19.62	4.26
TC (cm)	9	42.00	52.50	46.70	3.74	9	28.00	52.50	38.22	7.58

BMI: Body Mass Index, TC: Thigh circumference, SD: Standart Deviation, N: Number of subjects, kg: kilogram, m^2: meter square, cm: centimeter

Table 2.Showed thigh circumference by measuring the thigh loop at the distal third of the line connecting the SIAS with the mid patella in-unit cm [11].Before and after intervention there was sinificant difference of thigh circumference p≤0.001.

Table 2. The thigh circumference before and after intervention

Thigh circumference	Intervention mean (SD)	Control mean (SD)	p
Pre (cm)	46.7 (3.7)	38.2 (7.5)	0.008
Post (cm)	50.9 (4.0)	38.5 (7.6)	-
Alteration (cm)	4.2 (1.5)	0.3 (0.)	<0.001
P	<0.001	0.247	
Note: significance level at p <0.05, cm: centimeter			

SD: Standart Deviation,

Table 3.Showed Pax7, MyoD1, and Myogeninexpression in satellite cells before and after intervention, there were a significant different of myogenesis factors before and after intervention p≤ 0.05.

Table3.Pax7, MyoD1, and Myogeninexpression in satellite cells.

Variable	Intervention (SD)	Control (SD)	p
Pax7 (mean cells/field of view)	0.34 (0.14)	0.11 (0.10)	0.002
MyoD1 (mean cells/field of view)	0.30 (0.13)	0.10 (0.10)	0.010
Myogenin (mean cells/field of view)	0.53 (0.21)	0.25 (0.15)	0.007
Note: significance level at p <0.05, SD: Standart Deviation			

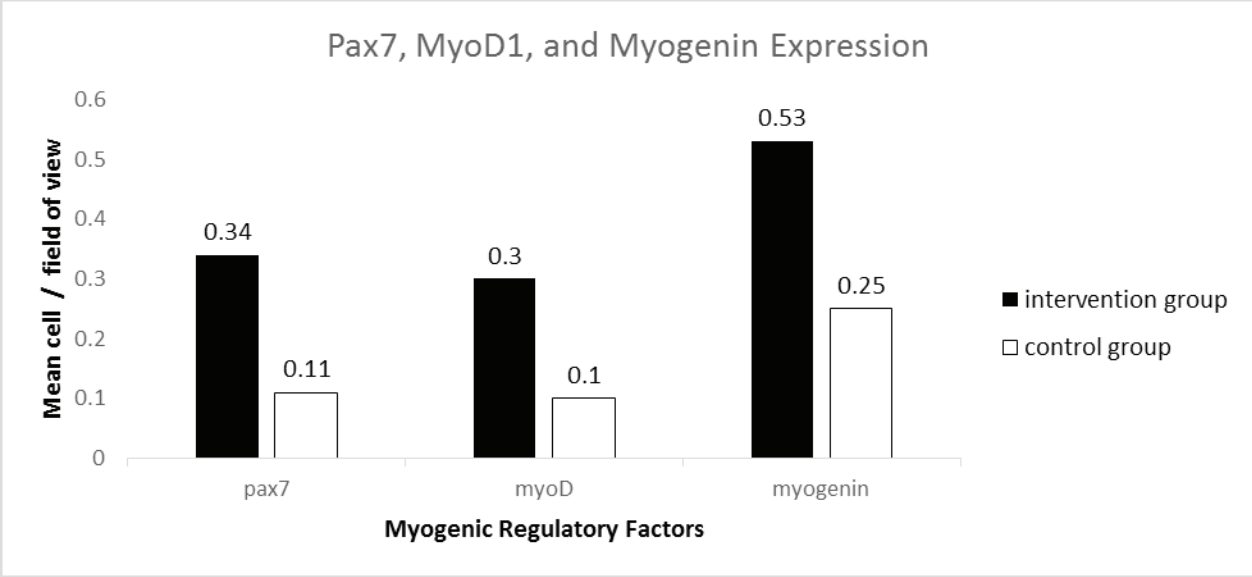

Figure 1. Showed the result of Pax7, MyoD1, and myogeninexpression of the intervention group obtained from the rightvastuslateralis muscle biopsies after HIVA physical exercise followed for eight weeks, and the control group after eight weeks of observation.

Discussion

Skeletal muscle regeneration is a highly regulated process, very complicated. It involves many factors in the process, among other specific muscle genes, satellite cells, and extracellular matrix was instrumental in muscle fibers reconstruction[12].The synthesis and degradation of proteins unbalanced also leads to the loss of muscle mass. Research shows that more than a standard protein diet: 0.8 g / kg/day reduces skeletal muscle mass and the condition of negative protein balance [13]. Malnutrition in HIV infection causes a decrease in the immune response and progression of HIV[14]. The state of malnutrition causes endocrine changes, which led to the mobilization of amino acids, especially skeletal muscles that affect skeletalmuscle regeneration [13].

Skeletal muscle regeneration is mediated by satellite cells, although generally in calm conditions. Satellite cells become active due to muscle damage, subsequently undergo proliferation, differentiation, and fusion to form new muscle fibers, resulting in tissue regeneration and restoration to normal function[15].Satellite cells closely associated with Pax7, and together with MyoD, determine the fate of satellite cells. Pax 7 expression on calmsatellite cells, and the muscle injury, will stimulate satellite cells to proliferate and rapidly induces the expression of MyoD (Pax7 + / MyoD +). Most satellite cells active, differentiated, ensued repression Pax7 and increased expression of Myogenin, and MRF4, a small portion back to the conditions (Pax7 + / MyoD-) who responded the next muscle injury, and repair[16].Muscle regeneration via the activation of many signaling pathways. Activation ofNotch signaling increase satellite cells to self-renew and inhibits differentiation by suppressing the expression of MyoD. Notch and Wntsignaling play an essential role in myogenesis, and Wntsignaling stimulates the expression ofPax7[17].

Physical exercise as a non-pharmacological therapy in people living with HIV in Indonesia has not become standard therapy. There is no formal physical training for people living with HIV on the intensity, duration, frequency, and type. The treatment given to people living with HIV in this study is regular physical exercise as aerobics HIVA, 2x per week frequency, duration of 23 minutes of physical exercise, moderate-intensity 60-70% of maximal heart rate, type of training with weight-bearing activities, for eight weeks or 16 times.The profile of HIV infection is continually changing, and some people regard it as a progressive disease and lethal.

Most people living with HIV who get antiretroviral therapy showed a chronic illness and treatable. Chronic conditions reflect an increase in disability in people living with HIV[18], but physical exercise is a crucial strategy for overcoming disabilities.

Paired box transcription factor (Pax7) activate transcription and controls the expression ofa myogenic regulatory element in the quiet and active satellite cells [19]. Paired box transcription factor (Pax7) expression increased within 24 hours after muscle injury and when the satellite cells have been heterogeneous. Research McKay et al. [20] showed that satellite cell humans have always shifted in the cell cycle, from the phase G_0 / G_1 to S and G_2 / M within 24 hours after contractions that cause muscle injury. In the first 24 hours after muscle contraction, obtained Pax7 increase (36% in phase G_0 / G_1), then Pax7 + (59% in S phase), and the next satellite cells obtained Pax7 + (202% in G_2 / M phase). These data indicate that the development of satellite cells in the cell cycle lasted 24 hours until at least 72 hours after muscle injury[21]. This condition means that the active satellite cells did not coincide but in varying cell cycle phases[20]. In adult muscle under physiological conditions, satellite cells express Pax7. Satellite cells are ready to respond to molecular stimuli from physical training, injuries, and diseases. They can self-renew, extend themselves, proliferationas myoblast or myogenicdifferentiation for fusion, and restore muscle damage. Satellite cells are the primary contributors to the growth, maintenance, and repair of skeletal muscle after birth [22, 23].Some studies in mice have shown that Pax7- will lose satellite cell lineage in all muscle groups[16, 24] by contrast, research Lepper et al.[25] reported that Pax7 does not need the normal function of satellite cells anymore after adolescence.

Increased Pax7expression in this study because muscle contractions produce IGF-1, which stimulates anabolic and myogenic processes and plays a role in modulating muscle growth's size. Insulin-like growth factor-1 stimulates the metabolism of protein in muscle fibers and increases myonuclei's number through the proliferation, differentiation, and fusion of satellite cells[26].Liu et al.[27] show that muscle activity and weight-bearing wereinfluential in increasing the level of IGF-1 muscle.

This study indicated that satellite cells' activation increased significantly in the intervention group (Pax7, $p = 0.002$, and MyoD1, $p = 0.010$). Biopsy in this study was conducted 24 hours after the lastphysical exercise. Each marker of active satellite cell Pax7 and MyoD1 showed an increase that is not the same. These data concluded the phase difference, some satellite cells in a phase of activation and are already experiencing proliferation. Increased expression of Myogenin showed differentiation and fusion.The myogenesis process is dynamic so that the steps of activation, proliferation, differentiation, and fusion are always changing. These data showed the most significant increase in myogenin expression, which means there is already a fusion of new muscle fibers in the biopsy 24 hours after moderate-intensity*HIVA* physical exercise for eight weeks.

Myogenic differentiation factor D (MyoD) is a protein that plays a vital role in regulating muscle differentiation [28].MyoD is one of the early markers of myogenic commitment, expressed at low levels in quietsatellite cells and satellite cell fusion. This study showed that the expression of MyoD1 in the intervention group is higher than in the control group. These results are consistent with Charge and Rudnicki's [22] research, which shows an increased expression of MyoD in satellite cells and muscle fibers mature in cell proliferation and differentiation for subsequent cell regeneration hypertrophy.

Several myogenic regulators could examine muscle regeneration evaluation after the physical exercise in the vastuslateralis muscle. Satellite cell activation is an essential key in muscle regeneration. During regeneration, satellite cells undergo proliferation, differentiation, and fusion to form new muscle fibers, and it is necessary to compensate for muscle hypertrophy[29, 30].The MRF controlled Muscle regeneration (MyoD, Myogenin, and MRF5). MRF plays a role in the regulation of muscle response to changes in physical exercise or activity.

This study increased MyoD1 and Myogenin after HIVA physical exerciseas monitor satellite cell activation to physical activity.Myogenic differentiation D (MyoD) is an important regulator involved in skeletal muscle adaptation to *HIVA* exercise. Research by Kosek et al. [31] shows increased expression of MyoD after physical activity. On activation of satellite cells, MyoD increased within 12 hours of activation or physical exercise. Increased MyoD until more than 36 hours, and would be significantly reduced after one week. In this study, increased expression of MyoD has occurred 24 hours after the last physical exercise. Pislander et al.[32] also show similar results that find an increase in temporary (transient) in the expression of MyoD in response to muscle strengthening exercises.

Contrary to the role of MyoD, Myogenin plays an essential role in muscle differentiation, consistent with a subsequent regulatory function in the process of particular types of muscle fibers[33]. Myogenin expression increased significantly from the results of the biopsy were performed after eight weeks of physical exercise. This research shows that Myogenin increased within one week after physical therapy; these data are similar to those reported by Carson and Booth [34].

Physical exercise can influence the muscle fibers' function, modify the structure, metabolism, and increase growth factors. Other signalsare paracrine for the activation of satellite cells[35]. These data increased expression of Pax7 and MyoD1 so that the *HIVA* physical exercise, going on satellite cell activationcauses an increase in skeletal muscle regeneration. Increased Pax7, MyoD1, and Myogenin mean that the provision of HIV A physical exercise repeatedly improved the regeneration of skeletal muscle indicated with activation and proliferation (increased expression of Pax7 MyoD1) and satellite cell differentiation (increased expression of Myogenin).

Conclusions

Moderate-intensity *HIVA* physical exercise in a clinical-stageII HIV infection increases skeletal muscle regeneration. The addition ofmuscle mass to people living with HIV prevents HIV progression of muscle wasting.

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References

1. Kementrian Kesehatan RI (Ministry of Health of the Republic of Indonesia). StatistikKasus HIV/AIDS di Indonesia. 2014. Cited on January 20th, 2021.Available from: https://pusdatin.kemkes.go.id/article/view/17020100001/situasi-penyakit-hiv-aids-di-indonesia.html
2. Gaidhane A, Zahiruddin QS, Waghmare L, Zodpey S, Goyal RC and Johrapurkar SR. Assessing self-care component of activities, and participation domain of the international classification of functioning, disability, and health (ICF) among people living with HIV/AIDS. AIDS Care. 2008;20(9):1098–104.
3. Grinspoon S and Mulligan K. Weight loss, and wasting in patients infected with human immunodeficiency virus. Clin Infect Diss. 2003;36(2): S69–78.
4. Bueter C, McKinzey M, Salzmann C, and Zorniak M. Pathways of Skeletal Muscle Atrophy: HIV as a Model System. Eukaryon 9. 2006;28–33.
5. Hand GA, Phillips KD, Dudgeon WD, Lyerly GW, Durstine JL, and Burgess SE. Moderate-intensity exercise training reverses functional aerobic impairment in HIV-infected individuals. AIDS Care. 2008;20(9):1066–74.
6. Hayot M, Michaud A, Koechlin C, Caron MA, LeBlanc P, Prefaut C, and Maltals F. Skeletal muscle micro biopsy: a validation study of minimally invasive technique. EurRespir J. 2005;25:431–40.
7. Costamagna D, Costelli P, Sampaolesi M and Penna

F. Role of Inflammation in Muscle Homeostasis and Myogenesis. [Internet]. Mediator of Inflammation. 2015 [cited 2016 Feb 15]. Available from: http://dx.doi.org/10.1155/805172

8. Tipton KD and Wolfe RR. Exercise, protein metabolism, and muscle growth. Int J Sport NutrExercMetab 11. 2001;109–32.
9. Rantanen T, Avlund K, Suominen H, Schroll M, Frandin K, and Pertti E. Muscle strength as a predictor of the onset of ADL dependence in people aged 75 years. Aging ClinExp Res. 2002;14(3):10–5.
10. World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-related Disease in Adults and Children [Internet]. 2007. p. 12. Available from: http://www.who.int/hiv/pub/vct/hivstaging/en/
11. Ezaki C, Murata S, Miyazaki J, Horie J, Murata J, Otao H. Relationship between Thigh Circumference, Quadriceps Femoris Thickness and Quadriceps Femoris Muscle Strength in Community-Dwelling Elderly. Rigakuryoho Kagaku. 2010;25(5):673–6
12. Karalaki M, Fili S, Philippou A and Koutsilieris M. Muscle Regeneration: Cellular, and Molecular Events. In Vivo (Brooklyn). 2009;23:779–96.
13. Carbone JW, McClung JP, and Pasiakos SM. Skeletal Muscle Responses to Negative Energy Balance: Effects of Dietary Protein. AdvNutr. 2012;3:119–26.
14. Duggal S, Chugh TD, and Duggal AK. HIV, and Malnutrition: Effect on Immun System. ClinDevImmunol. 2012;
15. Yin H, Price F, and Rudnicki MA. Satellite Cells, and the Muscle Stem Cell Niche. Physiol Rev. 2013;93(1):23–67.
16. Kuang S and Rudnicki MA. The emerging biology of satellite cells, and their therapeutic potential. Trends Mol Med. 2008;14(2):82–91.
17. Bjornson, C.R., Cheung, T. H., Liu, L., Tripathi, P. V., Steeper, K. M., &Rando TA. Notch Signaling Is Necessary to Maintain Quiescence in Adult Muscle Stem Cells. Stem Cells. 2012;30(2):232–42.
18. World Health Organization. International Classification of Functioning, Disability, and Health: ICF Short Version. 2011. p. 19–53
19. Zammit PS. All muscle satellite cells are equal but are more equal than others. J Cell Sci. 2008;121(18):2975–82.
20. McKay BR, Toth KG, Tarnopolsky MA, and Parise G. Satellite cell number, and cell cycle kinetics in response to acute myotrauma in humans: immunohistochemistry versus flow cytometry. J Physiol. 2010;588(Pt 17):3307–20.
21. O'Reilly C, McKay B, Phillips S, Tarnopolsky M and PG. Hepatocyte Growth Factor (HGF), and the satellite cell response following muscle lengthening contraction in humans. Muscle Nerve. 2008;38(5):1434–42.
22. Charge SB and Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev. 2004;84(1):209–38
23. Bentzinger CF, Wang YX, and Rudnicki MA. Building Muscle: Molecular Regulation of Myogenesis. Cold Spring HarbHarbPerspect Biol. 2012;4(2):a008342.
24. Relaix F, Montarras D, Zaffran S, Gayraud-Morel B, Rocancourt D, Tajbakhsh S, and Buckingham M. Pax3 Pax7 have distinct and overlapping functions in adult muscle progenitor cells. J Cell Biol. 2006;172(1):91–102.
25. Lepper C, Conway SJ, and Fan CM. Adult satellite cells and embryonic muscle progenitors have distinct genetic requirements. Nature. 2009;460(7255):627–31.
26. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. Int J Biochem Cell Biol. 2005;37:1974–84.
27. Liu M, Stevens-Lapsley JE, Jayaraman A, Ye F, Conover C, Walter GA, Bose P, Thompson FJ, Borst SE and Vandenborne K. Impact of treadmill locomotor training on skeletal muscle IGF1, and myogenic regulatory factors in spinal cord injured rats. Eur J Appl Physiol. 2010;
28. Olson EN and Klein WH. bHLH factors in muscle development: Deadlines, commitments, what to leave in, and what to leave out. Genes Dev.

1994;8:1–8.

29. Adams GR. Role of insulin-like growth factor-I in the regulation of skeletal muscle adaptation to increased loading. Exerc Sport Sci Rev. 1998;26:31–60.
30. Bellamy LM, Joanisse S, Grubb A, Mitchell CJ, McKay BR, Phillips SM, Baker S and Parise G. The Acute Satellite Cell Response and Skeletal Muscle Hypertrophy following Resistance Training. PLos ONE 9(10):e109739. Doi:10.1371/journal.pone.0109739
31. Kosek DJ, Kim JS, Petrella JK CJ, and Bamman MM. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. J Appl Physiol. 2006;101(2):531–44.
32. Pislander N DR and Pillegaard H. Resistance Exercise alter MRF and IGF-1 mRNA content in human skeletal muscle. J Appl Physiol. 2003;95:1038–44.
33. Verdijk LB, Gleeson BG, Jonker RAM, Meijer K, Savelberg HHCM, Dendale P, and van Loon LJC. Skeletal Muscle Hypertrophy Following Resistance Training Is Accompanied by a Fiber Type-Specific Increase in Satellite Cell Content in Elderly Men. J Gerontol A BiolSci Med Sci. 2009;64A(3):332-9.
34. Carson JA and Booth FW. Myogenin mRNA elevated during rapid, slow, and maintenance phases of stretch-induced hypertrophy in chicken slow-tonic muscle. Pflügers Arch. 1998;435(6):850–8.
35. Magaudda L, Mauro DD, Trimarchi F and Anastasi G. Effects of Physical Exercise on Skeletal Muscle Fiber: Ultrastructural and Molecular Aspects. Basic ApplMyol. 2004;14(1):17–21.