Effects of cutaneous receptor stimulation on muscular atrophy developed in hindlimb unloading condition

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Abstract

The aim of this study was to investigate whether stimulation of the cutaneous mechanoreceptors of the rat foot sole can partially or totally prevent the soleus muscle atrophy developed after 14 days in hindlimb unloading conditions. Final experiments were achieved under deep anesthesia using pentobarbital sodium (60 mg/kg, ip injection). Atrophy was characterized by a significant decrease in muscle weight, fiber size, maximal twitch and tetanic tensions, contraction kinetics, and histochemical and electrophoretical changes. Our data demonstrate that the stimulation of these mechanoreceptors partially prevents the decrease in muscle weight (53%) and cross-sectional area of the soleus muscle (36%) and in all fiber types (type I: 31%; type Ic: 40%; type IIc: 49%; and type IIa: 44%) and also prevented the reductions in peak twitch tension (31%); peak tetanic tension: 25%). However, the decrease in contraction kinetics was not counteracted. Thus our results suggest that stimulation of these receptors in the development of muscular atrophy in HU situations has never been examined. Therefore, the aim of this study was to determine whether intermittent stimulation of the cutaneous mechanoreceptors of the rat sole has preventive effects on the modifications of the morphological, mechanical, histochemical, and electrophoretical properties of the soleus muscle observed in HU conditions.

METHODS AND MATERIALS

Animal groups. Twenty-four male Wistar rats (Iffa Credo L'arbresle) weighing 280–300 g were randomly divided into four groups of six rats each: control (Con), HU only, HU with stimulation of cutaneous receptors (HU-S) (wearing inflated shoes), and HU without stimulation of cutaneous receptors (HU-NS) (wearing noninflated shoes). Muscular mechanical properties, histochemical typing (ATPase staining), and electrophoretical determination [expression of myosin heavy chain (MHC) isoforms] of the right soleus muscle were measured.

All rats were housed in identical, individual cages and were allowed food and water ad libitum. The rats were acclimatized at a 25°C room temperature with a 12:12-h light-dark cycle for 1 wk before the experiments began. An-
imals in the HU, HU-S, and HU-NS groups were hindlimb unloaded for 14 days, using the model of Morey (21). The experiments, as well as the maintenance conditions of the animals, received authorization from both the Agricultural and Forest Ministry and the National Education Ministry (Veterinary Service of Health and Animal Protection, authorization 03805).

Stimulation of cutaneous receptors. Stimulation of the cutaneous receptors was performed by fitting a latex balloon to the two plantar surfaces of hindfeet (Fig. 1A) and then applying an alternating cycle of inflation and deflation. The pressure inside the balloon was maintained at 40 mmHg using a sphygmomanometer. Stimulation was applied for 5 s, followed by 10 s of rest, for 10 min/day during the 14 days of HU (Fig. 1B). This specific pressure was chosen because it stimulated the cutaneous mechanoreceptors of the rat sole (assessed by electromyogram (EMG), see EMG analysis) without inducing nociceptive reaction in the animals.

Before and during sole stimulation, the anesthetized rats (80 mg ketamine and 8 μg acepromazine per gram body weight) were kept in an experimental setup, developed in our laboratory, that allowed maintenance of both the unweighted position of the animal’s body and the soleus muscle in a neutral position (angle between ankle and foot = 90°).

Animals of the HU-S group were maintained in the experimental setup, and stimulation of the cutaneous receptors was applied during the 14 days of the experiment. The HU-NS group was submitted to the restraints of the experimental chamber but not to the stimulation.

EMG analysis. To verify the changes in muscular activity levels, soleus EMG activity was recorded in four separate animal groups, each composed of two rats (Con’, HU’, HU-NS’, and HU-S’) not used in the terminal physiological experiments. The rats were anesthetized via an injection of pentobarbital sodium (60 mg/kg, ip injection). The right soleus muscle was exposed for implantation of bipolar electrodes (7 strands, AM System, Cooner Wire, Chatsworth, CA) under sterile conditions. An incision was made in the back skin, near the base of the tail. Electrode wires were then subcutaneously led up to the right soleus, with one loop at the upper proximal end of the soleus and another loop in the sacral region, so as to prevent the electrodes that were inserted in the muscle from being displaced when the animal moved. Recording electrodes with 0.5 mm of the Teflon insulation removed were inserted in the midbelly of the right soleus (1.5 mm between the two electrodes) using a 23-gauge, 1.5-in. hypodermic needle. The position of the electrodes was tested by electrical stimulation (Grass Instruments S88, Quincy, MA). Then the recording surface of each electrode was secured in the muscle by a suture at the entry and another at the exit of the electrode wires from the right soleus. Under antiseptic conditions, all incisions were sutured. After surgery, animals were placed in individual cages to recover. Three days after surgery, EMG recordings began at 10 AM and were made every second day of the 14-day HU period. EMG was recorded successively for 10 min in the different conditions: Con’, HU’, HU-NS’, and HU-S’. Each condition was followed by 2 min of rest. The raw EMG of each muscle was amplified, recorded, and then analyzed on a personal computer through interactive software (Spike 2, Cambridge Electronic Design). This program rectified the EMG signal, and the mean EMG was expressed in millivolts per second. The mean EMG was calculated and averaged in Con’, HU’, HU-NS’, and HU-S’ conditions for rats. Fourteen days of HU caused a decrease in the EMG amount by 87.5% (EMG HU = 0.37 ± 0.03 mV/s) compared with the EMG of the Con group (EMG Con = 2.96 ± 0.05 mV/s). The stimulation of the animal’s sole (HU-S group) triggered a transitory elevation of the EMG amount by ~110% (EMG HU-S = 6.2 ± 0.1 mV/s). No statistical difference was observed between HU and HU-NS groups.

Dissection and mechanical parameters. The rats were anesthetized using pentobarbital sodium (60 mg/kg, ip injection), and anesthesia was prolonged with further injections of 30 mg/kg, when necessary. Briefly, the right soleus muscle was exposed while care was taken not to damage the main blood supply or the soleus nerve trunk. The hindlimb of the rat was then immersed in a bath filled with paraffin oil maintained at 37°C, so as not to modify the morphological properties of the soleus fibers. The knee and the foot were rigidly fixed to respect isometric recording conditions. The distal tendon of the soleus muscle was severed and connected to a force transducer (FT10, 700 Hz, Grass Instruments).

The initial muscle tension was determined to allow production of the peak muscle twitch tension (Pᵢ). Contractions of the soleus muscle were induced by stimulation of the sciatic nerve trunk (0.2 ms pulses) through bipolar platinum electrodes, at twice the minimum voltage required to obtain the Pᵢ response.

The following mechanical parameters were determined: Pᵢ, peak tetanic tension at 100 Hz (Pᵢ), the ratio of subtetanic tension at 20 Hz relative to Pᵢ (P₀f/Pᵢ), which was used as an indicator of muscle type (33), time-to-peak [(TTP) defined as the time from the initiation of force until peak force], half-relaxation time [(HRT) measured from the peak force to the moment when force decreased to half its highest value], and the fatigue index (FI). FI was defined after application of the fatigue test of Burke and colleagues (5).
At the end of the fatigue test, the right soleus muscles of the four experimental animal groups were removed, weighed for determination of muscle wet weight (MWW), frozen in isopentane precooled to its freezing point by liquid N₂, and stored at −80°C until histochemical and electrophoretical analysis were performed.

**Histological analysis.** The muscular fiber types were classified following the method of Guth and Samaha (14). The right soleus muscles of the Con, HU, HU-NS, HU-S groups were cut in serial transverse sections (10-μm-thick), perpendicular to their longitudinal axis at the midbelly of the muscle, by using a cryostat microtome (Leica CM 1800, Heidelberg, Germany) set at −20°C and were stained for myofibrillar ATPase activity with acid (pH 4.3 and 4.45) and alkali (pH 10.4) preincubations. This histochemical method allowed the characterization of four types of soleus fibers: type I (slow), type IIa (fast), and types Ic and IIc (intermediate). Soleus composition was expressed as the percentage of each fiber type in an examined section (450 fibers from each section). Moreover, the cross-sectional area (CSA) of both the whole muscle and fibers was measured using an image analyzer (SAMBA 2005, Alcatel, Grenoble, France). Fiber measurements were made in 80 fibers for type I and IIa fibers and in all type Ic and IIc fibers.

**Electrophoretical analysis of MHC isoforms.** Fifteen transverse sections of each right soleus were treated using the method of Carraro and Catani (7). The quantity of proteins in each tube was determined, and 1 μg of protein per sample was loaded into each electrophoretical lane. MHC composition was determined by using vertical SDS-PAGE. Polyacrylamide gels were constituted of a 4.5% (wt/vol) acrylamide stacking gel and a 7.5% (wt/vol) separating gel (15). The migration buffer contained (in mM) 25 Tris (pH 7.9), 190 glycerine, and 3.5 SDS. Gels were run at constant voltage (180 V) for 24 h and then stained with a solution containing (in percentage) 1.7 ammonia, 20.2 NaOH (90 mM), and 3.8 AgNO₃ (1.17 M). A laser scanning densitometer (Quantiscan, Micrivial Systems, Biosoft) was used to determine the relative proportions of MHC isoforms expressed in each sample of soleus muscle.

Cryostat sections of the extensor digitorum longus (EDL) muscles of the Con group were used as indicators for the electrophoretical mobility of the fast-twitch MHC Ila, IIX, and IIB isoforms.

**Statistical analysis.** All results are expressed as means ± SD and were analyzed using a one-way ANOVA. Significant differences between the four experimental groups were determined by using a Bonferroni t-test. Statistical significance was accepted at P < 0.05.

### Table 1. Morphological parameters

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>HU</th>
<th>HU-NS</th>
<th>HU-S</th>
<th>Prevent, %</th>
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<tr>
<td>MWW, mg</td>
<td>145.9 ± 12.5</td>
<td>83.6 ± 2.8*</td>
<td>87.1 ± 10.4*</td>
<td>116.8 ± 6.2†‡</td>
<td>53</td>
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<td>MWW/BW, mg/g</td>
<td>0.49 ± 0.03</td>
<td>0.28 ± 0.02*</td>
<td>0.29 ± 0.03*</td>
<td>0.38 ± 0.02* †‡</td>
<td>48</td>
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<tr>
<td>Soleus muscle CSA, mm²</td>
<td>8.9 ± 0.6</td>
<td>4.2 ± 0.2*</td>
<td>4 ± 0.1*</td>
<td>5.9 ± 0.4* †‡</td>
<td>36</td>
</tr>
<tr>
<td>Fiber CSA, μm²</td>
<td>3.606 ± 207</td>
<td>1.698 ± 168*</td>
<td>1.651 ± 197*</td>
<td>2.281 ± 145†‡</td>
<td>31</td>
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<tr>
<td>Type I</td>
<td>2.536 ± 52</td>
<td>1.144 ± 116*</td>
<td>1.131 ± 243*</td>
<td>1.700 ± 20* †‡</td>
<td>40</td>
</tr>
<tr>
<td>Type Ic</td>
<td>2.683 ± 447</td>
<td>1.531 ± 354*</td>
<td>1.253 ± 155*</td>
<td>2.092 ± 233†‡</td>
<td>49</td>
</tr>
<tr>
<td>Type IIa</td>
<td>3.111 ± 425</td>
<td>1.563 ± 316*</td>
<td>1.593 ± 19*</td>
<td>2.235 ± 236†‡</td>
<td>43</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 for all groups. Morphological parameters of rat soleus muscle in control (Con), hindlimb unloaded (HU), hindlimb unloaded without stimulation of cutaneous receptors (HU-NS), and hindlimb unloaded with stimulation of cutaneous receptors (HU-S) groups. Percentages of change prevention (Prevent) were obtained by adapting the equation for percentage atrophy prevented in Table 3 of Ref. 18 as follows: (HU-S value − HU value)/(Con value − HU value). MWW, muscle wet weight; MWW/BW, ratio of MWW to body wt; CSA, cross-sectional area. *Significantly different from Con; †significantly different from HU; ‡significantly different from HU-NS.
determined by ATPase staining on serial cross sections, is shown in Fig. 2. Only the ATPase staining of Con, HU, and HU-S is shown because the soleus of the HU-NS group appeared to have the same fiber type composition as the soleus of the HU group. Acid preincubation (pH 4.3) is illustrated by Fig 2, A–C, whereas alkaline preincubation (pH 10.4) is presented Fig. 2, D–F. The slow-twitch, type I fibers, designated by I in Fig. 2, were characterized by a high ATPase activity at pH 4.3 and by a low activity at pH 10.4. Fast-twitch, type IIa fibers (IIa in Fig. 2) exhibited inverse histochemical behavior (alkali stable and acid labile). The intermediate type Ic fibers (notated as Ic) had a stable activity at pH 4.3 and a moderately stable activity at pH 10.4. The intermediate type IIc fibers showed an inverse histochemical activity (alkali stable and acid moderately stable). Type IIb and IIx fibers were never detected in any group at pH 4.45; therefore, we voluntarily chose to focus our illustrations on pH 4.3 and 10.4.

HU muscles were characterized by a large degree of atrophy and a decrease in the number of type I fibers, which was associated with an overexpression of type IIa fibers (Fig. 2, B and E).

The animals of the HU-S group showed a degree of atrophy that was less marked than that of the HU group and a higher expression of fast IIa fibers, which was associated with a greater decrease in slow type I fibers.

The distribution histograms of the different fiber types expressed in the soleus muscles are shown in Fig. 3. In the HU condition, a decrease in type I fibers (−8.5%), associated with a 93% increase in IIa fibers, was observed. The HU-NS group showed a similar fiber type repartition when compared with the HU group. On the other hand, compared with the Con group, HU-S animals showed a decrease (−26.4%) in type I fibers, which was associated with an overexpression of IIa fibers (+170%).

Electrophoretical characteristics. An example of the electrophoretical profile of migration of the different MHC isoforms constituting the soleus muscle of all four groups is illustrated in Fig. 4A. The MHC isoforms were identified by comparing the electrophoretical profile of the soleus to that of a fast muscle, the EDL.

Con soleus muscles contained two MHC isoforms: the fast MHC IIa and the slow MHC I, with a clear predominance of the MHC I expression. After a period of HU, the MHC I isoform decreased, whereas the MHC IIa isoform increased. Moreover, new MHC isoforms, the fast MHC IId/IIX and the fast MHC IIb, appeared. The profile of electrophoretical migration of the HU-NS group was similar to that of the HU group; therefore, we voluntarily showed only the HU electrophoretical profile. In the HU-S group, the amount of MHC I isoform was reduced and the expressions of MHC IIa and MHC IId/IIX isoforms were clearly denser than those in the HU group. However, HU-S animals did not express the fast MHC IIb isoform.

Figure 4B shows the distribution histogram of MHC isoforms expressed among the four experimental groups. Compared with Con animals, the HU, HU-NS, HU-S groups showed a reduction of MHC I by 28.8, 27.6, and 39.8%, respectively, and a rise in MHC IIa by 44.3, 45.1, and 127.9%, respectively. Moreover, the MHC IId/IIX isoform appeared in the HU (11%), HU-NS (12%), and HU-S (18%) groups. However, only the HU and HU-NS groups expressed the fast MHC IIb isoform (8.6 and 6.5%, respectively). Compared with the HU group, the HU-S group showed that stimulation of the mechanoreceptors of the rat sole induced a greater diminution in MHC I (−15.5%) and a higher expression of MHC IIa and MHC IId/IIX isoforms (increases of 57.9 and 68.8%, respectively).

DISCUSSION

The aim of this study was to determine whether the effects produced by stimulation of cutaneous receptors either partially or totally counteracted the changes in morphological, mechanical, histochemical, and electrophoretical properties of rat soleus muscles observed after HU.

Effects of 14 days in HU situation. The effects produced by HU were in agreement with those obtained by other authors (9, 29). A period of 14 days of HU induced, in soleus muscle, a decrease in muscle and fiber CSA and a reduction in P0. Compared with Con, the HU, HU-NS, and HU-S groups showed a decrease in MHC I by 28.8, 27.6, and 39.8%, respectively, and an increase in MHC IIa by 44.3, 45.1, and 127.9%, respectively. Moreover, the MHC IId/IIX isoform appeared in the HU (11%), HU-NS (12%), and HU-S (18%) groups. However, only the HU and HU-NS groups expressed the fast MHC IIb isoform (8.6 and 6.5%, respectively). Compared with the HU group, the HU-S group showed that stimulation of the mechanoreceptors of the rat sole induced a greater diminution in MHC I (−15.5%) and a higher expression of MHC IIa and MHC IId/IIX isoforms (increases of 57.9 and 68.8%, respectively).

Table 2. Mechanical parameters

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>HU</th>
<th>HU-NS</th>
<th>HU-S</th>
<th>Prevent, %</th>
</tr>
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<tbody>
<tr>
<td>P0, N</td>
<td>0.25 ± 0.03</td>
<td>0.09 ± 0.02*</td>
<td>0.09 ± 0.01*</td>
<td>0.14 ± 0.04†‡</td>
<td>31</td>
</tr>
<tr>
<td>P0, N</td>
<td>1.51 ± 0.30</td>
<td>0.30 ± 0.05*</td>
<td>0.30 ± 0.01*</td>
<td>0.60 ± 0.14*‡‡</td>
<td>25</td>
</tr>
<tr>
<td>Pt/P0</td>
<td>0.69 ± 0.05</td>
<td>0.46 ± 0.05*</td>
<td>0.48 ± 0.07*</td>
<td>0.55 ± 0.09*</td>
<td></td>
</tr>
<tr>
<td>TTP, ms</td>
<td>39.3 ± 6.4</td>
<td>24.7 ± 3.3*</td>
<td>26.8 ± 1.8*</td>
<td>25.7 ± 4.5*</td>
<td></td>
</tr>
<tr>
<td>HRT, ms</td>
<td>43.7 ± 4.6</td>
<td>33.7 ± 2.9*</td>
<td>34.1 ± 2.1*</td>
<td>28.3 ± 3.7*</td>
<td></td>
</tr>
<tr>
<td>FI, %</td>
<td>94 ± 3.8</td>
<td>95 ± 2.2</td>
<td>93.8 ± 5.9</td>
<td>95.8 ± 2.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Contractile parameters of rat soleus in Con, HU, HU-NS, and HU-S groups. P0, maximal twitch tension; Pt, maximal tetanic tension at 100 Hz; Pt/P0, ratio of tetanic tension at 20 Hz relative to P0; TTP, time to peak; HRT, half relaxation time; FI, fatigue index. *Significantly different from Con; †significantly different from HU; ‡significantly different from HU-NS.
formation because, after HU, MHC IIa was overexpressed at the expense of MHC I isoform. The MHC IId/IIX and MHC IIb isoforms, not detected in Con muscles, appeared (6, 27, 30).

Effects of cutaneous mechanoreceptor stimulation. Our results also showed that mechanoreceptor stimulation of the plantar surface of the hindfoot partially prevented some of the muscular modifications observed in HU. The decrease in MWW, MWW/BW, and muscle and fiber CSAs was counteracted by 53, 48, 36, and 31% for type I fibers, 40% for type Ic fibers, 49% for type IIc fibers, and 44% for type IIa fibers, respectively. The decreases in P\textsubscript{T} and P\textsubscript{O} were also partially prevented (31 and 25%, respectively). These prevention percentages, observed for stimulation occurring in only 0.23% of the HU period, should be a direct consequence

Fig. 2. Cross sections of rat soleus muscles used for morphological measurements and histochemical analyses. A–C: muscle samples from control (Con; A), hindlimb unloaded (HU; B), and HU with stimulation of cutaneous receptors (HU-S; C) groups that were preincubated in acid (pH 4.3). D–F: preincubation in alkali at pH 10.4 of Con (D), HU (E), and HU-S (F) group muscle samples. Bar = 100 μm. Ic, IIc, and IIa, muscle fiber types found within samples after ATPase staining.
of the muscular activity developed during stimulation of the mechanoreceptors. In fact, a significant increase in the EMG soleus activity was always observed in the HU-S group, recorded in parallel with the HU-S group. In our conditions, we therefore supposed that, in the HU-S group, this stimulation could prevent the decrease in MWW and the strength loss normally observed in HU groups. The transitional increase in the EMG activity (enhanced neuromuscular activation) probably resulted from the stimulation of mechanoreceptors sensitive to pressure, which play an important role in controlling normal body balance (17). In normal conditions, the reflexes produced by stimulation of the mechanoreceptors are defined as polysynaptic reflexes (1). Thus stimulation of the right plantar surface leads to an ipsilateral flexor muscle activation and a simultaneous ipsilateral extensor muscle inhibition via medullar interneurons. Moreover, the same stimulation also produces an inhibition of the contralateral flexor muscles and an activation of the contralateral extensor muscles. Consequently, the final physiological movements consist of a flexion of the right stimulated hindlimb, with a concomitant extension of the left hindpaw.

In the present study, stimulation of the cutaneous receptors was simultaneously performed on the two plantar surfaces. In this condition, an apparent paradox emerged, as stimulation of the left plantar surface produced a right soleus activation, whereas stimulation of the right plantar surface caused a right soleus inhibition. Because we still observed EMG activity in the right soleus muscle after each stimulation, our hypothesis is that the activation level of the soleus muscle was higher than the inhibition level, and, accordingly, the soleus muscle remained temporarily activated.

However, at this stage of study, we are not able to differentiate between the effects produced on the slow adapting receptors (Merkel and Ruffini end-organs), those produced on the rapidly adapting receptors (Meissner’s end-organs), and those produced on Pacinian corpuscles. According to the results of Leem et al. (19), the activation thresholds of these receptors were 5.69 ± 3.28 mN for Meissner, 0.97 ± 0.56 mN for Pacini, 8.2 ± 4.41 mN for Ruffini, and 6.44 ± 2.49 mN for Merkel receptors. It is classically admitted that a pressure of 7.5 mmHg corresponds to a strength of 1 kN/m². The mean area of the rat soles of the four animal groups was measured and equaled 400 mm². Thus the level of pressure that we used corresponded to 5.3 kN/m² (i.e., 2,120 mN) for the entire plantar surface of the hindlimb, which corresponded to a strength of 5.3 mN/mm². Therefore, the pressure theoretically stimulated all cutaneous mechanoreceptors (Merkel, Ruffini, Meissner, and Pacini) of the plantar surface of hindlimb because the thresholds for all of these receptors were exceeded.

In HU condition, the nervous motor message (tonic) received by the soleus muscle becomes more phasic (2). Our hypothesis is that plantar surface stimulation produces a phasic nervous message that adds to the phasic motor message in HU. This can explain why the...
were similar to those reported by Shaw et al. (25) after the prevention of decrease in soleus muscle weight. HU partially prevented atrophy of rat soleus muscles showed that 2 or 4 hours of daily ground support for 4 wk in microgravity was more effective in atrophy prevention than some other countermeasures. Indeed, Thomason and co-workers (30) suggested that the reflex contraction of postural muscles is induced by maintenance of either posture or locomotion activity (26). In HU conditions, because weight bearing is abolished, the eccentric and isometric contractions are very reduced or disappear. Different types of exercise have been used to prevent some modifications of muscular tissue. It has been demonstrated that climbing (primarily a concentric resistance exercise) a 1-m grid at an 85% incline, with a load equal to 75% body wt, for 8 repetitions, 4 times a day (total = 6 min/day), results in a 43% prevention of soleus MWW decrease after 1 wk in an HU situation (16).

The application of eccentric exercises in HU conditions could prevent both the atrophy and the loss of muscular strength that appears in real microgravity. In fact, intermittent stimulation of soleus muscle, applied during muscle stretch, is an effective means of partially preventing the atrophy observed after 10 days of HU (18). This previous study showed a 77% prevention of atrophy during an eccentric exercise that represents only 0.035% of the HU period. Falempin and Fodili In-Albon (12) also observed that the intermittent application of brief daily tendon vibration for 14 days in HU condition (soleus muscle stretched during application of vibrations) partially prevented atrophy (75%) and loss of twitch and maximal muscular strength (93 and 59%, respectively). These percentages were obtained with an eccentric muscular activity of 0.23% in the HU period.

In our experimental conditions, the ankles were locked in a neutral position (90° angle between foot and ankle). Therefore, we suggest that the reflex contraction produced by stimulation of the cutaneous mechanoreceptors is an isometric contraction. If we compare our results with those obtained by Kirby and colleagues (18) and Falempin and Fodili In-Albon (12), we observe that periods of eccentric exercise are more effective countermeasures than those of isometric exercise. However, our exercise program was more effective in atrophy prevention than some other countermeasures. Indeed, Thomason and co-workers (30) showed that 2 or 4 h of daily ground support for 4 wk in HU partially prevented atrophy of rat soleus muscles (32 and 35% of muscle weight decrease, respectively, vs. 53% in the present study). Our results regarding the prevention of decrease in soleus muscle weight were similar to those reported by Shaw et al. (25) after running on a treadmill at 20 m/min at a 30% grade for 1.5 h/day for 4 wk in HU (50 vs. 53% of prevention in our study). However, our exercise program had a much shorter duration than that of Shaw et al. (10 vs. 90 min/day, respectively). However, the countermeasure used by Pierotti et al. (22) was more effective than our exercise protocol. They showed that, for rats kept 7 days in HU conditions, walking slowly on a treadmill at 0.2 m/s on a 19% incline for 10 min every 6 h (total of 40 min/day exercise) counteracted the decrease in the soleus MWW/BW by 60% (vs. 48% in our study) and counteracted P0 and P1 loss by 57.6 and 54.3 vs. 31 and 25%, respectively, in the present study.

To summarize, although eccentric exercises seem more effective as means of countermeasure, the percentages of prevention remained partial. By associating an isometric exercise with an eccentric exercise, the prevention of atrophy and loss of strength could be higher than that induced by either of these exercises applied alone during the HU period. This supposition is confirmed by the study of Stump et al. (26). In their suspension model, one hindlimb was placed on a platform with the leg in a position similar to that observed during standing. This platform provided a base against which the animal could contract or stretch the supported limb at any time during HU. In these conditions, the soleus muscle produced combinations of isometric, concentric, and eccentric contractions. This model prevented 95% of muscle weight decrease in the supported hindlimb after 14 days of head-down suspension. Moreover, it appeared that short, intermittent bouts of exercise, interspersed throughout the day, may be more efficient countermeasures than a single, long bout of exercise (4, 16, 23). We hypothesize that modifications in the stimulation of cutaneous receptors of rat foot soles (increases in time and number of applications) would provide a more effective means of preventing muscular atrophy and the associated post-flight motor control deficits experienced by astronauts.

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REFERENCES