

# Influence of brief daily tendon vibration on rat soleus muscle in non-weight-bearing situation

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**Falempin, Maurice, and Soumeya Fodili In-Albon.** Influence of brief daily tendon vibration on rat soleus muscle in non-weight-bearing situation. *J. Appl. Physiol.* 87(1): 3–9, 1999.—The purpose of this study was to investigate whether tendon vibration could prevent soleus muscle atrophy during hindlimb unloading (HU). Mechanical vibrations with a constant low amplitude (0.3 mm) were applied (192 s/day) with constant frequency (120 Hz) to the Achilles tendon of the unloaded muscle during the 14-day HU period. Significant reductions in muscle mass (–41%), fiber size, maximal twitch (–54%), and tetanic tensions (–73%) as well as changes in fiber type and electrophoretic profiles and twitch-time parameters (–31% in the contraction time and –30% in the half relaxation time) were found after 14 days of HU when compared with the control soleus. Tendon vibration applied during HU significantly attenuated, but did not prevent, 1) the loss of muscle mass (17 vs. 41%); 2) the decrease in the fiber cross-sectional area of type IIA (–28 vs. –50%) and type IIC (–29 vs. –56%) fibers; and 3) the decrease in maximal twitch (–3 vs. –54%) and maximal tetanic tensions (–29 vs. –73%) and the half relaxation time (1 vs. –30%). Changes in the contraction time and in histological and electrophoretic parameters associated with HU were not counteracted. These findings suggest that tendon vibration can be used as a paradigm to counteract the atrophic process observed after HU.

hindlimb unloading; muscular atrophy; countermeasure

IT IS WELL KNOWN that simulated weightlessness when the hindlimb-unloading animal model is used (29, 30) produces structural and functional deficits in skeletal muscles comparable to those described after a period of real microgravity during spaceflight (12, 39). The magnitudes of these alterations (muscular atrophy, decreases in muscle strength and twitch contraction time, modifications in fiber type and electrophoretic profiles) are specific to the muscle and fiber type. These changes are greatest in the ankle extensors, such as the soleus muscle, that normally perform antigravity postural functions (12, 39). Several different training programs have been tested for their ability to reverse or prevent muscle atrophy during unloading (11, 19, 23, 36, 40). However, no exercise countermeasure has completely prevented soleus muscle atrophy during unloading.

Muscle spindle primary endings (type Ia fibers) are sensitive to tendon vibration (6, 7, 27, 28, 35). Moreover, when vibrations are applied during passive stretching of the soleus muscle, the response of the primary

sensory endings is increased. In contrast, this response decreases when vibrations are applied during muscle shortening (6). We hypothesized that stimulation of the muscle spindle primary endings via combined vibration and passive stretching movements of the rat soleus muscle during hindlimb unloading transiently provides a reflex increase in electromyographic (EMG) and contractile activity. The objectives of this study were thus to 1) test the effects of tendon vibration on the morphological, mechanical, and histological properties of the unloaded rat soleus muscle; and 2) demonstrate the practicality of using tendon vibration as a countermeasure for preventing atrophy and loss of muscle function in microgravity.

## MATERIALS AND METHODS

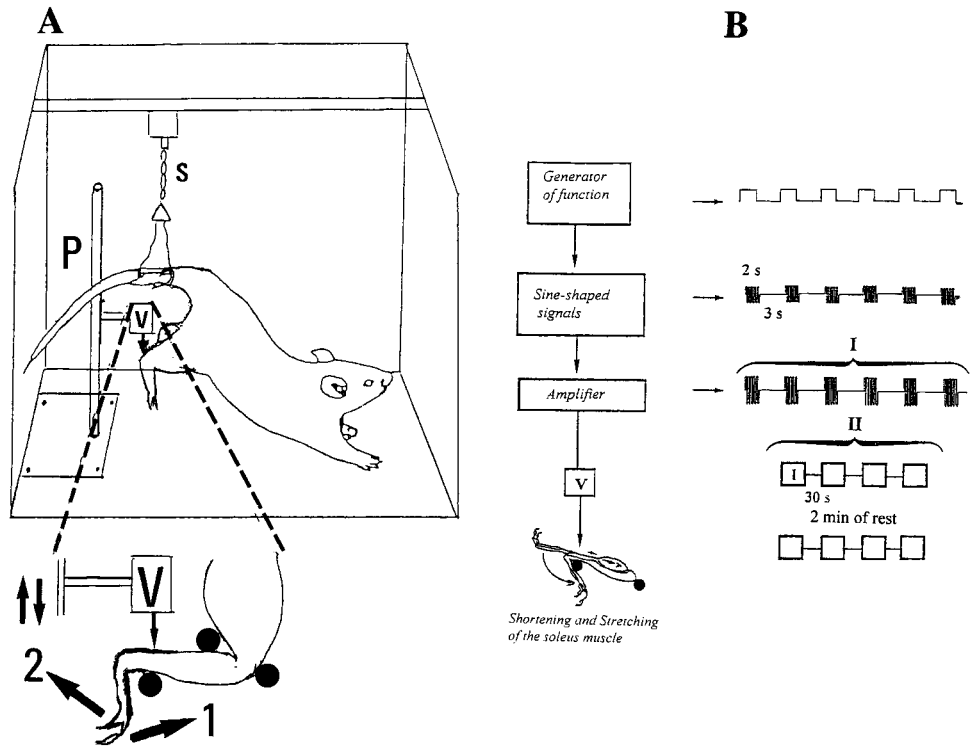
**Animal groups and maintenance.** Twenty-four male Wistar rats weighing 240–250 g at the beginning of the experiments were divided randomly into three groups: control (Con;  $n = 7$ ), hindlimb unweighted (HU;  $n = 7$ ), and hindlimb unweighted submitted to daily intermittent tendon vibration (HU-TV;  $n = 10$ ). The rats were housed individually in conventional plastic cages and had access to pellet food and water ad libitum. The rats were acclimatized at a 23°C room temperature and with a 12:12-h light-dark cycle for 1 wk before they were used in the experiments. The maintenance conditions of the animals and the experiments received authorization from both the Agricultural and Forest Ministry and the National Education Ministry (Veterinary Service of Health and Animal Protection; authorization 03805).

**Hindlimb-unloading protocol.** Both unloaded groups (HU and HU-TV) were subjected to a suspension period of 14 days. The suspension model used was the tail-suspension model introduced by Morey (29). Briefly, the tail of each rat was washed, cleaned, dried, and wrapped in an antiallergic orthopedic tape-adhesive plaster. This cast, covering less than half of the tail, was secured to an overhead swivel, mounted at the top of the cage and permitting free 360° rotation of the animals. The rats were unloaded at ~30° head-down angle to mimic fluid shifts characteristic of weightlessness.

**Tendon vibration and movement sequence paradigm.** The apparatus used to induce tendon vibration and the protocol of vibrations are illustrated in Fig. 1, A–B. Vibrations were applied to the Achilles tendon of the right hindlimb. For application of the vibrations, anesthetized rats (80 µg ketamine and 8 µg acepromazine/g body wt) of the HU-TV group were placed in a custom-built system, which allowed the maintenance of the unweighted position but kept the animal's body and the knee of the right hindlimb in a firm position while leaving the ankle free to traverse its normal range of motions (plantar flexion at 160–180° to dorsiflexion at 30–35°). Mechanical vibrations were applied perpendicularly to the Achilles tendon of the right hindlimb by means of a Teflon probe (3 mm in diameter) connected to the moving coil of an electromechanical vibrator (Bruel and Kjaer minishaker 4810). The electromagnetic device was fixed in an indepen-

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Fig. 1. *A*: model of tendon vibration application in rat soleus muscle during hindlimb suspension. Soleus reflex contractions were obtained by application of vibration on Achilles tendon by a vibrator (V) with a 120-Hz frequency during stretching of soleus muscle. Stretching (→1) was performed by manually advancing right ankle through its full range of motion (plantar flexion at 160–180° to dorsiflexion at 30–35°) (2←, shortening movements). *B*: sequence paradigm of tendon vibration. Sine-shaped signal generator was coupled with a power amplifier. After amplification, 4 series of 6 periods of vibration were applied during stretching (→1). Three-second intervals separated 2-s periods of vibration, and 2 min of rest separated 2 sessions of vibrations. P, holder used to maintain vibrator; s, swivel for fixation of the rat; ↑↓, movements for application of vibrator; ●, metallic device to maintain the knee or to allow passive movements of the ankle.



dent holder (Fig. 1A; P). The vibrating device was driven by a sinusoidal signal generator (ITT Instruments,  $6 \times 239$ , Metrix, France) coupled with a power amplifier. On the basis of data collected in animals (27, 28) and humans (6, 35), relating to muscle spindle sensitivity to vibration, the amplitude of the vibrations was 0.3 mm peak to peak. The frequency of the vibrations was 120 Hz. The right ankle joint was centered on the axis of rotation of a metallic device fixed on the customized system (Fig. 1A). In this position, the ankle joint could be subjected to passive plantar flexion and dorsiflexion movements. These movements were performed by the experimenter. Tendon vibration was applied when the soleus muscle was being stretched.

During the 14 days of unloading, two sessions of 48 s of vibrations (Fig. 1B; diagram I) associated with stretching, separated by 2 min of rest, were carried out every day (Fig. 1B). Four series of six 2-s periods of vibration (Fig. 1B; diagram I) were applied during stretching at 3-s intervals. After each series, the soleus muscles were maintained in a shortened position. For the HU-TV group, we used this protocol twice a day, the first application in the morning (11 AM) and the second in the afternoon (5 PM).

Three rats, as a sham group, were hindlimb unloaded and submitted to daily stretching without vibration in the same experimental conditions as were the rats of the HU-TV group. After 14 days, the morphological, contractile, and histochemical properties as well as electrophoretic profiles of this sham group were similar to those of the HU group.

**Isometric contractile properties.** For the final experiments, the right soleus muscle was surgically prepared for measurement of isometric contractile responses to sciatic nerve stimulation *in situ*. In pentobarbital sodium-anesthetized rats (35 mg/kg ip), the sciatic nerve and the distal end of the soleus muscle were isolated from the hamstrings and surrounding tissue. Care was taken to avoid damage to the blood supply. The right hindlimb was immersed in a bath filled with mammalian Ringer solution, oxygenated by a 95%  $O_2$ -5%  $CO_2$  mixture, and kept at  $37 \pm 0.5^\circ C$ . The body temperature of the

rat was regulated at  $\sim 37.5^\circ C$  by using radiant heat. The limb was stabilized by using a combination of pins, clamps, and bars so that the muscle was tested in a horizontal position. The thigh and the leg were at a  $120^\circ$  angle. The leg and the foot were at a  $90^\circ$  angle. A short 3–0 nylon ligature connected the severed distal muscle tendon to a transducer (ENTRAN, model ELP 1200, 0.393 mV/g, band pass 0–3 kHz, Les Clayes sous Bois, France).

Contractions of the soleus muscle were elicited by stimulation of the severed sciatic nerve via two platinum electrodes using pulses of 0.1-ms duration and supramaximal voltage (Grass S88, Quincy, MA). Muscle force was 1) displayed on a thermal recorder (Ankersmit, WR 7900, Graphtec Yokohama, Japan), 2) sampled (Axon Instrument 125 kHz Labmaster Dema TL-1–125 Interface, Foster City, CA), and 3) analyzed online with a computer (Zephyr 386, 25 MHz, Villeneuve d'Ascq, France). Isometric tension parameters included the maximal twitch ( $P_t$ ) and tetanic ( $P_o$ ) force. Two parameters were used to characterize the speed-related properties: 1) twitch contraction time ( $t_c$ ) and 2) the half relaxation time ( $RT_{1/2}$ ) from the maximal twitch.

At the end of the contractile testings, the rats received an overdose of pentobarbital sodium, and the soleus muscle was quickly removed and weighed (muscle wet weight).

**EMG activity.** To draw conclusions about changes in activity levels, the soleus EMG activity was recorded in four animals of the HU-TV group. Compared with the other six animals in this group, no obvious variations in the morphological and mechanical properties were observed for the four implanted animals. This indicated that the presence of electrodes did not induce tissue damage and did not alter soleus muscle changes due to unloading. The 10 rats in the HU-TV group thus constituted a homogeneous group. The four rats were anesthetized via an injection of pentobarbital sodium (35 mg/kg ip) and were prepared for implantation of bipolar electrodes (7 Strands, A. M. System) under sterile conditions. The soleus muscle of the right hindlimb was exposed after a

1-cm incision had been made on the lateral surface of the limb.

The electrode wires were led subcutaneously from a skin incision low on the back at the base of the tail to the soleus, with two loops positioned as follows: one at the upper proximal end of the soleus, near the knee joint, and the other on the sacral region, to avoid direct pulling on the muscle while the rats moved. The common ground wire was placed in the back musculature. The midbelly of the muscle was exposed, and two electrodes (with a 0.5-mm portion of the Teflon insulation removed) were inserted in each muscle with a 23-gauge 1.5-in. hypodermic needle. The spacing between the wires was ~1.5 mm and was checked under a dissecting microscope. The effectiveness of the implant was tested by back-stimulating the soleus muscle through the electrode wires (0.1-ms square wave, delivered by Grass S88). After this verification, the recording surface of each electrode was secured in the muscle belly with a suture at its entry and exit from the muscle. The ends of the wires (distal parts) were also tied with a suture. All the incisions were closed, and an antiseptic (Betadine) was applied to the incision areas. After surgery, the animals were placed in a cage to recover. At the conclusion of the experiment, the position of each electrode was checked and was found to be as originally placed.

Recordings began 3 days after surgery, during the light cycle at 11 AM and 5 PM. The purpose of these experiments was not to study the muscle activity level in the soleus during hindlimb unloading, since it has been very well described in the literature (1, 3, 34). The EMG activity was recorded only to determine the effectiveness of the tendon vibration protocol. The following experimental time line was used. On *day 1* before hindlimb unloading, two sessions of forty-eight 2-s periods of EMG recordings, separated by 2 min of rest, were carried out when the rats were in a quiet quadrupedal standing position in the cage. On *day 1* and *day 10* during the hindlimb-unloading period, the recording scheme was as follows: two sessions of forty-eight 2-s periods of EMG recordings (interval between each period: 3 s), separated by 2 min of rest when the rats were in a quiet position in the hindlimb-unloading situation (i.e., without hindlimb pedaling); two sessions of forty-eight 2-s periods of EMG recordings, separated by 2 min of rest during stretching without vibration; and two sessions of forty-eight 2-s periods of EMG recordings, separated by 2 min of rest during stretching plus vibrations. A period of 2-min rest separated each of these three recording sessions. EMG activity was analyzed for each rat and for each recording day. The EMG signals were digitized at 2,000 Hz into a 486 processor-based personal computer. The burst selection was determined with an interactive software (Spike 2, Cambridge Electronic Design, UK). The analysis program located EMG burst onset and offset times by applying user-determined criteria for amplitude threshold and burst duration. The rectified EMG signal was integrated over that period. The mean EMG (mEMG) for a burst was obtained by dividing the integrated area of a burst by its duration. Each value was finally expressed per minute (mV/min). The mEMG was calculated and averaged for all four implanted rats in the HU-TV group. On *day 1* before hindlimb unloading, the soleus muscles of these rats were tonically active during the recording session. The mEMG was  $3.2 \pm 0.3$  mV/min. On *day 1* of the hindlimb-unloading period, mEMG was  $0.40 \pm 0.06$  mV/min when the rats were not moving. During stretching of the soleus muscles (HU-ST), mEMG increased and was on average  $10.8 \pm 0.08$  mV/min. On the contrary, when vibration plus stretching (HU-TV) were applied, mEMG average value was  $52.7 \pm 0.6$  mV/min. Values obtained on

*day 1* and *day 10* during the hindlimb-unloading period were the same ( $P > 0.05$ ).

**Histochemical and morphological analyses.** After the contractile measurements, the soleus muscles of the Con, HU, and HU-TV groups were frozen at normal length in isopentane, precooled to its freezing point by liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until further analysis. At the midbelly, the muscle was cut perpendicularly to its longitudinal axis into serial 10- $\mu\text{m}$ -thick cross sections in a cryostat microtome at  $-30^{\circ}\text{C}$ . The muscular fiber types were classified according to the terminology of Brooke and Kaiser (5). Briefly, sections were stained for myofibrillar ATPase after acid (pH 4.35, pH 4.6) or alkaline (pH 10.4) preincubation, by using the method of Padykula and Hermann (31), modified by Guth and Samaha (16). This histochemical method allowed the distinction between three types of soleus fibers (I, IIA, and IIC). With the myosin ATPase reaction, intermediate type IIC fibers displayed an intermediate behavior to pH sensitivity between type I and IIA fibers. Fiber-type composition was determined by evaluating 250 fibers in each section in all the muscles of the Con, HU, and HU-TV groups. Distribution was expressed as the number of fibers of each type relative to the total number of fibers. The cross-sectional area of each type of fiber (FCSA) was measured by using an image processor (Samba 2500, Alcatel, Grenoble, France).

**Electrophoretic analysis of myosin heavy chain (MHC) isoforms.** Myosin electrophoresis was performed according to the method described by Carraro and Catani (9). Muscles were cut perpendicularly to their longitudinal axis into 10- $\mu\text{m}$ -thick cross sections in the same microtome cryostat. These sections were obtained through the midbelly close to the sections taken for ATPase staining. They were solubilized in 100  $\mu\text{l}$  of SDS sample buffer [containing 62.5 mM Tris (pH 6.8), 1% SDS, 15% glycerol, and 5%  $\beta$ -mercaptoethanol]. For the majority of the experiments, 3  $\mu\text{l}$  of the sample buffer were loaded on gels consisting of a 3% (wt/vol) acrylamide stacking gel and a 6% (wt/vol) separating gel (6). The migration buffer contained 32.5 mM Tris, 288 mM glycine, and 0.1% SDS (wt/vol). Gels were run at constant voltage (250 V) for 6 h and then stained with Coomassie blue, as described by Giulian et al. (15). Laser scanning densitometry (Quantiscan, Microvial Systems, Biosoft, Cambridge, UK) was used to determine the relative proportion of the different types of MHC isoforms in each muscle section. All sections expressed type I and fast type MHC isoforms. However, the electrophoretic technique used in this study did not allow the consistent separation of the so-called fast MHC-IIX type from the fast MHC-IIA type, as the two proteins comigrated; the upper band was therefore called MHC-IIA-IIX. Cryostat sections of an extensor digitorum longus muscle were used as an indicator for the electrophoretic motility of the fast-twitch myosin isoforms called IIA-IIX and IIB.

**Statistical analysis.** Results are presented as means  $\pm$  SE. Significant differences between the three experimental groups, i.e., Con vs. HU, Con vs. HU-TV, and HU vs. HU-TV, were determined with a one-way analysis of variance and a Bonferroni *t*-test applied as a post hoc test. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

**Morphological properties.** After the 14-day period of hindlimb unloading, no significant difference was observed between the body weights of HU, HU-TV, and Con rats (Table 1). The mean absolute and relative soleus wet weights were significantly lower ( $-41$  and  $-40\%$ , respectively) in the HU than in the Con group.



Table 1. *Morphological properties of soleus muscle from control rats and from hindlimb-unloaded rats without or with tendon vibration*

	Con ( <i>n</i> = 7)	HU ( <i>n</i> = 7)	%ΔCon	HU-TV ( <i>n</i> = 10)	%ΔCon	%Atrophy Prevented
BW, g	256 ± 12	255 ± 5		248 ± 10		
MWW, mg	126.6 ± 5.2	74.2 ± 4.8*	-41	104.6 ± 3.6*†	-17	+58
MWW/BW, mg/g	0.49 ± 0.04	0.29 ± 0.02*	-40	0.44 ± 0.03†	-10	+75
FCSA I, μm <sup>2</sup>	2,402 ± 115 ( <i>x</i> = 1,470)	1,023 ± 121* ( <i>x</i> = 1,260)	-57	1,384 ± 190* ( <i>x</i> = 1,750)	-42	+28
FCSA IIA, μm <sup>2</sup>	1,604 ± 62 ( <i>x</i> = 155)	795 ± 81* ( <i>x</i> = 260)	-50	1,150 ± 104*† ( <i>x</i> = 330)	-28	+43
FCSA IIC, μm <sup>2</sup>	2,036 ± 102 ( <i>x</i> = 140)	889 ± 54* ( <i>x</i> = 220)	-56	1,442 ± 145*† ( <i>x</i> = 410)	-29	+48

Values are means ± SE; *n*, no. of rats per group. Con, control group; HU, hindlimb-unloaded group; HU-TV, HU rats with tendon vibration; BW, body weight; MWW, muscle wet weight; FCSA, fiber cross-sectional area; %ΔCon, %change from control; %atrophy prevented = [(countermeasure group - HU group)/(Con group - HU group)]; *x*, no. of cells in each of the fiber type groups. \*Significant difference ( $P < 0.05$ ) between Con and HU or HU-TV groups; †significant difference ( $P < 0.05$ ) between HU and HU-TV groups.

Tendon vibration during hindlimb unloading produced significantly greater absolute and relative wet weights in the HU-TV group than in the hindlimb-unloaded muscles. However, our data showed that tendon vibration did not completely counteract the soleus muscle atrophy. A significant decrease in the FCSA of each kind of fiber type (-57% for type I, -50% for type IIA, and -56% for type IIC fibers) was observed after hindlimb-unloading conditions. Tendon vibration (HU-TV group) significantly limited the effects of hindlimb unloading in the FCSA of type IIA and IIC fibers. The decreases in the FCSA were 28% (type IIA) and 29% (type IIC), respectively.

**Contractile properties.** Hindlimb-unloading condition resulted in a significant reduction in  $P_t$  (-54%) and in absolute and relative  $P_o$  (-73 and -55%, respectively), compared with the Con group (Table 2). After tendon vibration,  $P_t$  value was not modified (no significant difference between the HU-TV and Con groups), whereas  $P_o$  absolute values only showed a decrease of 29% instead of 73%. Relative  $P_o$  values were the same in the Con and HU-TV groups.

Hindlimb unloading of 14 days resulted in a reduction in the isometric twitch duration in the soleus muscle (Table 2). The  $t_c$  and  $RT_{1/2}$  of the HU rats were significantly decreased by 31 and 30%, respectively, when compared with the Con group. In the HU-TV group, the  $RT_{1/2}$  values were the same ( $P > 0.05$ ) compared with the Con group. However, the  $t_c$  values were not statistically different in the HU and the HU-TV groups.

**Histochemical properties.** In the control solei, 83.2 ± 1.0% of muscle fibers were type I, 8.9 ± 1.1% were type

IIA, and 7.9 ± 1.1% were type IIC. Hindlimb unloading decreased ( $P < 0.05$ ) type I fibers to 72.6 ± 2.1%, whereas an increase ( $P < 0.05$ ) in type IIA (14.8 ± 1.0%) could be observed. No significant change was detected for type IIC fibers (12.6 ± 2.8%). Compared with hindlimb-unloading values, tendon vibration induced no change ( $P > 0.05$ ) in fiber-type distribution, since the percentages of type I, type IIA, and type IIC fibers were 70.0 ± 2.5, 13.4 ± 1.9, and 16.6 ± 3.8%, respectively.

**MHC isoform distribution.** In the control solei, the mean percentages of the MHC type I and of the MHC type IIA-IIX were 90.6 ± 1.1 and 9.4 ± 0.8%, respectively. After hindlimb unloading, the percentage of the slow type MHC I isoform was significantly decreased (81.3 ± 3.0%) while a concomitant increase in the percentage of the fast type MHC IIA-IIX was observed (18.7 ± 3.0%). Proportions of the MHC isoforms in the HU-TV solei were comparable to those in the HU solei ( $P > 0.05$ ).

## DISCUSSION

In agreement with previous studies (see Refs. 12 and 39 for reviews), 14 days of hindlimb unloading induced significant alterations in the rat soleus muscle. These changes included 1) muscle and fiber atrophy; 2) a decrease in the maximal force developed during the twitch and the tetanus; 3) decreases in the isometric contraction time parameters, composed of  $t_c$  and  $RT_{1/2}$ ; 4) a shift toward a higher percentage of fast fibers; and 5) changes in the MHC isoforms. In the present study, hindlimb-unloaded rats were subjected to an intermit-

Table 2. *Contractile properties of soleus muscle from Con, HU, and HU-TV rats*

	Con ( <i>n</i> = 7)	HU ( <i>n</i> = 7)	%ΔCon	HU-TV ( <i>n</i> = 10)	%ΔCon	%Atrophy Prevented
$P_t$ , mN	236 ± 8	107 ± 6*	-54	228 ± 14†	-3	+93
$P_o$ , mN	1,298 ± 54	338 ± 17*	-73	912 ± 70*†	-29	+59
$P_o$ /MWW, mN/mg	10.6 ± 0.6	4.7 ± 0.3*	-55	8.6 ± 0.9†	-18	+66
$t_c$ , ms	39.6 ± 0.9	27.2 ± 1.3*	-31	31.6 ± 2.4*	-20	+35
$RT_{1/2}$ , ms	37.5 ± 1.6	25.9 ± 1.5*	-30	36.8 ± 1.1†	-1	+93

Values are means ± SE; *n*, no. of rats per group.  $P_t$ , twitch tension;  $P_o$ , maximal tetanic tension;  $t_c$ , twitch contraction time;  $RT_{1/2}$ , half relaxation time. \*Significant difference ( $P < 0.05$ ) between Con and HU or HU-TV groups; †significant difference ( $P < 0.05$ ) between HU and HU-TV groups.

tent protocol with associated tendon vibration and stretching. When tendon vibration was applied, the soleus muscle was being stretched. This exercise protocol constituted, in fact, an eccentric exercise training. Because loaded eccentric contractions occur during normal daily activity but are suppressed during hindlimb unloading, we hypothesized that the return of eccentric contractions during hindlimb unloading might provide an effective countermeasure to muscle atrophy. As shown in Tables 1 and 2, this protocol partially prevented the changes in absolute and relative muscle weight, the decrease in the FCSA in the fast-type muscle fibers (IIA-IIC), the decrease in  $P_t$ , and in the absolute and relative maximal  $P_o$  values.

It can be suggested that the stretching imposed during the application of vibration may participate in the prevention of a significant decrease in the muscle mass and in the force output level. In fact, it has been shown that 0.5 h/day of passive stretch of the soleus muscle caused a significant reduction in muscle atrophy (26). In the same way, continuous passive stretching of the soleus muscle prevented changes in mass and protein metabolism produced by a 6-day period of unloading (20). Scientists in our laboratory (25) have also found that stretching of the soleus muscle during hindlimb unloading totally prevented the loss of muscular mass and force output and also counteracted the slow-to-faster shift in contractile and phenotypical parameters, normally associated with hindlimb unloading. However, the morphological and contractile properties in the sham group (hindlimb unloaded and submitted to daily stretching without vibration) and in the HU group were identical. Therefore, we suggest that the stretch duration that we used was too short (0.05 h/day) to play a role in the prevention of the soleus atrophy.

Our data showed that the decreases in absolute and relative soleus muscle wet weights were prevented by 58 and 75%, respectively. These values were comparable to those obtained for the prevention of the decrease in absolute (59%) and relative (66%) maximal tetanic forces. To our knowledge, the effects of tendon vibration on either muscle protein metabolism or force production have never been described in the literature, particularly concerning the rat. This absence of investigations makes it difficult to hypothesize a mechanism that could explain our results. We can only speculate that our eccentric resistance exercise provided a potent stimulus to attenuate the decrease in the percentage of protein content observed in HU rats. This concept was supported by the fact that an eccentric exercise training during hindlimb unloading attenuates the loss of noncollagenous protein by 44% (23). However, our quantitative data concerning the activity level showed that mEMG values increased significantly when tendon vibration was applied during soleus muscle stretching in the HU animals. It has been demonstrated that the muscle spindle primary endings are recruited by stretching alone (6) or by tendon vibration alone (6, 7). We suggest that the number of IA afferent impulses of the activated muscle spindle during the application of our protocol increased the recruitment of the soleus

motor units and enhanced the force they developed. The spinal proprioceptive stimulation augmented the excitation of the respective  $\alpha$ -motoneurons, thus increasing the muscular tension, which seemed to have the beneficial effect of counteracting the loss of muscle mass and force observed during hindlimb unloading. Moreover, our results showed that tendon vibration applied during hindlimb unloading had a specific effect on type IIA and IIC fibers. The FCSA of these fibers, in the HU-TV group, were significantly increased compared with the values obtained in the HU group. This specific mechanism could, therefore, participate in the prevention of the loss of muscle wet weight and, consequently, in the prevention of the decrease in  $P_o$ .

Many scientific results have been published assessing the effectiveness of different exercise modalities in counteracting the atrophy of the rat soleus muscle during unloading (11, 12, 19, 23, 36, 40). All these protocols of exercise and/or muscle loading attenuate soleus atrophy to some degree. For example, 60 min of stationary ground support prevented up to 118% of the absolute muscle mass atrophy and 61% of the atrophy in muscle mass relative to body mass (11). From all these data, it appears that exercise in the form of resistance training is the most effective countermeasure to rat soleus atrophy induced by hindlimb unloading. Furthermore, short periods of exercise are very effective: 6 min of daily ladder climbing (primarily concentric resistance exercise) prevent the absolute and relative soleus muscle mass atrophy by 75 and 43%, respectively (19); 1 min of eccentric exercise every day also prevents absolute (74%) and relative (80%) muscle mass atrophy produced by 10 days of hindlimb unloading (23).

In our conditions, as the length of the soleus muscle increased during the application of tendon vibration, the reflex contraction induced by our protocol was an eccentric contraction. Our results showed that the percentages of atrophy prevented in the absolute and relative muscle mass were 58 and 75%, respectively. This eccentric exercise training obtained with our noninvasive method was very effective, since muscle activation was performed only 0.22% of the total unloading time. Furthermore, tendon vibration prevents the decrease in the  $P_t$  and  $P_o$  by 93 and 59%, respectively. Quantitatively, our results are different from those of Herbert et al. (19), who found that ladder climbing (primarily concentric exercise) applied during hindlimb unloading attenuated, in the soleus muscle, decreases in  $P_t$  and  $P_o$  by 23 and 39%, respectively. Our data thus indicate that resistance training with eccentric actions may participate in muscle mass and muscle force maintenance and suggest that this resistance exercise should be developed for use during spaceflight in association with concentric and isometric exercises.

Our results showed that hindlimb unloading shifted the contractile time parameters ( $t_c$  and  $RT_{1/2}$ ) and the histological and electrophoretic profiles toward those observed in fast muscles, confirming earlier findings (see Ref. 12 for review). In the rats in the HU-TV group, the decrease in  $t_c$  was not counteracted. In contrast, the

decrease in  $RT_{1/2}$  was completely counteracted. Concerning  $t_c$ , the finding was not surprising, since this parameter has generally been found to change only in normal animals after prolonged and severe exercise programs (14, 33). A striking correlation between the speed of muscle contraction and relaxation and the extent of the sarcoplasmic reticulum (SR) function, and a neural influence on the SR amount and composition had been reported in the literature (24). Our results thus suggested that tendon vibration could counteract some changes in SR functioning. The effect appeared more pronounced on the proteins implied in the relaxation process, i.e., chiefly in the  $Ca^{2+}$  uptake by the SR. This uptake is known to be increased in disuse conditions (22, 38) and can be attributed either to a larger surface of SR per volume of muscle, as usually described after experiments inducing slow-to-fast changes in fiber composition (17, 21), or to a higher activity of the  $Ca^{2+}$  ATPase protein itself. We could speculate that these changes were prevented by tendon vibration.

A very rapid atrophy has been described in humans in space. After a 5-day flight, mean FCSAs were, respectively, 11 and 24% smaller in type I and II fibers (13). According to Edgerton et al. (13), these morphological changes are qualitatively similar to those observed in animals after real or simulated spaceflight conditions for short periods. The validity of extrapolating from unweighted muscles to humans in spaceflight is likely to prove qualitatively correct. Muscular vibration has been used to 1) study the contribution of muscle spindles to kinesthetic sensitiveness (35); 2) study the perceptions of position and velocity of an isometric limb (37); and 3) investigate how the central nervous system uses the muscle spindle information to coordinate movements (10). No systematic study of the effects of tendon vibration on the mechanical properties of atrophied muscles has been reported in the literature. Our results provide evidence for a therapeutic effect of tendon vibration on unloaded muscles. The data are even more convincing when we consider that 1) tendon vibration was applied for only 192 s in every 24-h period during the unloading time and 2) it was a noninvasive method to prevent any change in the spinal proprioceptive regulation by the muscle spindle reflex during spaceflight. Consequently, this countermeasure could be applied in humans during a mission. However, further studies are necessary to test whether changes in the characteristics of this exercise protocol, i.e., duration, intensity, and frequency of the vibratory stimulus, can prevent totally all of the changes in the soleus muscle properties observed in disuse conditions.

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