Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures

N. D. Reeves, C. N. Maganaris, G. Ferretti, and M. V. Narici

Institute for Biophysical & Clinical Research into Human Movement, Manchester Metropolitan University, MMU Cheshire, Alsager Campus, Cheshire ST7 2HL, UK

Submitted 9 November 2004; accepted in final form 4 February 2005

Influence of 90-day simulated microgravity on human tendon mechanical properties and the effect of resistive countermeasures. J Appl Physiol 98: 2278–2286, 2005. First published February 10, 2005; doi:10.1152/japplphysiol.0266.2004.—While microgravity exposure is known to cause deterioration of skeletal muscle performance, little is known regarding its effect on tendon structure and function. Hence, the aims of this study were to investigate the effects of simulated microgravity on the mechanical properties of human tendon and to assess the effectiveness of resistive countermeasures in preventing any detrimental effects. Eighteen men (aged 25–45 yr) underwent 90 days of bed rest: nine performed resistive exercise during this period (BREx group), and nine underwent bed rest only (BR group). Calf-raise and leg-press exercises were performed every third day using a gravity-independent flywheel device. Isometric plantar flexion contractions were performed by using a custom-built dynamometer, and ultrasound imaging was used to determine the tensile deformation of the gastrocnemius tendon during contraction. In the BR group, tendon stiffness estimated from the gradient of the tendon force-deformation relation decreased by 58% (preintervention: 124 ± 67 N/mm; postintervention: 52 ± 28 N/mm; P < 0.01), and the tendon Young’s modulus decreased by 57% (postintervention: 66 N/mm; P < 0.01). In the BREx group, tendon stiffness decreased by 37% (preintervention: 136 ± 66 N/mm; postintervention: 86 ± 47 N/mm; P < 0.01), and the tendon Young’s modulus decreased by 38% (postintervention: P < 0.01). The relative decline in tendon stiffness and Young’s modulus was significantly (P < 0.01) greater in the BR group compared with the BREx group. Unloading decreased gastrocnemius tendon stiffness due to a change in tendon material properties, and, although the exercise countermeasures did attenuate these effects, they did not completely prevent them. It is suggested that the total loading volume was not sufficient to completely prevent alterations in tendon mechanical properties.

unloading; ultrasound; flywheel exercise

UNLOADING OF THE MUSCULOSKELETAL system due to actual or simulated microgravity exposure is associated with a number of adverse musculoskeletal alterations. Skeletal muscle atrophy and strength decrements have been reported following both relatively short (10–17 days) (14, 49) and longer (>5 wk) periods of unloading (11, 24, 33–35). While the deterioration of muscle mass and function with unloading is of obvious clinical relevance, there are other important functional structures within the musculoskeletal system that may also be adversely affected. A typical example is the tendon in-series with the muscle. Tendons provide the structural link between muscle and bone, and as such they play an integral role in the transmission of contractile forces to the skeleton. Via their primary role as force transmitters, tendons have the capacity to influence the length and thus the force of the in-series contractile element, depending on the degree of tensile deformation they undergo (54, 69). The extent of tendon deformation in response to a given level of tensile force generated by muscle contraction is dependent on the tendon’s mechanical properties and dimensions (38, 41–43). The tendon is not an inert structure, and just as skeletal muscle displays plasticity to changes in the level of physiological loading, there is evidence that tendon mechanical properties may be adaptable to changes in loading level. For example, tendons of both animals (18, 66, 68) and humans (53) have been shown to respond to loading levels higher than those experienced habitually (running in animals and resistive training in humans), by increasing their tensile stiffness. In some of these studies, increased loading resulted in tendon hypertrophy (66, 68), whereas in others no change in tendon size was observed (18, 53). In contrast to these reports, 9 mo of running training were shown to have no effect on the mechanical properties of the human Achilles tendon (23), which may reflect a tendon-specific effect or interstudy differences in the type of loading. Much less is known regarding the response of tendons to chronic unloading. Animal models, however, suggest that collagenous tissue stiffness is reduced following periods of unloading, causing greater deformations for the same given load (50, 51, 66).

If unloading were to cause reductions in tendon tensile stiffness as suggested by animal studies, it would be important to know whether the intermittent loading provided by exercise training could prevent these potentially detrimental effects. In most investigations on the effectiveness of physical activity countermeasures against unloading of the musculoskeletal system in ground-based studies, the exercise procedure/device has been inappropriate for use in space. However, in the present study, a gravity-independent exercise device designed specifically for use in space is tested in a ground-based simulation. This device has previously been shown to increase muscle size and strength in ambulatory subjects (60) and to prevent muscle atrophy, while maintaining strength in subjects undergoing unilateral lower limb unloading (61). Flywheel exercise performed during 28 days of bed rest prevented the loss of muscle mass and function in the knee extensors, while attenuating the decline in the plantar flexors (7).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In light of the previously mentioned issues, the aims of this study were to investigate the effects of simulated microgravity (bed rest) on tendon mechanical properties and to assess the effectiveness of exercise countermeasures using the flywheel device in attenuating the potentially detrimental effects on the tendon induced by unloading. It was hypothesized that unloading would cause a reduction in tendon stiffness and that the specific exercise countermeasures performed would prevent this detrimental effect.

METHODS

Study design. The long-term bed rest (LTBR) study 2001–2002 was a ground-based investigation designed to simulate the physiological effects of microgravity exposure. This study was organized by the European Space Agency, together with the Centre National d’Etudes Spatiales and the Japanese National Space Development Agency and was conducted at the MEDES Institute for Space Medicine and Physiology in Toulouse, France. Volunteers underwent a period of 90 days of bed rest in a 6° head-down tilt position. This time period was selected because it represents the projected minimum duration of future International Space Station missions. During this period, the volunteers remained in the Space Clinic, which provided an environment similar to a hospital setting. Subjects performed all activities and duties in the 6° head-down tilt position. Physical activity was not permitted at any time during the period of bed rest, but passive joint mobilization was performed daily. Subjects were closely monitored by nurses and cameras positioned in each room to ensure that no physical activity was performed. Subjects followed a carefully controlled diet with the caloric intake determined relative to the subject’s body mass, following recommendations from the World Health Organization. Liquid intake was at least 30 ml·kg\(^{-1}\)·day\(^{-1}\) (to minimize renal stone risk) with a maximum of 2.5 l/day, while beverages such as tea, coffee, and cola were not permitted. The volunteers were allocated to two experimental groups: a group that underwent bed rest only (BR) and a group that underwent bed rest while performing exercise countermeasures (BREx). All study procedures complied with the declaration of Helsinki and were approved by the local Ethics Committee in Toulouse.

Subjects. Subject selection was based on a screening evaluation consisting of a detailed medical history and physical examination. All subjects were recreationally active, but not involved in sporting activities at a competitive level. At the time of the study, no participant was taking any medication, and all were nonsmokers. Eighteen men between 25 and 45 yr of age gave their written, informed consent to participate in the study after they had been informed of all of the procedures and possible risks. The BREx group \([n = 9; \text{age: } 32.6 (4.9) \text{ yr}; \text{body mass: } 70.6 (5.5) \text{ kg}; \text{height: } 1.75 (0.05) \text{ m}; \text{body mass index: } 22.9 (1.5) \text{ kg/m}^2; \text{means (SD)}]\) performed exercise countermeasures during the bed rest period, while the BR group \([n = 9; \text{age: } 31.9 (3.6) \text{ yr}; \text{body mass: } 71.7 (5.4) \text{ kg}; \text{height: } 1.73 (0.03) \text{ m}; \text{body mass index: } 23.8 (1.6) \text{ kg/m}^2]\) underwent bed rest only.

Exercise intervention. Exercise was performed every third day in the 6° head-down tilt position using a gravity-independent flywheel resistive exercise device. This exercise device enables loading in both concentric and eccentric contraction phases via the inertia of rotating flywheels (Fig. 1) and was selected for use in this microgravity simulation study because it has been specifically developed for use in space (13, 15, 59). This gravity-independent exercise device was recently shown to be effective for inducing strength gains and muscle hypertrophy at 1 G in the knee extensors (60). Subjects were familiarized with the exercise device on two occasions before the start of bed rest. Two exercises were performed: 1) the leg press for the hip, knee, and ankle extensors, and 2) the calf raise for the ankle extensors. Following a progressive warm-up, 4 sets of 7 repetitions were performed for the leg press, while 4 sets of 14 repetitions were performed for the calf raise. The range of motion at the ankle joint was \(\sim 50°\) (~30° of plantar flexion to ~20° of dorsiflexion) during the calf-raise exercise with the knee joint almost in full extension. In each set, two submaximal contractions were performed initially, followed by the 7 or 14 repetitions in the leg-press and calf-raise exercises, respectively. Coupled concentric and eccentric muscle actions were executed with maximal effort throughout the range of movement, except for a short submaximal effort during the first ~20° of the eccentric phase to allow an increase in flywheel velocity. Two-minute rest was introduced between sets and 5 min between the different exercises. A coach was present during all training sessions to provide verbal encouragement for subjects to exert maximal effort during each contraction. The flywheel device enables subjects to exert maximal effort throughout all contractions, even when torque output may decline slightly due to fatigue. This is in contrast to conventional resistive exercise devices, where a fatigue-induced decline in torque may likely result in failure to lift the constant external load and, therefore, cause the subject to cease exercising. Example traces of the force, flywheel velocity, etc., during training and further details regarding the flywheel apparatus have been recently described by Alkner and Tesch (7).

Fig. 1. A participant performing the calf-raise exercise on the flywheel device in the 6° head-down tilt position.
Measurement of isometric force. Isometric plantar flexion force was measured using a custom-built dynamometer (Fig. 2), similar to the device described by Marsh et al. (45). The dynamometer used enables a very stable fixation of the lower leg, thus minimizing ankle joint rotation during isometric contraction. The subject was seated with the knee joint at 90° (0° = full extension) and the ankle joint at 20° of dorsiflexion (0° = right angle between foot and tibia). The rationale underlying this joint configuration is that the gastrocnemius muscle-tendon unit becomes relatively slack when the knee joint is flexed; hence, to compensate for this effect, the ankle joint was dorsiflexed by 20°. The change in length of the gastrocnemius muscle-tendon unit with changes in knee and ankle joint angle can be estimated from the following equation:

\[
\Delta L_{MT} = \Delta JA \cdot MA \tag{1}
\]

In Eq. 1, \(\Delta L_{MT}\) is the change in length of the gastrocnemius muscle-tendon unit, \(\Delta JA\) is the change in knee/ankle joint angle (rad), and \(MA\) is the tendon moment arm length of the gastrocnemius muscle at the knee/ankle. The calculations below detail the length changes in the gastrocnemius muscle-tendon unit as a result of ankle joint rotation from the neutral position (0°) to 20° of dorsiflexion (Eq. 2) and by knee joint rotation from full extension (0°) to 90° of knee flexion (Eq. 3). In the following equations, gastrocnemius tendon moment arm length values at the knee and ankle joints have been taken from the literature (39, 63). The symbols + and − indicate an increase and decrease in muscle-tendon unit length, respectively.

\[
\Delta L_{MT} = 0.35 \text{ rad} \cdot 5 \text{ cm} = +1.8 \text{ cm} \tag{2}
\]

\[
\Delta L_{MT} = -1.57 \text{ rad} \cdot 1.2 \text{ cm} = -1.9 \text{ cm} \tag{3}
\]

The similarity in magnitude between the two \(\Delta L_{MT}\) values provides confidence that the length of the gastrocnemius muscle-tendon unit in our measurements with the knee flexed and the ankle joint in 20° of dorsiflexion approximates the length of the muscle-tendon unit with the knee joint in full extension and the ankle joint in the neutral position. Further confirmation is provided by the fact that our calculations are consistent with a report that the maximum voluntary plantar flexion torque is approximately equivalent between these two conditions (55). Measurements were performed on the right leg of all subjects and were taken before and after (2 days following reambulation) the 90 days of bed rest. Postintervention measurements were conducted 2 days after reambulation in all subjects because only testing performed in the 6° head-down tilt position was permitted during the 90-day bed rest period, and it was not possible to perform the dynamometry measurements in this position. Force was measured during an isometric voluntary contraction using a precalibrated force transducer located under the footplate. Two maximal isometric plantar flexion contractions were performed, and, if the force from these two contractions varied by >5%, a third contraction was performed.

Ultrasound scanning. B-mode ultrasound (Honda HS-2000, Toyohashi City, Japan) was used to scan the gastrocnemius myotendinous junction at the site of the medial head using a 10-MHz linear-array transducer (Fig. 3). Ultrasound measurements were conducted under the conditions described above in the Measurement of isometric force section. Sagittal plane scans were acquired in the resting state and during isometric plantar flexion contraction at 20, 40, 60, 80, and 100% of maximal voluntary force of 2- to 3-s duration. The ultrasound transducer was placed over a marker fixed to the skin, which cast a line on the ultrasound image and served as a reference position to measure tendon tensile displacement. The relevant scans were identified, and tendon displacement was measured using digitizing software (NIH Image version 1.61, National Institutes of Health, Bethesda, MD).

Estimation of tendon forces. The external moment arm of the ankle joint was measured as the distance from the center of rotation of the ankle joint to the distal head of the first metatarsal bone. The Achilles tendon force was estimated by multiplying the force measured at the footplate by the previously reported ratio (2.67) of force measured at the foot to the force developed at the Achilles tendon (25). The gastrocnemius tendon force was estimated based on its physiological cross-sectional area (PCSA) relative to that of the entire plantar flexor muscle group, from the data of Fukunaga et al. (22).

MRI. Axial-plane scans of the Achilles tendon were acquired using Magnetic resonance imaging (MRI) before and after the 90 days of bed rest. Baseline measurements were taken before bed rest, and postintervention measurements were taken before reambulation. Subjects rested in the horizontal supine position for 1 h before scanning to avoid the influence of potential fluid shifts that would induce interstitial and/or intracellular volume changes (12). Subjects refrained from excessive muscular exercise for 24 h before scanning. The MRI scans were acquired using a 1.0-T scanner (Siemens Somatom Impact 1.0 T, Erlangen, Germany). Scans were acquired using a proton density sequence with the following scanning parameters: repetition time, 2,000 ms; echo time, 20 ms; slice thickness, 8 mm;
values are stated in RESULTS.

Fig. 4. Gastrocnemius tendon force-deformation relation for the bed rest (BR) group and the bed rest exercise (BREx) group. Values are means; maximal SD values are stated in RESULTS.

Estimation of tendon stress and strain. The gastrocnemius tendon CSA was assumed to occupy a proportion of the Achilles tendon CSA, equal to the relative PCSA of the gastrocnemius muscle with respect to the triceps surae muscle group PCSA (20, 22, 64). The theory of Ker et al. (29) predicts that the muscle PCSA-to-tendon CSA ratio is constant, a notion that has been supported experimentally (9), but further experiments are needed to confirm the applicability of these findings to highly stressed tendons, including the plantar flexor tendons. Gastrocnemius tendon forces were divided by the estimated gastrocnemius tendon CSA to obtain tendon stress. Gastrocnemius tendon strain was estimated from the ratio of tendon deformation to the initial unloaded gastrocnemius tendon length.

Estimation of tendon stiffness and Young’s modulus. The gastrocnemius tendon force-deformation data beyond 60% of the maximum force were fitted with a linear function. From the fitted data points, the gastrocnemius tendon stiffness was estimated as the distance between the osteotendinous junction at the calcaneous and the myotendinous junction at the gastrocnemius.

Effect of unloading on tendon mechanical properties. Tendon deformation was 12.1 (3.5) mm at a maximum tendon force of 572.4 (185.4) N at baseline; deformation increased to 15.3 (3.3) mm (P < 0.05; 26% increase in tendon deformation) at a tendon force of 409.4 (161.6) N (P < 0.01; 28% reduction in tendon force) following unloading (Fig. 4). Tendon strain was 5.5 (1.5)% at a maximum tendon stress of 16.8 (4.7) MPa at baseline; strain increased to 7 (1.7)% (P < 0.05; 27% increase in tendon strain) at a tendon stress of 12.2 (4.4) MPa (P < 0.01; 27% reduction in tendon stress) after bed rest (Fig. 5). Tendon stiffness decreased by 58% (P < 0.01) over the force interval of 250–500 N following the period of unloading (Fig. 6). The corresponding tendon Young’s modulus decreased by 57% from 266.3 (137.5) to 113.6 (62.4) MPa after bed rest (Fig. 7; P < 0.01). Tendon length [pre-bed rest: 221.3 (10.6) mm; post-bed rest: 219.6 (13.9) mm] and CSA [pre-bed rest: 102 (13.5) mm²; post-bed rest: 100.3 (10.5) mm²] remained unchanged by the period of unloading (P > 0.05).

Effect of exercise countermeasures during unloading on tendon mechanical properties. Tendon deformation was 11.6 (2.7) mm at a maximum tendon force of 545 (166.1) N at baseline; following the intervention period, deformation increased to 13.5 (3.2) mm (P < 0.05; 16% increase in tendon deformation) at a tendon force of 466.8 (187.4) N (P < 0.01; 14% reduction in tendon force; Fig. 4). Tendon strain was 5.3 (1.3)% at a maximum tendon stress of 16.7 (5.5) MPa at baseline; strain increased to 6.2 (1.7)% (P < 0.05; 17% increase in tendon strain) at a tendon stress of 14.3 (6.1) MPa (P < 0.01; 14% reduction in tendon stress) following unloading combined with exercise training (Fig. 5). Following the intervention period, tendon stiffness decreased by 37% (P < 0.01) over the force interval of 250–500 N (Fig. 6). The corresponding tendon Young’s modulus decreased by 38% from 303.4 (150.8) to 187.2 (100.5) MPa after unloading combined with exercise training (Fig. 7; P < 0.01). Tendon

RESULTS

There were no significant baseline differences between the BR and BREx groups for any of the reported variables.
length [pre-bed rest: 220.4 (19.6) mm; post-bed rest: 218.7 (18.8) mm] and CSA [pre-bed rest: 99.3 (13.1) mm²; post-bed rest: 99 (11) mm²] remained unchanged following unloading, in combination with exercise countermeasures (P > 0.05). The relative decline in tendon stiffness and Young’s modulus was significantly greater in the BR group compared with the BREx group (Figs. 6 and 7; P < 0.01). There was no significant difference between the two groups in the relative decline in maximum tendon force.

DISCUSSION

The present study aimed to elucidate the effects of chronic unloading on the mechanical properties of human tendon and to examine the potential preventive effects of resistive exercise performed during the period of unloading on tendon mechanical properties. Our findings show that 90 days of unloading resulted in a reduced structural and material stiffness of human tendon, and, although the exercise regimen performed did attenuate these detrimental effects, it did not completely prevent them. The present study may be considered unique in terms of the duration of unloading (90 days); few studies have investigated physiological adaptations to longer periods of unloading (35, 36).

Effect of unloading on tendon mechanical properties. Actual or simulated microgravity has been previously shown to cause marked atrophy of skeletal muscle (10, 11, 24, 34, 35, 49). Together with alterations in neural drive, this morphological deterioration is largely responsible for the considerable loss of strength experienced at the level of the whole joint system (10, 11, 27, 61). By virtue of their anatomical location (in series with skeletal muscle), tendons have the capacity to influence joint system function. Although the deterioration in muscle size and activation with unloading are clearly major factors, the present study has shown that tendon is also adversely affected and may play a role in determining this functional decline.

After 90 days of simulated microgravity, we observed a 58% decrease in gastrocnemius tendon stiffness in the absence of any tendon atrophy (Fig. 6). The tendon stiffness value of ~130 N/mm found at baseline in the present study is comparable to values previously reported for the gastrocnemius and tibialis anterior tendons of 150 and 161 N/mm, respectively (41, 42). Similarly, we found a 57% decrease in the normalized tendon stiffness, the Young’s modulus, indicating that the reduction in structural stiffness was exclusively due to changes in the material properties of the tendon (Fig. 7). These findings are consistent with animal data showing reduced structural and material stiffness of collagenous structures following 8–9 wk of immobilization (50, 51, 66). A reduced stiffness of tendon structures has also been reported to occur in humans following 20 days of bed rest (30, 31). The present findings do not enable elucidation of the mechanism(s) accounting for the change in tendon material properties following unloading. However, data from animal studies suggest that unloading may alter the mechanical properties of collagenous tissues through changes in both the extracellular matrix and the fibrous structures. Reductions in the concentration of ground substances such as water, hyaluronic acid, and glycosaminoglycans have been observed following periods of unloading (1–3, 57). Although the amorphous ground substance does not primarily fulfill a mechanical function, artificial removal of this component has been shown to reduce the stiffness of human tendon (46). The arrangement, thickness, and the cross-linking of collagen fibers can be affected by unloading (3, 67), all of which are factors that would adversely affect the tissue’s mechanical properties.

In terms of the functional implications of the present findings, the reduced tendon stiffness following unloading means that, for any given level of contractile force production, the deformation of the tendon would be greater postintervention (Fig. 4), implying that muscle fibers would shorten more. The
gastrocnemius muscle acts on the ascending limb of the sarcomere length-tension relation (26, 37). Theoretically, if all other conditions remained constant by unloading, the reduced tendon stiffness would result in a left shift of the length-tension relation, thus causing a decline in force. Therefore, independent of morphological changes to skeletal muscle, the decrease in tendon stiffness occurring after 90 days of simulated microgravity could potentially reduce maximal tendon force and joint torque output.

Clearly however, it is not only tendons that are affected, it is well known that human muscle size declines considerably during actual or simulated microgravity (11, 14, 24, 33–35, 49). While unloading has been shown to cause muscle atrophy, little is known about how changes at the whole muscle level will affect the internal structure. It has been reported that fascicle lengths and pennation angles are reduced in the gastrocnemius muscle of patients affected by unilateral lower limb muscle disuse atrophy (48). In line with these observations, animal models have shown that immobilization at a short muscle length reduces the number of sarcomeres in series (58, 65). Changes in the number of sarcomeres in series may affect the degree to which muscle fibers shorten during contraction (illustrated in Fig. 1 of Ref. 54). It may be the case that adaptations occurring in muscle and tendon compensate each other to maintain the muscle’s operating range constant, an adaptation strategy shown to occur following increased loading levels in elderly humans (54). The increased tendon strain for any given level of tendon stress following unloading may predispose the tendon to strain injury. Although the increased susceptibility of strain injury post-unloading may be partially compensated for by the lower tendon stresses generated via voluntary muscle contraction, externally imposed eccentric loads are likely to represent the greatest risk.

In many daily situations, while the ability to produce high muscle forces or joint torques is important, the capacity to generate torque rapidly may be even more essential. The rate of torque development (RTD) depends on a number of factors: 1) the duration of the excitation-contraction coupling process, 2) the force-velocity characteristics of the muscle fibers (even during an isometric contraction due to the deformation of tendon structures), and 3) the stiffness of the series elastic component. Thus it seems likely that the reduction in tendon stiffness after unloading might be associated with a decrease in the RTD. In a subsample of five participants from the BR group, the RTD was tested during a maximal isometric plantar flexion effort performed as rapidly as possible, and it was found that the RTD decreased by 38% following the period of unloading. This seems to support the theoretical association between a decrease in tendon stiffness and a slowing in the rate of contractile force transmission.

Effect of exercise countermeasures during unloading on tendon mechanical properties. Exercise training during the period of bed rest did not prevent the detrimental effects on the gastrocnemius tendon. Following unloading combined with exercise countermeasures, tendon stiffness decreased by 37% and the tendon Young’s modulus by 38% (Figs. 6 and 7). Consistent with the lack of any change in tendon dimensions, these findings indicate that the decrease in stiffness was attributed exclusively to changes in tendon material properties. The exercise training did, however, attenuate to a certain extent the decline in tendon stiffness and the Young’s modulus compared with unloading without countermeasures (Figs. 6 and 7).

The flywheel resistive exercise device used in the present study has been previously shown to increase strength and cause muscle hypertrophy in the knee extensors of ambulatory subjects (60) and to prevent muscle atrophy and weakness in the knee extensors of individuals undergoing unilateral lower limb unloading (61). In ambulatory humans, resistive training using conventional exercise devices has been shown to increase tendon stiffness (32, 53). Therefore, given the known beneficial effects of exercise loading on the tendon and the established effectiveness of the flywheel device in terms of its influence on skeletal muscle, it may be considered surprising that the detrimental effects on the tendon were not completely prevented by the present regimen. However, it should be noted that, with few exceptions (6, 56), the majority of previous unloading studies, including those involving the flywheel device, have shown these positive effects on the knee extensor muscles (5, 7, 27) but not on the plantar flexors. The antigravity extensor muscle groups at the knee and ankle are the most severely affected by exposure to actual or simulated microgravity (5, 24, 34, 35) due to the high levels of loading imposed on these muscle groups under normal gravitational conditions on Earth. Although both of these muscle-tendon units act to oppose gravity, the plantar flexors are habitually subjected to higher loads and might, therefore, be affected to a greater extent by unloading compared with the knee extensors (5, 7), thus requiring a greater loading volume to maintain their size and function. This is supported by data from the same study (LTBR 2001–2002) showing that, following 90 days of bed rest combined with exercise training, knee extensor muscle atrophy was completely prevented, whereas plantar flexor muscle volume decreased by 15% (8). In further support of this concept, exercise training during 20 days of bed rest involving both the knee and ankle extensors actually increased knee extensor PCSA, whereas plantar flexor PCSA declined to the same extent (~12%) as in the subjects undergoing bed rest without exercise countermeasures (5). It should also be considered that differences in protein synthesis and degradation rates might contribute to the reduced responsiveness of the plantar flexors compared with the knee extensors. For instance, despite having similar resting rates, the soleus muscle shows a much smaller increase in the rate of protein synthesis compared with the vastus lateralis muscle in response to a single bout of high-intensity resistive exercise (16, 52, 62).

In the present study, the exercise training was performed every third day, which meant that, in some instances, only two sessions were performed in 1 wk. Although the number of repetitions performed for the calf-press exercise (14 repetitions) was increased with respect to that for the leg-press exercise (7 repetitions), the total volume of loading appears to have been insufficient to maintain gastrocnemius tendon mechanical properties. During gravitational loading experienced on Earth, the plantar flexor tendons are subjected to high repeated loads associated with a “springlike” action due to the continuous application and removal of muscle forces required to withstand body weight and to propel the body forward (21). It is, therefore, likely that during unloading, the total exercise volume (loading level, frequency, and duration) needs to exceed a threshold level to completely prevent alterations in tendon mechanical properties. In the present study, during calf
HUMAN TENDON RESPONSES TO SIMULATED MICROGRAVITY

raises performed using the flywheel device, the Achilles tendon stress can be estimated as \(\sim 68 \text{ MPa} \) [calculated by using flywheel forces reported in Ref. 7, external moment arm lengths and tendon CSAAs measured in the present study, and Achilles tendon moment arm length values from the literature (39)]. During walking, it has been estimated using the fiber-optic technique that the loads generated correspond to an average Achilles tendon stress of \(\sim 21 \text{ MPa} \) (19). For a 70-kg person, simply raising and lowering their body mass at 1 G would result in an Achilles tendon stress in the region of \(\sim 20 \text{ MPa} \). If it is assumed that habitual walking is a sufficient stimulus to maintain tendon mechanical properties under normal gravitational conditions on Earth, the threshold level of loading required to prevent any deterioration during unloading may need to approximate or exceed body mass. Thus it appears the level of exercise loading used in the present study may have been sufficient; however, the exercise regimen performed during unloading was well below the frequency of loading due to habitual walking. It is, therefore, likely that the frequency of loading needs to be increased to exceed a threshold volume. This concept is supported by a recent 20-day bed-rest study showing that plantar flexor strength and muscle size are maintained when exercise was performed almost daily (16 of the 20 days) with loads approximating body mass (6). Placing the present results in the wider context of musculoskeletal adaptations, muscular contraction generates the forces acting on the tendon, and unloading-induced muscle atrophy would result in a decline in these forces and hence the stress imposed on the tendon. Given that alterations in tendon stress are likely to be the stimulus for adaptation in tendon mechanical properties, it is likely that changes in muscle and tendon will occur in the same direction. However, little is currently known regarding the time course of muscle and tendon adaptations with alterations in loading levels. The present findings have implications for patients confined to bed following musculoskeletal surgery; the recommendation would be to reamputate patients as soon as possible to avoid deterioration of tendon mechanical and material properties, which may confound any existing muscle/joint function problems.

Relating to the approach followed to estimate tendon mechanical properties in the present study, a number of assumptions have been made. First, it has been assumed that the relative CSA of the gastrocnemius muscle remained unchanged with respect to the entire plantar flexor CSA post-unloading. If this assumption is invalid, it may result in errors in the estimation of tendon stiffness. Second, we have assumed that the gastrocnemius tendon occupies the same relative proportion of the Achilles tendon in both pre- and postconditions. If this assumption is invalid, it would introduce errors in the estimation of the tendon Young’s modulus. In support of the first assumption, it has been shown that, with aging, a phenomenon involving a large component of reduced loading, the decline in the relative CSA of the constituent muscles of the triceps surae, remains constant (47).

Tendon forces have been estimated neglecting the influence of cocontraction from antagonist muscles. To assess the degree of error introduced by this simplification, the level of antagonist cocontraction was assessed in a subsample of participants \((n = 8)\) using electromyographic measurements taken from the tibialis anterior muscle by following previously applied methods (e.g., Refs. 28, 40, 53). The level of cocontraction in this sample was \(\sim 5\% \) (expressed relative to the level of activity from the same muscle when acting as an agonist during maximal dorsiflexion) and was unaltered postintervention. Furthermore, at the specific joint position studied in the present investigation (20° of dorsiflexion), the maximum dorsiflexion torque was very low, and hence, any given level of cocontraction, however large, would have a very minimal impact on the resultant plantar flexion torque and hence the tendon force. Thus the tendon forces presented are only a small underestimate of the true values, but more importantly the pre- and postintervention comparison is valid.

In the present study, tendon stiffness was estimated from tensile displacement measurements of the myotendinous junction. An alternative approach could have been to measure the displacement of an intramuscular anatomical point. With the present approach, it can be assumed that the forces generated by the whole muscle are acting to displace the measured point. In contrast, when measuring the displacement of an intramuscular anatomical point, it may only be fibers proximal to the specific site selected that are causing its displacement. This may complicate the estimation of forces acting on this point, especially given that submaximal human muscle contraction is compartmentalized (4, 70), likely causing a heterogeneous force and displacement along the length of the aponeurosis as contraction intensity increases up to the maximum.

The dynamometer used in this study requires the knee joint to be flexed at 90° to compensate for the slack gastrocnemius muscle-tendon unit length in this position, the ankle joint was dorsiflexed by 20°. This joint configuration ensures that the gastrocnemius muscle-tendon unit length is similar to when the knee joint is in full extension with a neutral ankle position (see METHODS for calculations relating to this joint configuration), but the Achilles tendon passive tension may be affected by dorsiflexing the ankle, because it is composed of both the gastrocnemius and the soleus (a uniarticular muscle) tendons. Although the anatomical reference point tracked in the present study (the gastrocnemius myotendinous junction) was proximal to the Achilles tendon, the contraction-induced displacement of this point will be influenced by any distal structures fused with this tendon. It has been shown that there is a differential displacement between the tendon-aponeuroses of the gastrocnemius and soleus muscles during isometric plantar flexion contractions, both with the knee joint flexed and extended (17). These results, in combination with observations during other studies on the Achilles tendon (44), indicate a certain degree of shear within the Achilles tendon, suggesting some independent movement of the gastrocnemius and soleus tendons.

It has been shown that, after 17 days of spaceflight, electrically evoked plantar flexion torque continues to decline, even after the return to Earth, up until the 8th day of reambulation (49). In contrast to joint torque, muscle CSA does not continue to decline further during reambulation, but it progressively recovers to preflight size (35, 49). This suggests that the continuing strength decline observed during reambulation may be related to muscle damage induced by reloading at 1 G, a concept supported by the elevated MR T2 transverse relaxation time (T2) observed to persist for several weeks of reambulation following both short- (17 days) and longer term (16–28 wk) spaceflight (35). Conducting postintervention measurements 2 days after reambulation in the present study may have induced
a slightly greater decrement in joint torque compared with the situation immediately after unloading. However, tendon stiffness is measured as the slope of the tendon force-deformation relation, and hence the possibility of slightly lower tendon forces induced by reambulation would not affect the tendon stiffness values. Nevertheless, the effects of reambulation following unloading on the tendon remain an issue for future investigation.

In conclusion, 90 days of simulated microgravity resulted in a reduction of gastrocnemius tendon stiffness due to changes in the material properties of the tendon, and, although these adverse effects were attenuated, they were not completely prevented by the countermeasures performed using a gravity-independent exercise device. It is, therefore, suggested that the exercise volume did not exceed a threshold level required to completely prevent alterations in tendon mechanical properties.

ACKNOWLEDGMENTS
The long-term bed rest study 2001–2002 was organized by the European Space Agency, together with the Centre National d’Études Spatiales, and the Japanese National Space Development Agency. Many thanks to all of the very dedicated staff at the MEDES Institute for Space Medicine and Physiology in Toulouse, France, and, in particular, to Dr. Marie-Pierre Bareille, Dr. Alain Mailet, and Dr. Jacques Bernard. The authors are very grateful to the volunteers for their excellent dedication to the study. The investigators principally responsible for the flywheel testing and training were Prof. P. Tesch and Dr. B. Alkner. The photograph of the flywheel (Fig. 1) was provided courtesy of these investigators.

GRANTS
This study was partially supported by funds from the Italian Space Agency. Support by the Swiss National Science Foundation is acknowledged (Grant 31–64267.00, G. Ferretti).

REFERENCES