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

Karin Alev, PhD,[a] Arved Vain, PhD,[b] Maire Aru, MSc,[a] Ando Pehme, PhD,[a] Priit Purge, PhD,[a] Priit Kaasik, PhD,[a] and Teet Seene, PhD[a]

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

[a] Institute of Sport Sciences and Physiotherapy, University of Tartu, Tartu, Estonia.
[b] Institute of Physics, Univeristy of Tartu, Tartu, Estonia.
Corresponding author: Karin Alev, PhD, Ravila 14a, 50411 Tartu, Estonia. Tel.: + 372 7375364. (e-mail: *karin.alev@ut.ee*).
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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/