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Research article

The Application of Neuromuscular Electrical Stimulation Training in Conditions of Gravitational Unloading

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Abstract

The effect of a 7-day "Dry" Water Immersion (DI) with countermeasures (Neuromuscular Electrical Stimulation-NMES) on the function and architecture of the human triceps surae muscle was studied in six healthy young men subjects. During DI, subjects performed NMES 4 muscle groups of both lower extremities. NMES continued for six days, during which daily five days on end (from Monday to Friday inclusive) including one day of rest (Saturday). Architectural properties of the triceps surae muscles was measured *in vivo* by use of B-mode ultrasonography. The ankle was positioned at 15° dorsiflexion (-15°) and 0, +15, and +30° plantar flexion, with the knee set at 90°. At each position, longitudinal ultrasonic images of the Medial (MG) and Lateral (LG) Gastrocnemius and Soleus (SOL) muscles were obtained while the subject was relaxed and performed 50% from maximal voluntary isometric plantarflexion, from which lengths and angles of fascicles with respect to the aponeuroses were determined. After DI with by NMES maximal plantar flexion torque increased by 11%. In the passive condition, fascicle lengths decreased by 16%, 37%, and 24%; pennation angle increased by 38%, 35%, and 34% for MG, LG, and SOL, respectively. Decreases in muscle thicknesses in leg muscles were not prevented by the present exercise protocol, suggesting a need for specific exercise training for these muscles. Trained muscles showed significant changes in pennation angles and fibre length after DI with by NMES, suggesting that muscle architecture does change remarkably by muscle atrophy. These findings suggest that rapid muscle architecture remodeling occurs in lower limb in humans, with changes occurring within of unloading of the musculoskeletal system. These adaptations might protect from a larger loss of muscle force.

Keywords: "Dry" water immersion; Neuromuscular electrical stimulation; Ultrasonography; Muscle architecture; Triceps surae muscle; Muscle contraction

INTRODUCTION

A number of studies have indicated that sudden exposure to microgravity environment causes a decrease in the tone/stiffness of the skeletal muscles [1-4], reduction of muscle volume and muscle strength [5-9], perceptual and coordination disorders in the neuromuscular systems [6,10-12], shift of the spinal reflex mechanisms [5,13], and degradation of joint position sense [14]. There are alterations in muscle activity, proprioception and posture [15], disc hyperhydration associated with fluid shifts [16] and cardiovascular changes [17].

It is accepted that the major factor responsible for all of these changes is the sudden elimination of the proprioceptive information from the muscle and tendon in response to absence of load-bearing. Gravitational loading appears to be necessary for the maintenance of human lower limb skeletal muscle size and force [18,19]. Studies simulating microgravity have shown that exercise countermeasures can attenuate, but not completely prevent the loss of muscle mass and force [18,19]. The muscle groups most affected by exposure to microgravity appear to be the antigravity extensors of the knee and ankle [20]. Among these, the plantarflexors seem to be the most affected [21], likely due to their greater mechanical loading under normal gravitational conditions. Most notable after exposure to microgravity is a disproportionate loss of force as compared to that of muscle size [21,22], indicating that factors other than atrophy contribute to muscle weakness. The internal architecture of a muscle is an important determinant of its functional characteristics (force-velocity relationships, force-length, and maximum isometric force [23,24].

The purpose of the present study was to investigate the internal architecture of the triceps surae [Medial (MG) and Lateral (LG) Gastrocnemius and Soleus (SOL) muscles] in relation to the functional characteristics of the plantarflexors after 7 days of "Dry" Water Immersion (DI) with exercise countermeasures [term-long low-frequency Neuromuscular Electrical Stimulation (NMES)].

METHODS

Subjects

The subjects in this study were six healthy men-volunteers. Their average age, height, body mass were 22.8 ± 0.8 years, 1.84 ± 0.1 m, and 79.3 ± 4.2 kg, respectively. Selection of subjects was based on a screening evaluation that consisted of a detailed medical history, physical examination, complete blood count, urinalysis, resting and cycle ergometer electrocardiogram, and a panel of blood chemistry analysis, which included fasting blood glucose, blood urea nitrogen, creatinine, lactic dehydrogenase, bilirubin, uric acid, and cholesterol. All of the subjects were evaluated clinically and considered to be in good physical condition. No subject was taking medication at the time of the study, and all subjects were nonsmokers. Each subject served as her own control. Each subject performed two sets of experiments 2-1 days before DI (baseline data collections) and immediately after DI.

None of them had a habit of exercise on a regular basis. Prior to the experiment, details and possible risks of the protocols were explained to the subjects, and written informed consent was obtained from each of them. The experimental protocol was approved by the Russian National Committee on Bioethics of the Russian Academy of Sciences and was in compliance with the principles set forth in the Declaration of Helsinki.

"Dry" water immersion

DI was used to simulate microgravity as described by Shulzhenko and Vil-Villiams [25]. Each subject was positioned horizontally in a special bath on fabric film that separated him from the water (Figure 1). During DI, the subjects remained in a horizontal position (angle which make the body and horizontal line, e.g. 5° head-up position) continuously for all including excretory function and eating. The water temperature was constant (33.4°C) and maintained automatically at this level throughout the experiment. The duration of the DI was 7 days. A nursing staff was present for subjects transportation, maintenance of hygiene including toilet and shower, provision of food and medical care, as well as support of subjects needs within the constraints of the protocol. The subjects were supervised 24 $h \cdot d^{-1}$.

Neuromuscular electrical stimulation

Principle of training: NMES is applied to 4 muscle groups of both lower extremities. After careful preparation of the skin, two "dry" electrodes (Ltd. «*Axelgaard*», USA) were placed on the skin above the quadriceps femoris muscles, the hamstrings, the tibialis anterior, the perinea, and the triceps surae muscles. The synchronous stimulation of antagonistic muscle groups prevents unwanted joint movements.

The electrical stimulus was provided by the «STIMUL LF-1» stimulator (Russia). The technical equipment consists of electrode trousers carrying stimulation electrodes for the 12-channels, and 2 interconnected 6-channel stimulators carried on a belt.

Subjects were carefully instructed in the use of the stimulator and the placement of the electrodes. During DI, subjects executed a NMES «training» during 3 hours per day with 1 s « on » and 2 s « off » and a frequency of 25 Hz and amplitude of stimulus from 0 up to 45 V. The intensity of the stimulation was adjusted according to patient tolerance. NMES «training» of muscles of the examinee was carried out directly in a bath (Figure 1). Dynamics of changes stimulus pulse of everyone subjects during daily training shown (Figure 2).

Ultrasound scanning

Joint position settings and torque measurement: Each subject's right foot was firmly attached to an isokinetic dynamometer («Biodex», USA), and the lower leg was fixed to a test bench. The ankle joint was fixed at 15° deg dorsiflexion (-15°) and 0°, +15°, and +30° plantar flexion. The knee joint was positioned at ~ 90. Thus the following measurements were performed in 4 conditions. In each condition, the subject was asked to relax the plantar flexor muscles (passive condition), and passive plantar flexion torque was recorded from the output of the dynamometer by a PC computer. After performance in the passive condition, the subject was encouraged to perform maximal voluntary isometric plantar flexion (active condition), and torque output was recorded (isometric maximal voluntary contraction – MVC). Each subject was then asked to maintain the stronger leg contractions for at least 2-3 s at 50% of MVC at the neutral ankle position (0°). Subjects were given visual feedback of the target and elicited force on a computer screen.

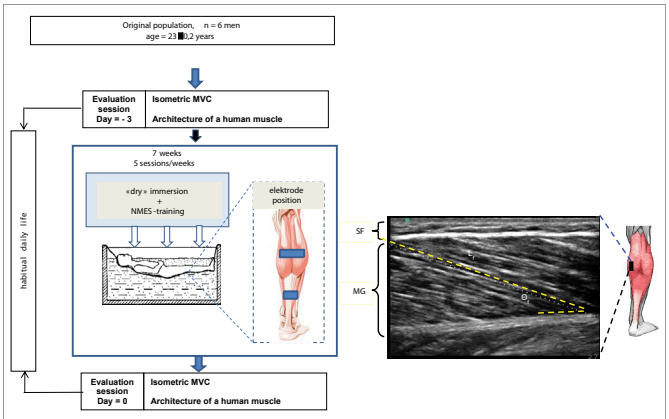

Figure 1: Experimental design
All of the enrolled participants were older than 20 years and have never used NMES. SF - subcutaneous fat layer; MG – Medial Gastrocnemius muscle

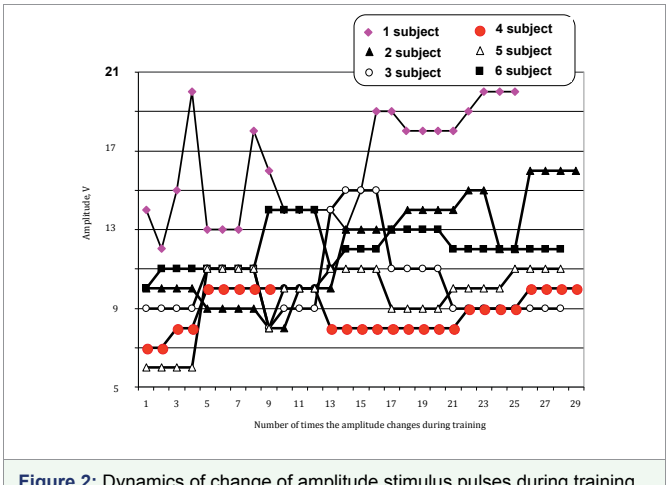

Figure 2: Dynamics of change of amplitude stimulus pulses during training.

Measurement of lengths, and angles of fascicles, and thickness muscle: Fascicular lengths and pennation angles of human triceps surae muscles were measured *in vivo* from sonography taken during rest (passive) and active (contracting) conditions. A real-time B-mode ultrasound apparatus («SonoSite MicroMaxx», USA) with a 7·5 MHz linear-array probe, and length of a scanning surface 60 mm was used to obtain sagittal images of the MG, LG and SOL at rest and at 50% of plantarflexor MVC (active) at the neutral ankle position. In each position, longitudinal ultrasonic images of the MG, and LG, and SOL were obtained at the proximal levels 30% (MG and LG) and 50% (SOL) of the distance between the popliteal crease and the center of the lateral malleolus. Each level is where the anatomic cross-sectional area of the respective muscle is maximal [26]. At rest and during contractions, the probe was firmly held against the skin at the same site over the muscle belly.

The scanning head of the probe was coated with transmission gel to obtain acoustic coupling, and oriented along the mid-sagittal axis of each muscle. Sonographs were taken after having adjusted the depth gain compensation to optimize image quality.

The fascicle pennation angle (Θ_f) was measured from the angles between the echo of the deep aponeurosis of each muscle and interspaces among the fascicles of that muscle [27] (Figure 1).

The length of fascicles (L_f) across the deep and superficial aponeurosis was measured as a straight line [28] (Figure 1).

Shorter fascicle lengths and steeper fascicle angles in the active compared with the rest (passive) conditions show internal shortening of fascicles by contraction (ΔL_{muscle}).

The ΔL_{muscle} [29] was estimated by the following formula, i.e. $\Delta L_{muscle} = L_r \cdot \cos \Theta_r - L_s \cdot \cos \Theta_s$

Where, L_r and L_s - are fascicle lengths in rest (passive) and active conditions (strength 50% MVC); Θ_r and Θ_s — are fascicle angles in rest (passive) and active conditions, respectively.

The distance between aponeuroses (muscle thickness) was estimated from the fascicle length and pennation angle using the following equation:

muscle thickness = L_f x sin α

where L_f, and α is the pennation angle of each muscle determined by ultrasound.

In the present study, ultrasonic measurement was repeated three times for each subject and averaged values were used. The coefficients of variation of three measurements were in the range of 0-2%. All ultrasonic images were processed with use of the software package Dr. ReallyVision (Ltd. «Alliance – Holding», Russia).

Statistics

Data are presented as the mean values ± standard error (± SE) of the mean. Differences in pennation angles, fibre lengths and thicknesses between rest and 50% MVC and between different ankle angles were tested using two-way analysis of variance tests. Tukey's

test was used to determine significant difference between mean values. One-Way Analysis Of Variance (ANOVA) was used for comparison of muscle thickness, pennation angles, and fibre lengths. A level of $p < 0.05$ was selected to indicate statistical significance.

RESULTS

After DI with NMES maximal plantar flexion torque were significantly higher with respect to baseline by 11.3% ($p < 0.05$).

Figure 2 shows average L_f, and Θ_f of MG, LG, and SOL. L_f were longest when the ankle joint angle was -15°. After DI, in the passive condition, L_f in the MG, and LG, and SOL has decreased for 12 (from 32 ± 2 to 28 ± 1 mm), 13 (from 36 ± 2 to 31 ± 2 mm), and 13% (from 36 ± 3 to 32 ± 2 mm) but in the active condition by 18 (from 26 ± 3 to 22 ± 2 mm), 22 (from 36 ± 3 to 28 ± 2 mm), and 21 % (from 32 ± 2 to 26 ± 2 mm), respectively (Figure 3).

The degree of fascicle length change was not identical for the three muscles. The Θ_f, in the passive condition, was decreased by 22, 20 and 16%; but in the active condition by 17, 22 and 17%, respectively (Figure 3).

Shorter fascicle lengths and steeper fascicle angles in the active compared with the passive condition show internal shortening of fascicles by contraction. Before DI ΔL_{muscle} the MG has found 7.9 mm after has decreased and has made 7.8 mm, and in SOL 5.9 *vs* 5.6 mm. The ΔL_{muscle} increase from 0.9 to 3.3 mm ($p < 0.05$) were found by LG.

DISCUSSION

This study describes, for the first time, the architecture of the human triceps surae [MG, and LG, and SOL] *in vivo,* both at rest and during graded (50% MVC) isometric plantar flexions. The results obtained *in vivo* indicate that human MG,

LG, and SOL architecture drastically changes both as a function of ankle joint angle at rest and as a function of the force developed during isometric contractions at a fixed joint angle. At rest, when changing the ankle joint angle from -15° to +30°, SOL – from 22.8° to 34°; fibre length decreased from 35.5 mm to 26.8 mm, LG – from 46.8 mm to 31.2 mm, and SOL – from 39.2 mm to 28.2 mm. These results indicate that fibre length and pennation angle of the human triceps surae cannot be assumed to remain constant with changing muscle length [30]. The decrease in fibre length and increase in pennation angle with increasing muscle length may be ascribed the taking up of the slack characterizing these structures [30]. In the present study, the decrease in fibre length occurring from -15° to +30° of passive plantar flexion also suggests that muscle fibre became progressively slack with increasing ankle joint angles. The major findings of this study were that, after 7 day DI with of NMES «training», isometric maximal voluntary torque by the plantar flexor muscles increased. Previous studies have documented decrease of the contractile properties of skeletal muscles during DI [6-8,31]. The present exercise training resulted small increased (~ 11%) in maximal voluntary plantar flexion torque in the triceps surae muscle what is antigravitational muscle whereas absence of preventive actions results in decrease in MVC more than on 50% [6-8,31,32] and in P_o more than on 30% [7,19,31,32].

Efficacy of NMES for increased the contractile properties of skeletal muscles has been suggested in previous studies [33-35]. The insignificant increase in force of contraction in the present study can be assumed it is defined by slack intensity impulses.

It is well known that the smaller motoneurons innervating muscles are more readily activated than the larger cells innervating units [36,37], as the strength of the contraction increases progressively. The smaller units consist of slow twitch muscle fibres (type I) and the larger units consist of fast twitch fibres (type II). In submaximal voluntary contractions, type I fibres the motor units are activated by the synaptic current impinging on the motor neuron. The situation is completely different in contractions triggered by NMES, because the muscle fibres of the motor units are activated by an electric current which is applied extracellularly to the nerve endings, and larger cells with lower axonal input resistance are more excitable [38,39]. In fact, when the stimulus is applied from outside the cell, the electric current

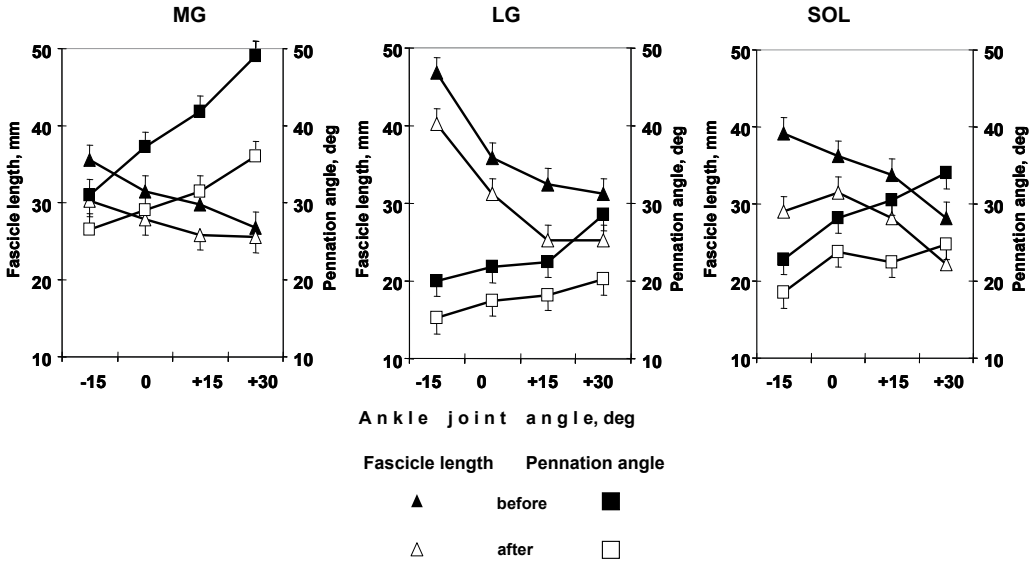

Figure 3: Changes in the triceps surae complex architecture. Medial (MG), and Lateral (LG) Gastrocnemius, and Soleus (SOL) muscles fascicle Length (L) and pennation ankle (Q) as a function of changes joint ankle at rest. Values presented are means ± SD (n = 5).

must first enter through the membrane before it depolarises the cell, but the extracellular medium shunts the current, and the smaller motor units will not be activated during submaximal NMES because of their higher axonal input resistance. Therefore, the smaller motor units do not adapt to training with submaximal NMES. However when use electrical stimulation high training intensity, larger force NMES to be more efficient exercise [40].

Internal architecture of the GM, LG, and SOL muscle was altered and this was only partially prevented by exercise countermeasures. Both fascicle length and pennation angle were reduced after DI with NMES, this strongly suggests a loss of both in-series and in-parallel sarcomeres, respectively. The functional consequence of the decreased fascicle length was a reduced shortening during contraction. The loss of in-series sarcomeres would mean that this is likely to have implications both on the force-length and force-velocity relationships of the muscle. The observation of a smaller pennation angle during contraction after DI with NMES will partially compensate for the loss of force, because of a more efficient force transmission to the tendon. The reduced initial resting pennation angle probably, grows out reduction decreased tendon stiffness or of the muscle-tendon complex that finds confirmation in substantial growth ΔL_{muscle} of LG (with 0.9 up to 3.3 mm after DI) during contraction. This observation is consistent with the findings of Kubo et al. [41]. In conclusion, NMES-training was partially successful in mitigating the loss of function and architecture induced by prolonged DI. Apparently, by ascending during NMES a flow muscular afferentation [42].

In summary, from the present results, follows, first, that the architecture different lead the triceps surae muscle considerably differs, reflecting, probably, their functional roles, second, various changes fibre length and pennation angle between different muscles, probably, are connected to distinctions in ability to develop force and elastic characteristics of sinews or muscle-tendon complex and, at last, in the third, NMES has preventive an effect on stimulated muscles: in part reduces loss of force of reduction of the muscles, the caused long unloading. The received data, allow concluding, that use of NMES renders the expressed preventive action, essentially reduces depth and rate of atrophic processes in muscles.

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