Glucocorticoid-Induced Changes in Rat Skeletal Muscle Biomechanical and Viscoelastic Properties: Aspects of Aging

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Abstract

Objectives: The purpose of this study was to estimate the state of tension (tone) and the biomechanical and viscoelastic properties of skeletal muscle in aging rats during the administration of different doses of dexamethasone and to find the relationships among the state of muscle atrophy, muscle strength, and the abovementioned muscle properties.

Methods: Muscle state of tension, biomechanical (elasticity, dynamic stiffness) and viscoelastic (mechanical stress relaxation time, Deborah number) properties (using MyotonPRO, Myoton Ltd, Tallinn, Estonia), lean body mass (BM), and hind limb grip strength were measured before and after the administration of a 10-day treatment with dexamethasone 100 μg/100 g BM (young and old group) and 50 μg/100 g BM (old group).

Results: Muscle elasticity (logarithmic decrement) was lower in old animals (1.86 ± 0.03) in comparison with young adult rats (1.38 ± 0.04) ($P < .01$). After the 10-day treatment with dexamethasone 100 μg/100 g BM, young adult rats had 10% lower muscle elasticity ($P < .01$). The same dose of dexamethasone in old rats increased tone (frequency of natural oscillation) from 29.13 ± 0.51 Hz to 38.50 ± 0.95 Hz ($P < .001$). There were dose-dependent differences in dynamic stiffness and tone of muscle; changes in elasticity were independent of the dose in old animals. In old rats, the muscle's viscoelastic properties decreased after dexamethasone administration. Significant correlation was found between changes in muscle logarithmic decrement and stiffness ($r_s = 0.90$; $P < .05$) in old animals.

Conclusions: Biomechanical and viscoelastic properties of skeletal muscle indicate changes in the main function of muscle during glucocorticoid-induced muscle atrophy and are in agreement with changes in hind limb strength. The myometric measurements indicate the direction and magnitude of change in muscle tissue after different doses of dexamethasone administration easily and quickly. (J Manipulative Physiol Ther 2018;41:19-24)

Key Indexing Terms: *Myopathy; Muscular Atrophy; Muscle Tonus; Biomechanical Phenomena; Aging*

Introduction

Administration of pharmacologic doses of glucocorticoids as well as Cushing syndrome leads to reduction in muscle mass, wasting of muscle, loss of muscle strength, selective atrophy of fast-twitch muscle fibers, and the lower turnover rate of contractile proteins.[1-3] In both laboratory animals and humans, the synthesis rate of contractile proteins decreases with age.[4] After administration of glucocorticoids, muscle wasting was more rapid in the older group, and recovery of muscle mass took twice as long as in young adults[5] because of the diminished regenerative capacity.[6] It is not clear how biomechanical (stiffness and elasticity) and viscoelastic properties (creep [Deborah number] and mechanical stress relaxation time) change in atrophied elderly skeletal muscle. The question is whether there is a relationship between a dose of dexamethasone and biomechanical/viscoelastic properties and the state of muscle atrophy. The biomechanical properties of muscle are (1) dynamic stiffness (N/m), which characterizes the resistance to a contraction or to an external force that deforms a muscle's initial shape; and (2) logarithmic decrement of a muscle's natural oscillation, which indicates the muscle's elasticity.[7,8] Elasticity characterizes the ability to recover the initial muscle shape after a contraction or removal of an external force. Viscoelastic properties of muscle are (1) mechanical stress relaxation time (ms), which is the time for a muscle to restore its shape from deformation after a voluntary contraction or an external force is removed; and (2) creep, which is the gradual elongation of a muscle over time when placed under a constant tensile stress. This is characterized as the Deborah number—the ratio of the mechanical stress relaxation time and the time to maximum

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deformation caused by an external force.[9,10] The state of tension is characterized by oscillation frequency (Hz), which indicates the tone of a muscle in its passive or resting state without any voluntary contraction. The aging aspect of skeletal muscle's biomechanical and viscoelastic properties is unclear, but it is well known that glucocorticoids lead to the loss of the myofibrillar apparatus, changes in the extracellular matrix, and decrease of muscle strength and motor activity in the elderly.[11] After muscle fiber damage, the regeneration of the contractile apparatus is less effective in the aged in comparison with the young.[12]

The aim of this study was to estimate the tone and the biomechanical and viscoelastic properties of the skeletal muscle of aging rats during different doses of dexamethasone administration and to find the relationships among the state of muscle atrophy, muscle strength, and the abovementioned muscle properties.

We hypothesized that old rats are more sensitive to the dose of dexamethasone compared with young adults and that a relationship may exist between the state of muscle atrophy caused by dexamethasone and the tone and biomechanical/viscoelastic properties of skeletal muscle.

Methods

Animals and Dexamethasone Treatment

Laboratory animals were used in accordance with the European convention for the protection of vertebrate animals used for experimental and other scientific purposes. The experimental protocol was approved by the Animal Experimentation Committee at the Estonian Ministry of Agriculture.

In the study, female Wistar rats that were 24 weeks old (young adults group) and 72 weeks old (old group) at the beginning of the dexamethasone treatment were used. Dexamethasone (Dexafort 3 mg/mL; International B.V., Boxmeer, The Netherlands) was diluted to 200 μg/mL with 0.15 M NaCl and administered intraperitoneally daily for 10 days to all groups.

The young adults group received dexamethasone 100 μg/100 g body mass (BM) (n = 8); half of the old group animals received 50 μg/100 g BM (n = 5) and the other half 100 μg/100 g BM (n = 5). The animals were anaesthetized by intraperitoneal injection of ketamine (60 mg/1 kg BM) (Bioketan, Vetoquinol BIOWET Sp.z o.o.) and xylzine (9 mg/1 kg BM) (Xylapan, Vetoquinol BIOWET Sp.z o.o.) before dual-energy x-ray absorptiometry and MyotonPRO device scanning. No effect of anesthesia on the contractile behavior of rat skeletal muscles was observed. The rats were weighed at the beginning and at the end of the experiment. All the animals were housed in identical environmental conditions in polycarbonate type III cages, at 21°C, 2 per cage at 12/12 hours light/dark period and received food and water ad libitum.

Hind Limb Grip Strength

Hind limb grip strength was measured before anaesthetization, before and after 10 days of dexamethasone treatment, using a Grip Strength Meter 0167-004L (Columbus Instruments, Columbus, Ohio).

Lean BM

The body composition was evaluated before and after 10-day dexamethasone treatment only in old rats by dual-energy x-ray absorptiometry (Hologic Discovery W, Bedford, Massachusetts) equipped with small animal software. Each rat was anesthetized for the procedure, as described previously. Scans were analyzed with Hologic APEX, Version 3.3.01 software, and lean BM (g) was recorded.

Myometric Methods

The state of tension and the biomechanical and viscoelastic properties of muscle were measured using a handheld MyotonPRO device (MyotonPRO, Myoton Ltd, Tallinn, Estonia).[7,8,13,14]

The central part of the gastrocnemius muscle (the major calf muscle on the posterior surface of the lower hind limb) belly of the anesthetized rats was tested. Myometric measurement is impossible to perform in an animal model without anesthesia because muscle should be in a state of relaxation. The following characteristics of muscle were calculated by software of MyotonPRO: (1) frequency of natural oscillation (Hz) (the frequency of damped mechanical oscillation of muscle tissue that is a parameter of the tension in the muscle); (2) logarithmic decrement of damping oscillations amplitude (characteristics of elasticity or the ability of muscle to restore its initial shape after deformation. A lower level of decrement reveals better muscle elasticity); (3) dynamic stiffness of the muscle (N/m) (the ability of tissue resistance to a contraction or to an external force that deforms the muscle's initial shape); (4) mechanical stress relaxation time (ms); and (5) gradual elongation of a muscle over time when placed under constant tensile stress (creep characterized by Deborah number). For this study, a MultiScan pattern of 20 measurements was used, and the mean was considered. Pooled data for the right and left gastrocnemius muscle were calculated.

Statistics

Descriptive statistics was used to summarize data as means and standard errors (SEs). Paired t test was used for the determination of differences between the groups. Spearman's Rho correlation coefficient was calculated. Differences were considered significant at $P < .05$.

Results

Effect of Dexamethasone

All young adult rats tolerated the dexamethasone infusion of 100 μg/100 g BM during 10 days. In the old groups, only 60% of rats survived the dexamethasone treatment of 100 μg/

Table 1. *Comparison of BM Before and After Hormone Treatment*

Group	n	BM (g)		Lean BM (g)		Hind Limb Grip Strength (N)	
		Before	After 10 Days Hormone Treatment	Before	After 10 Days Hormone Treatment	Before	After 10 Days Hormone Treatment
Young adults							
DEX 100 μg/100g BM	8	275.0 (5.4)	222.1 (4.5) [a]	-		10.05 (0.24)	7.41 (0.19) [a]
Older adults							
DEX 100 μg/100g BM	5	316.6 (12.0)	231.6 (6.8) [a]	197.3 (9.3)	166.3 (8.7) [a]	8.59 (0.29)	5.85 (0.16) [a]
DEX 50 μg/100g BM	5	348.3 (10.5)	265.0 (9.6) [a]	197.0 (7.8)	177.6 (5.0) [a]	7.40 (0.15)	5.59 (0.21) [a]

BM, body mass; *DEX,* dexamethasone.
[a] $P < .001$ in comparison with the before hormone treatment.

100 g BM during the 10-day treatment period, and all old rats survived the dexamethasone treatment of 50 μg/100 g BM. According to changes in lean BM, significant muscle atrophy occurred during 100 μg and 50 μg/100 g BM dexamethasone administration (Table 1).

Effect of Dexamethasone on the Tone and Biomechanical and Viscoelastic Properties of Muscle

The comparison of skeletal muscle biomechanical properties of young adult rats and old rats before the experiment showed that the only difference was in logarithmic decrement (elasticity).

Logarithmic decrement in the young adults group was 1.38 ± 0.04 and in the old group 1.86 ± 0.03 ($P < .01$). Old rats had significantly lower elasticity compared with young adults. The difference in the logarithmic decrement in the young adults group before and after 100 μg/100 g BM dexamethasone administration was 10% ($P < .01$). Changes in logarithmic decrement after different doses (100 μg/100 g BM and 50 and 100 μg/100 g BM) dexamethasone administration in old rats were not significant (Figs 1 and 2). The high initial level of logarithmic decrement is probably the reason for the effect of 100 μg/100 g BM dexamethasone administration being only 2% in the old group (Fig 1). Dynamic stiffness increased subsequently by 140% and 61% in the old groups after 10 days 100 μg/100 g BM and 50 μg/100 g BM dexamethasone infusion (Figs 1 and 2).

In both old groups during the 10-day 100 μg and 50 μg/100 g BM dexamethasone infusion, the oscillation frequency, indicating the state of muscle tone, increased subsequently from 29.13 ± 0.51 Hz to 38.50 ± 0.95 Hz ($P < .001$) and from 28.26 ± 0.48 Hz to 34.42 ± 0.70 Hz ($P < .001$). A dose-dependent difference was apparent in muscle tone after 100 μg and 50 μg/100 g BM dexamethasone administration (38.50 ± 0.35 Hz and 34.42 ± 0.70 Hz, respectively) ($P < .05$).

In old rats, viscoelastic properties of muscle, such as mechanical stress relaxation time and Deborah number, decreased during dexamethasone administration (Figs 3 and 4). Figures 2 and 4 show that there are dose-dependent differences in the biomechanical and viscoelastic properties of skeletal muscle after 50 μg and 100 μg/100g BM dexamethasone administration to old animals.

Significant correlation was found between changes in muscle logarithmic decrement and stiffness ($r_s = 0.90$; $P < .05$) in old animals. There was also a correlation between muscle grip strength before and after 10 days of dexamethasone administration ($r_s = 0.90$; $P < .05$): The higher the grip strength value before dexamethasone administration, the higher it was after the 10-day dexamethasone administration as well.

Discussion

The catabolic effect of glucocorticoids on skeletal muscle has been studied for a long time, but this is the first study showing changes in the biomechanical and viscoelastic properties of skeletal muscle of small laboratory animals during glucocorticoid-induced muscle atrophy.

Our results show that of skeletal muscle biomechanical properties, elasticity was better in young adults than in the old group. Because of the higher initial level of logarithmic decrement in the old group, the changes in elasticity after dexamethasone administration were not significant. Muscle stiffness increased during dexamethasone administration, and higher hormone doses caused higher stiffness. Higher doses of hormone also caused higher muscle tone. The results of the present study show that higher doses of hormone cause significantly higher muscle atrophy. It has been shown earlier that lower elasticity was accompanied by the lower content of titin and nebulin in dexamethasone-caused atrophied skeletal muscle[15]; the higher the degree of muscle atrophy, the lower the muscle elasticity and the content of the MyHC IIb isoform.[15] The oscillation frequency indicated that the state of muscle tone—in other words, intrinsic tension in muscle—was higher in the old group after dexamethasone administration. The increased muscle tone that was observed in atrophied muscle might be related to changes in the innervation of the atrophic muscle. Ultrastructural studies have shown that neuromuscular synapses are destroyed in part of an atrophied muscle.[16] Skeletal muscle viscoelastic properties (mechanical stress relaxation time and creep) show that dexamethasone-caused muscle atrophy is accompanied with a decrease of relaxation time and elongation of muscle over time. There is also dependence on the dose of dexamethasone and the

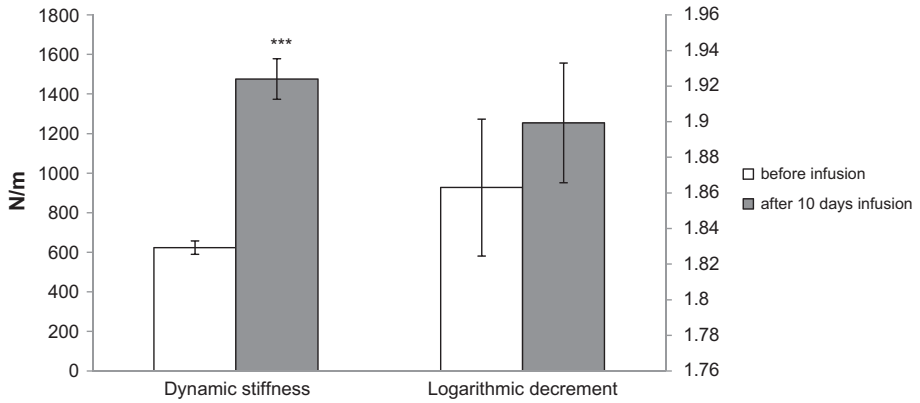

Fig 1. *Dynamic stiffness and logarithmic decrement before and after infusion. The asterisks indicate that* P < *.001 in comparison with the before infusion treatment.*

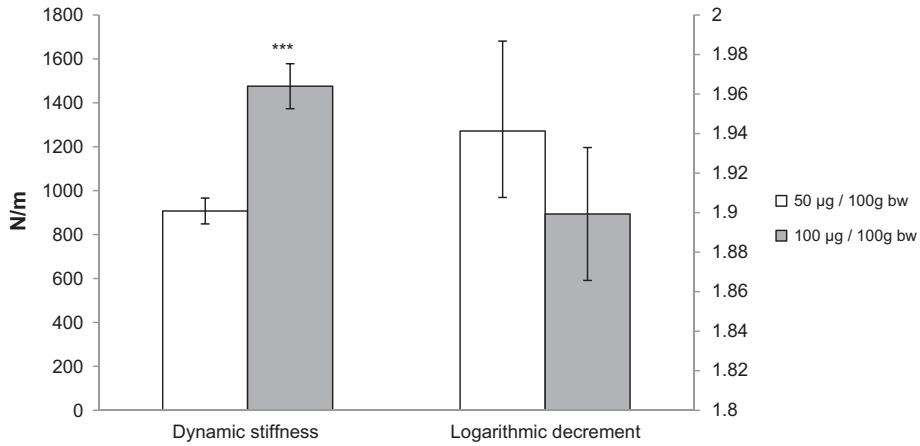

Fig 2. *Dynamic stiffness and logarithmic decrement under different dosages. The asterisks indicate that* P < *.001 in comparison with 50 μg/100 g BM.*

viscoelastic properties of muscle. It has been shown earlier that the disappearance of thick and thin myofilaments from about one-fifth of the cross-sectional area of myofibrils in glucocorticoid-induced myopathic glycolytic muscle fibers is the main reason there is reduced muscle strength and motor activity,[17] as well as the downregulation of collagen synthesis and changes of the extracellular matrix.[11] Increased myofibrillar protein degradation rate, decreased MyHC and actin synthesis rate, and a decrease in MyHC IIb isoform relative content in glycolytic muscle fibers are related to the qualitative remodeling of the myofibrillar apparatus in glucocorticoid-induced myopathic muscle.[3]

Decrease in hind limb grip strength of old rats after dexamethasone treatment in the present study showed that it was more significantly lower in the old group than in the young group. Decrease in the tone and biomechanical and viscoelastic properties of muscle after dexamethasone treatment is in good agreement with the decrease in hind limb grip strength.

If the changes in the ultrastructural level of dexamethasone-induced atrophic muscle described above show the deep mechanism of the decrease of muscle main function, then changes in the tone and biomechanical and viscoelastic properties of muscle reflect the changes of this function from the peripheral part of muscle. Increase of muscle stiffness and tone during muscle atrophy has been shown to depend most of all on the decrease of muscle microcirculation.[18] According to Kimura,[18] increase in soft tissue stiffness is associated with a rise of the internal pressure of soft tissue as a result of increase in blood volume. External low-pressure compression causes venous occlusion without arterial occlusion, and high-pressure compression occludes both veins and arteries. Therefore, low-pressure compression should increase the cross-sectional area and internal pressure, thereby increasing soft tissue stiffness. In the current study, the myometric measurement of biomechanical and viscoelastic properties of muscle was for the first time performed in small laboratory animals, and this seems to be

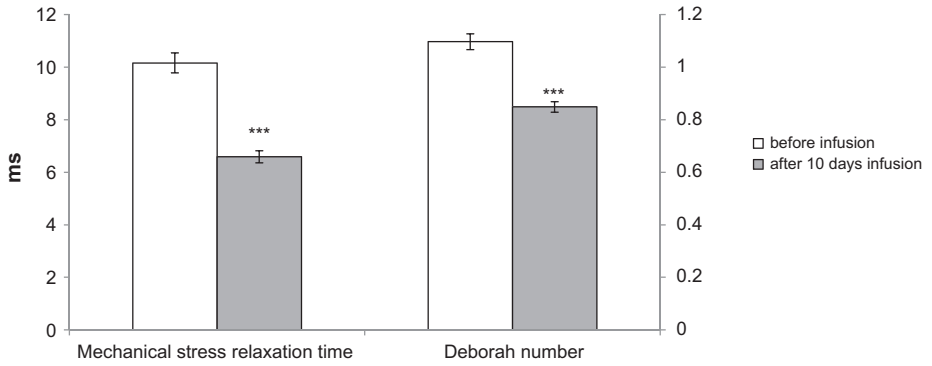

Fig 3. *Mechanical stress relaxation time and Deborah number before and after infusion. The asterisks indicate that* P < *.001 in comparison with the before infusion treatment.*

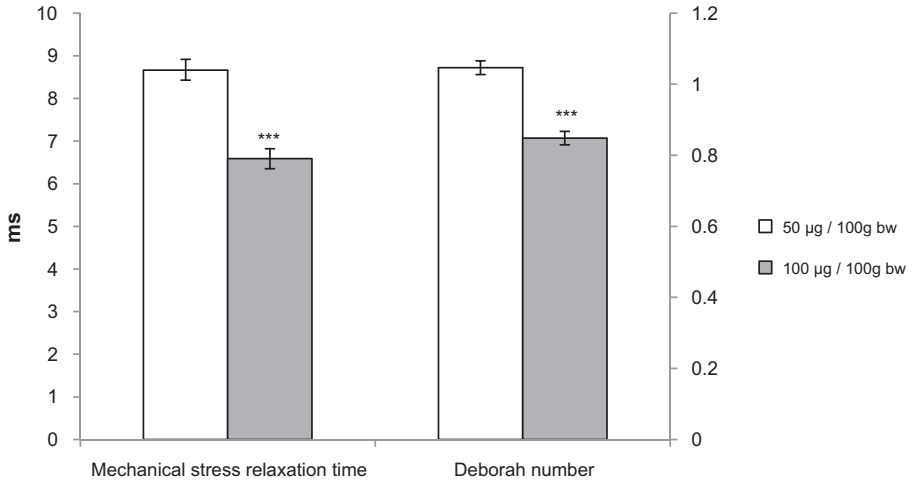

Fig 4. *Mechanical stress relaxation time and Deborah number under different dosages. The asterisks indicate that* P < *.001 in comparison with 50 µg/100 g BM.*

an accessible and quick method for measuring changes in muscle during glucocorticoid-induced muscle atrophy.

Limitations

Our results showed a dose-dependent difference in muscle biomechanical and viscoelastic properties in old rats. One limitation of this study is that we did not measure dose-dependent differences in muscle biomechanical and viscoelastic properties in young adult rats. The second limitation of the study is the small number of animals in the group of old rats. These factors can limit generalization of the results.

Conclusions

The results of the present study make a significant contribution to the literature. The biomechanical and viscoelastic properties of skeletal muscle indicate changes in the main function of muscle during glucocorticoid-induced muscle atrophy and are in agreement with changes in hind limb strength. Old rats are more sensitive to the dose of dexamethasone compared with young adult rats, as reflected in myometric measurements. The myometric measurement clearly and easily indicated the direction and magnitude of change in muscle tissue after different doses of dexamethasone administration.

Funding Sources and Conflicts of Interest

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Contributorship Information

Concept development (provided idea for the research): K.A., A.V., T.S.

Design (planned the methods to generate the results): P.K., A.P., P.P.
Supervision (provided oversight, responsible for organization and implementation, writing of the manuscript): A.V., T.S.
Data collection/processing (responsible for experiments, patient management, organization, or reporting data): K.A., A.V., M.A., A.P., P.P.
Analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): K.A., A.V., T.S., M.A., P.K., P.P
Literature search (performed the literature search): A.V., T.S.
Writing (responsible for writing a substantive part of the manuscript): T.S., K.A.
Critical review (revised manuscript for intellectual content, this does not relate to spelling and grammar checking): A.V., K.A., T.S.

Practical Applications

- Changes in muscle biomechanical and viscoelastic properties during the development of muscle atrophy reflect the changes in muscle contractile function.
- Myometric measurement of tone and biomechanical and viscoelastic properties is an accessible and quick method for measuring changes in the main function of muscle during the development of atrophy.

References

1. Goldberg A, Goodman H. Relationship between cortisone and muscle work in determining muscle size. *J Physiol.* 1969; 200(3):667-675.
2. Seene T. Turnover rate of skeletal muscle contractile proteins in glucocorticoid myopathy. *J Steroid Biochem Mol Biol.* 1994;50(1-2):1-4.
3. Seene T, Kaasik P, Pehme A, Alev K, Riso EM. The effect of glucocorticoids on the myosin heavy chain isoforms' turnover in skeletal muscle. *J Steroid Biochem Mol Biol.* 2003;86(2):201-206.
4. Attaix D, Mosoni L, Dardevet D, Combaret L, Patureau-Mirand P, Grizard J. Altered responses in skeletal muscle protein turnover during aging in anabolic and catabolic periods. *Biochem Cell Biol.* 2005;37(10):1962-1973.
5. Dardevet D, Sornet C, Taillandier D, Savary I, Attaix D, Grizard J. Sensitivity and protein turnover response to glucocorticoids are different in skeletal muscle from adult and old rats. Lack of regulation of the ubiquitin-proteasome proteolytic pathway in ageing. *J Clin Invest.* 1955;96(5):2113-2119.
6. Kaasik P, Umnova M, Pehme A, et al. Ageing and dexamethasone associated sarcopenia: peculiarities of regeneration. *J Steroid Biochem Mol Biol.* 2007;105(1-5):85-90.
7. Vain A. Estimation of the functional state of skeletal muscle. In: Veltink PH, Boom HBK, eds. *Control of Ambulation Using Functional Neuromuscular Stimulation*. Enschede, The Netherlands: University of Twente Press; 1995:51-55.
8. Vain A. Device and method for real-time measurement of parameters of mechanical stress state and biomechanical properties of soft biological tissue. World Intellectual Property Organization; 2012. 089221 A1.
9. Fung YC. Biomechanics. *Mechanical Properties of Living Tissues*. Berlin, Germany: Springer-Verlag; 1981:41.
10. Gregorová E, Pabst W, Štitina J. Rheology of ceramic suspensions with organic or bioorganic gelling additives - Part 1: Theory of linear viscoelasticity. *Ceramics-Silikáty.* 2004;48(3):93-99.
11. Seene T, Kaasik P, Riso EM. Review on aging, unloading and reloading: changes in skeletal muscle quantity and quality. *Arch Gerontol Geriatr.* 2012;54(2):374-380.
12. Kaasik P, Aru M, Alev K, Seene T. Aging and regenerative capacity of skeletal muscle in rats. *Curr Aging Sci.* 2012;5(2):126-130.
13. Jarocka E, Marusiak J, Kumorek M, Jaskolska J. Muscle stiffness at different force levels measured with two myometric devices. *Physiol Meas.* 2012;33(1):65-78.
14. Bizzini M, Mannion AF. Reliability of a new, hand-held device for assessing skeletal muscle stiffness. *Clin Biomech.* 2003;18(5):459-461.
15. Aru M, Alev K, Gapeyeva H, et al. Glucocorticoid-induced alterations in titin, nebulin, myosin heavy chain isoform content and viscoelastic properties of rat skeletal muscle. *Adv Biol Chem.* 2013;3:70-75.
16. Seene T, Umnova M, Kaasik P. The exercise myopathy. In: Lehman M, Foster C, Gastman U, Keizer H, Steinacker J, eds. *Overload, Performance Incompetence, and Regeneration in Sports*. New York, Boston, Dordrecht, London, Moscow: Kluwer Academic Publishers/Plenum Press; 1999:119-130.
17. Kaasik P, Umnova M, Alev K, Selart A, Seene T. Fine architectonics and protein turnover rate in myofibrils of glucocorticoid caused myopathic rats. *J Interdiscip Hist.* 2012;1:5-10.
18. Kimura K, Watanabe Y, Umeda M, Arima Y, Watsji T, Shinohara S. Quantitative analysis of the relation between soft tissue stiffness palpated from the body surface and tissue hemodynamics in human forearm. *Physiol Meas.* 2007; 28(12):1495-1505.