Title
Skeletal muscle unweighting: spaceflight and ground-based models

Journal
Journal of Applied Physiology, 95(6)

ISSN
8750-7587 1522-1601

Authors
Adams, Gregory R
Caiozzo, Vincent J
Baldwin, Kenneth M

Publication Date
2003-12-01

DOI
10.1152/japplphysiol.00346.2003

J Appl Physiol 95: 2185–2201, 2003;
10.1152/japplphysiol.00346.2003.

Skeletal muscle unweighting: spaceflight and ground-based models

Gregory R. Adams,[1] Vincent J. Caiozzo,[1,2] and Kenneth M. Baldwin[1]
Departments of [1]Physiology and Biophysics and [2]Orthopaedics, College of Medicine, University of California, Irvine, California 92697-4560

Adams, Gregory R., Vincent J. Caiozzo, and Kenneth M. Baldwin. Skeletal muscle unweighting: spaceflight and ground-based models. *J Appl Physiol* 95: 2185–2201, 2003; 10.1152/japplphysiol.00346.2003.—Long-term manned spaceflight requires that flight crews be exposed to extended periods of unweighting of antigravity skeletal muscles. This exposure will result in adaptations in these muscles that have the potential to debilitate crew members on return to increased gravity environments. Therefore, the development of countermeasures to prevent these unwanted adaptations is an important requirement. The limited access to microgravity environments for the purpose of studying muscle adaptation and evaluating countermeasure programs has necessitated the use of ground-based models to conduct both basic and applied muscle physiology research. In this review, the published results from ground-based models of muscle unweighting are presented and compared with the results from related spaceflight research. The models of skeletal muscle unweighting with a sufficient body of literature included bed rest, cast immobilization, and unilateral lower limb suspension. Comparisons of changes in muscle strength and size between these models in the context of the limited results available from spaceflight suggest that each model may be useful for the investigation of certain aspects of the skeletal muscle unweighting that occur in microgravity.

atrophy; unloading; bed rest; limb suspension; immobilization

WHEREAS MUCH OF THE CURRENT focus of various national space programs is on the International Space Station (ISS), the long-term intent is clearly to send manned missions to other planets (71). True interplanetary travel will represent a fundamental shift in the structure of manned spaceflight. To this date, the crews of various space vehicles or orbiting stations were seldom more than a few days' travel time away from Earth. An interplanetary mission will require flight crews to function autonomously for much greater time periods, on the scale of years, with no access to Earth. Before undertaking such missions, significant impediments to long-term spaceflight must be identified, understood, and overcome.

The general goals of this review will be to provide *1*) a brief examination of the unique physiological environment created during spaceflight; and *2*) a discussion of the results from the most prevalent ground-based models used to study spaceflight-related physiological adaptation. These two areas will be presented in the specific context of the adaptations of skeletal muscle that are thought to occur during spaceflight.

Address for reprint requests and other correspondence: G. R. Adams, Dept. of Physiology & Biophysics, Univ. of California, Irvine, Medical Sciences 1, Rm. D335, Irvine, CA 92697-4560 (E-mail: GRAdams@uci.edu).

The adaptation of skeletal muscle to microgravity can be studied at many different levels [e.g., capillary density, mitochondrial density, Ca^{2+} cycling by the sarcoplasmic reticulum, maximal isometric tension (P_o), muscle cross-sectional area (CSA), etc.]. From a conceptual perspective, perhaps the single best measure of muscle function in situ is the force-velocity relationship (see Fig. 1*A*). The reasons for this are as follows. First, this relationship describes the maximal force, work, and power that can be generated at any given shortening velocity. In a sense, this relationship represents a design constraint, such that skeletal muscle can only operate on or below this relationship: never above it. Additionally, it should be noted that the force-velocity relationship defines the metabolic costs of muscle performance. Second, there are important biochemical and molecular correlates with P_o and V_{max}. P_o is known to be highly correlated to the CSA of the muscle or, stated another way, the number of sarcomeres in parallel. V_{max} has been shown to be highly correlated with the myosin heavy-chain isoform composition of the muscle. Because of these important correlations, alterations in muscle function, as defined by the force-velocity relationship, can indirectly provide important insights regarding both the amount and types of contractile proteins. Third, the shape of the

 8750-7587/03 $5.00

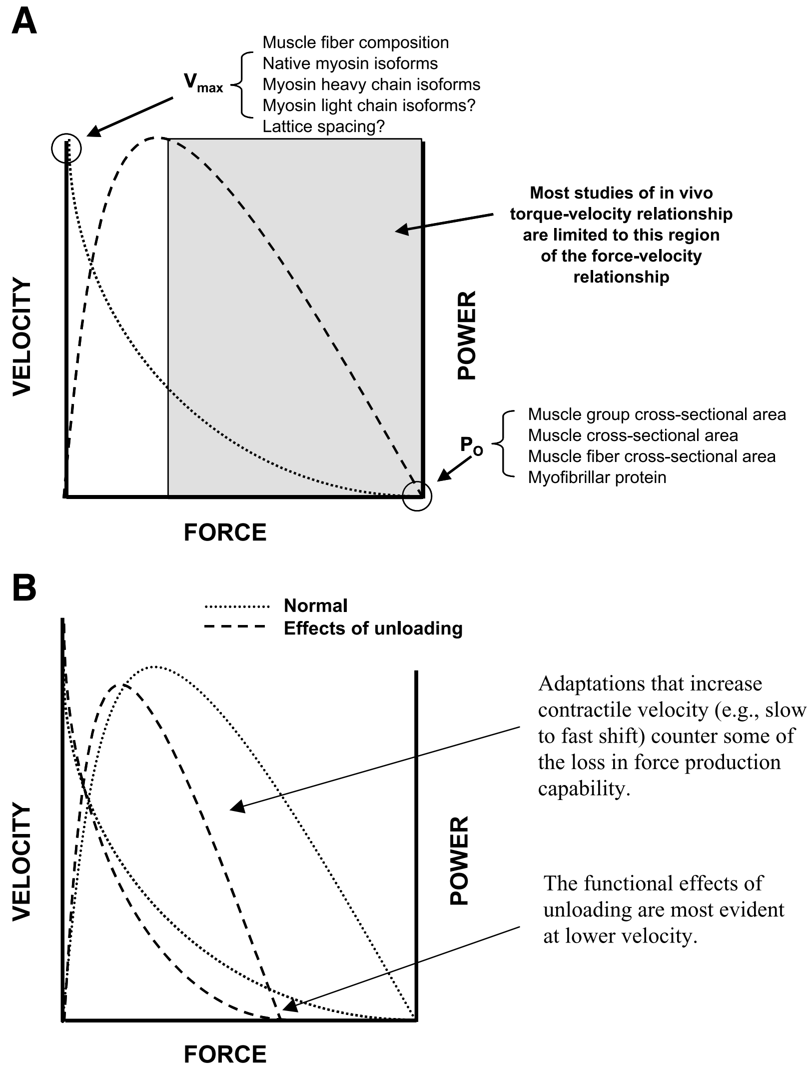

Fig. 1. *A*: force-velocity relationship (dotted line) and power curve (dashed line) for hypothetical muscle. The force-velocity relationship represents an important conceptual perspective regarding muscle function for several reasons. First, this relationship describes the amount of force that can be produced at any given shortening velocity. Therefore, interpretation of changes in muscle function is not restricted to a single point [e.g., maximal isometric tension (P_o)] along this continuum. Second, this relationship is determined with the muscle fully activated. Hence, the force-velocity relationship represents a design constraint, and all forms of activity occur either on or below the force-velocity relationship. Third, mechanical power is the mathematical product of 2 factors: force and velocity. Many activities are dependent on the production of mechanical power. Hence, the power curve has very important functional implications. *B*: alterations in the force-velocity relationship and power curves produced by the mechanical unloading of skeletal muscle that is associated with bed rest, immobilization, and unilateral lower limb suspension (ULLS). As noted in the text, each of these forms of mechanical unloading seems to result in a speed-specific loss of muscle function, as defined by the force-velocity relationship and power curve. The speed-specific loss of function is characterized by a large reduction in force production at slow shortening velocities. This has obvious effects on the production of mechanical power in this region of the force-velocity relationship. At higher shortening velocities, there is less loss of function (i.e., force), and, as a result, maximal power is partially or completely preserved. Shifts in myosin isoform composition induced by unloading result in an increase in maximal shortening velocity as identified in studies that have involved both human and nonhuman species.

force-velocity relationship reflects the intrinsic properties of cross-bridge cycling. Given this perspective, analogous measurements of muscle function (e.g., torque-velocity relationships, maximal isometric strength) will serve as the conceptual framework of this review, incorporating a discussion of alterations in the underlying cellular and molecular basis of muscle function. One might criticize such an approach on the basis that such measurements might not correlate with specific tasks. Clearly, the importance of using the force-velocity relationship is not to necessarily correlate it with specific tasks that require repetitive movements at submaximal forces. Force-velocity measurements will never satisfy such a criteria unless the specific task lies on or close to this relationship. Rather, the utility of the force-velocity relationship is that it provides a description of muscle function across a broad spectrum of loading conditions and easily definable cellular and molecular correlates. This relationship not only dictates the maximal force that can be generated at any given shortening velocity but also sets the limit of work and power that can be produced under any loading condition. Hence, analogous measurements of in vivo muscle function (e.g., torque-velocity relationships, maximal isometric strength) will serve as the conceptual framework of this review, incorporating a discussion of alterations in the underlying cellular and molecular basis of muscle function.

THE MICROGRAVITY ENVIRONMENT OF SPACEFLIGHT

During spaceflight, exposure to the microgravity environment induces a number of physiological adaptations in the human body. Of particular interest for this review, the relative unweighting experienced by various skeletal muscles in space results in structural and functional adaptations appropriate to the reduced loading demands imposed in this novel environment (0 g). Skeletal muscle adaptations include changes in the expression of metabolic, structural, and contractile proteins that fine-tune the function of this tissue. Because many adaptations occur on the time scale of days or weeks, the ability to move abruptly from microgravity to planetary gravity renders these otherwise appropriate muscle adaptations a potential liability. In contrast to this unique circumstance, the loss of muscle mass and function that occurs at 1 g when muscles are disused or unloaded as a result of illness or injury is generally recovered gradually as an individual returns to normal weight-bearing activities. However, when flight crews enter planetary gravity after spaceflight, the requirement for alteration in function is immediate.

In conjunction with its international partners, the US National Aeronautics and Space Administration (NASA) has identified a number of specific risk factors and attendant functional consequences that must be overcome to allow autonomous long-term spaceflight (71). Of the 12 major areas of concern detailed in the NASA Critical Path Roadmap, a number of the identified risks and potential impacts of long-term spaceflight were associated with skeletal muscle responses.

The risks germane to skeletal muscle that were identified in this report (71) included muscle atrophy, altered motor performance, decreased muscle strength and endurance, nutritional deficiencies, altered muscle phenotype, and hormonal imbalances.

The anticipated impacts of these risks were reported to include the following:[1] *1*) inability to perform emergency egress during landing and in partial gravity due to significant losses of muscle mass, strength, and/or endurance; *2*) inability to perform a variety of tasks associated with microgravity extravehicular activity and daily activities due to reduced motor performance, reduced muscle endurance, and disruptions in the structural and functional properties (including phenotype) of the soft and hard connective tissues of the axial skeleton (e.g., in intervertebral disk, lower back syndrome); *3*) the inability to sustain muscle performance levels to meet the demands of performing mission-specific activities of varying intensities, including deficits that may occur in the maintenance of gait and posture that are required to complete these activities, and an additional concern is that sufficient nutritional provisions may not be available to provide the necessary substrate energy to complete these tasks; *4*) deficits in skeletal muscle structure and function can impact the homeostasis of other systems including neurovestibular and motor performance, vascular compliance, and circulatory function impacting blood pressure regulation (e.g., skeletal muscle pump), soft and hard connective tissue integrity (e.g., tendon, bone, cartilage), and local vs. general (e.g., tendon-bone interaction).

It is clear that a number of the risks associated with skeletal muscle would have to be eliminated or at least substantially minimized before a long-term autonomous spaceflight mission could proceed. The integrated nature of muscle, bone, and other systems precludes approaches that would allow the atrophy to proceed and then rely on rehabilitation efforts at the end of missions.

[1]*Items 1–4* are directly excerpted from the CPR document.

SPACEFLIGHT AND SKELETAL MUSCLE: THE DATABASE

Formulation of the perceived risks associated with spaceflight was made in the context of previous observations from both spaceflight missions and ground-based studies. Some physiological consequences of spaceflight initially became evident following the 8- to 10-day Apollo missions (12). Systematic evaluation of spaceflight-induced skeletal muscle adaptation began with the Skylab missions in the 1970s.

Muscle Strength Measurements After Spaceflight

Both NASA and the Russian space programs have had space laboratories dedicated to scientific investigation of the microgravity environment from which a number of studies have been published. In addition, a number of more recent space transportation system (STS; i.e., shuttle) missions have included specific experiments designed to assess the effects of short-term spaceflight on skeletal muscle. Longer term muscle physiology experiments, including muscle performance, muscle size, and single muscle fiber analysis are currently slated for the ISS but have not been completed at this time.[2] As noted after the US Skylab series of missions (28, 56, and 84 days), exposure to microgravity generally resulted in decrements in skeletal muscle performance and size (94). Specifically, the strength of the knee flexors (peak torque, 45°/s), measured 5 days after landing, was decreased ~20% after the 28- and 56-day missions but was unchanged after the 84-day mission (see comments below) (37). The results from 140- and 175-day missions on space station Mir indicated that there was a "considerable decrease in gastrocnemius muscle strength characteristics, especially when working with the isometric and high rate (180°/s) regimens" (54). Another series of ~6-mo Mir missions found that isometric maximal voluntary contractions (MVC) of the triceps surae muscle group decreased by ~42%, whereas peak tetanic force (P_o) decreased ~25% (52). The results from 19 STS crew members who were in space for ~11 days indicated that maximal knee extension strength (concentric) decreased by ~10%, whereas trunk flexion strength was decreased by ~20% (34). In contrast to these reports, four crew members from the 17-day STS-78 mission [Life and Microgravity Sciences (LMS) mission] demonstrated no decrease in calf muscle strength (MVC) (68, 70, 97).

Taken together, the majority of the published results of strength testing after spaceflight indicate that significant declines in strength occur after exposure to microgravity. These results should be interpreted in the context of the fact that NASA flight rules require that all crew members must exercise on spaceflight missions of durations longer than 10 days, suggesting that the actual unweighting-induced deficits may have

[2]A number of measurements have been used after spaceflight to characterize muscle performance. We will use this general term to represent measurements related to muscle strength.

been underestimated. The type and volume of the countermeasure exercises used during these missions have been quite variable, but they have predominantly been more similar to Earth-based endurance types of activities (e.g., high repetition, low force). Interestingly, the results from Skylab 4 (84 days) indicated that dietary controls (e.g., caloric intake requirements) and a more comprehensive exercise package could significantly ameliorate skeletal muscle performance decrements (94). Consistent with this suggestion, the combination of flight crew voluntary exercise and multiple in-flight experiment-related muscle strength performance measurements appears to have acted as an effective countermeasure to unweighting-induced muscle performance decrements during the recent 17-day STS-78 mission (68, 70, 97).

Whole Muscle Size Measurements After Spaceflight

The P_o that a muscle fiber can generate is dependent, to a large extent, on the number of sarcomeres functioning in parallel (i.e., muscle fiber CSA). Extrapolation of this fundamental principle implies that the P_o that a whole muscle can produce is dependent on muscle CSA. In this context, a number of studies examined the effects of microgravity on muscle CSA as determined by various volume and imaging techniques.

Results from the Skylab missions included substantial decrements in leg volume (7–10%) (94). As with strength, the decrement in leg volume was partially ameliorated by the enhanced exercise and dietary regimens included on the Skylab 4 mission. In contrast, after 140- and 175-day Mir missions, Kozlovskaya et al. (54) found only small, transient decreases in leg circumference and, therefore, concluded that there was "no great muscular loss in flight." However, after 112–196 days on Mir, LeBlanc et al. (60, 88) found that crew members had significant decreases in leg muscle volume (e.g., 19% in the gastrocnemius and soleus and ~10% in the quadriceps muscle group). The results reported by LeBlanc et al. (60) were obtained by using MRI, suggesting that methodological differences could account for these divergent reports. In addition, the time elapsed for testing after the return to a 1-g environment has tended to vary significantly on Mir missions. It should also be noted that flight crews on various missions have participated in widely varying types and amounts of physical activity, including exercise intended as a countermeasure to muscle loss and general deconditioning.

After short-term STS missions (i.e., 8–17 days), decreases in gastrocnemius muscle CSA have been reported to range from 4 to 10% by using computed tomography (CT) or MRI (42, 60, 62, 70). Similarly, the CSA or volume of the quadriceps group has been reported to decrease between 5 and 15% after STS missions (30, 60, 62).

Muscle Fiber-size Measurements After Spaceflight

Several studies have examined muscle fiber characteristics after spaceflight. In considering these results, it must be emphasized that, as with both strength and whole muscle size measures, determinations of muscle fiber size have been made by using a number of different methodologies, including histological sections and single-fiber analysis. Using ATPase-based histochemistry on serial sections, Edgerton and colleagues (27, 108) found that the size of all muscle-fiber types from the vastus lateralis (VL) muscle decreased after 5–11 days of spaceflight [i.e., type I −16%, IIa −23%, and IIb −36%]. These authors also found that the percentage of type I myofibers decreased (6–8%). After the 17-day LMS Spacelab Mission spaceflight, Trappe et al. (97) found that there was no change in the size of single-muscle fibers from the soleus or gastrocnemius of four crew members. However, there was a great deal of between-subject variability in the single-muscle fiber characteristics from this mission, and some pre- to postflight decreases in the size of myofibers have been reported for individual LMS crew members (101, 104). As noted by the investigators, it is not clear whether these intersubject differences in myofiber response were attributable to spaceflight or were a function of the countermeasure exercise performed by the individual crewmembers. (97, 104).

Effects of Spaceflight on V_{max} and Power

It might be presumed that a 20% reduction in P_o and muscle CSA would translate proportionately throughout the force- or torque-velocity relationship, resulting in a similar decrease in the maximal power. In this context, however, it should be stressed that P_o only represents a single point along the force-velocity relationship and that a complete appreciation for functional alterations can only be obtained by making measurements throughout the entire force-velocity (or torque-velocity) relationship (Fig. 1). Additionally, it should be stressed that, whereas P_o is determined to a large extent by the number of sarcomeres in parallel, the opposite end of the force-velocity relationship (i.e., V_{max}) is largely dependent on fiber type and myosin isoform composition.

Due to technical limitations, it is not feasible to accurately describe the entire torque-velocity relationship of muscle groups, such as the knee extensors (KE) and flexors. Typically, the highest angular velocities used to test the KE approach 300°/s (~5 rad/s). The maximal angular velocity that the KE can generate has been predicted to be ~700–800°/s. Hence, isokinetic dynamometry for such muscle groups is restricted to ~40–45% of the in vivo torque-velocity relationship. Other muscle groups, such as the plantar flexors of the ankle, are not capable of reaching such high-angular velocities, and, as a result, the torque-velocity relationship of this muscle group can be studied more completely.

To date, the effects of microgravity (spaceflight) on the torque- and power-velocity relationships of key muscle groups remain poorly studied. Unfortunately, there has been a significant absence of any systematic study throughout the history of the space program.

This makes it difficult to determine whether there are so-called "speed-specific" or uniform alterations in the torque-velocity relationship. Kozlovskaya et al. (54) found that 175 days of spaceflight produced the greatest losses in ankle plantar flexor strength at high-angular velocities. Recently, Trappe et al. (97) examined the effects of 17 days of microgravity on the torque-velocity relationship of the ankle plantar flexors. These investigators reported that there were no differences pre- and postflight (please see earlier comments on the LMS mission).

As described above, it is not technically practical to describe the entire torque-velocity relationship of most muscle groups. Hence, our knowledge about the effects of spaceflight are limited to the high-to-moderate force regions of the torque-velocity relationship (Fig. 1). Importantly, it should be noted that mechanical unloading of skeletal muscle has been shown to produce slow-to-fast shifts in myosin isoform expression as described in rats, monkeys, and humans (16, 17, 46, 108). At moderate-to-high velocities, this has the effect of minimizing the functional consequences (e.g., reduction in maximal power) of muscle atrophy (see Fig. 1*B*).

In this context, the skinned single-fiber approach used by Widrick et al. (101, 104) provides potentially important insights about the effects of spaceflight on the V_{max} of skeletal muscle. The advantages of this approach are as follows: *1*) the confounding influence of neural drive is eliminated; *2*) the myofibrillar apparatus is fully activated independent of excitation and excitation-contraction coupling; and *3*) the entire spectrum of the force-velocity relationship can be examined, including measures of V_{max}. To our knowledge, only the LMS mission has included attempts to examine the effects of spaceflight on the contractile properties of human skinned single-muscle fibers (101, 104). Unfortunately, as noted earlier, the experimental design of this study makes it difficult to determine, with any certainty, the influence of spaceflight on specific tension (e.g., force/CSA), V_{max}, or maximal power of single fibers.

Some of the loss in muscle strength observed with mechanical unloading of skeletal muscle might also arise from decreases in neural drive. For instance, Lambertz et al. (56) recently reported that EMG activity of the plantar flexors was reduced by ~35–40% after 90–180 days of microgravity. Consistent with these findings, Koryak (50) observed a greater increase in MVC compared with electrically evoked contractions and interpreted this data to indicate a decrease in neural drive. If the unloading of skeletal muscle does produce a decrease in neural drive, it will be important to determine whether the loss is fiber-type dependent. If a decrease in neural drive occurs and is not fiber-type specific, then this should produce the speed-specific changes in the torque-velocity relationship noted above. In contrast, if the loss of neural drive is specific to fast fibers, then the greatest changes in the torque-velocity relationship should occur at higher angular velocities.

The Utility of The Spaceflight Physiological Database

It must be recognized that the spaceflight data discussed in the previous sections were collected over a relatively long period of time by using a rather heterogeneous mix of methodological approaches. However, the evaluation of legacy data is not a new problem for researchers. A more vexing problem is the lack of control that is inherent in the spaceflight data-gathering process. In general, spaceflight missions are not designed with the primary aim of conducting physiological studies. As a result, parameters that may significantly impact the data, such as the voluntary exercise conducted by crewmembers, can vary greatly. An example of this is the LMS mission (STS-78), launched by NASA in 1996. The LMS mission was specifically tasked to provide high-quality physiological observations. However, in practice, the sheer number of physiological measurements involving exercise, possibly coupled with an unrecorded volume of voluntary exercise activities on the part of the crew members, appear to have significantly interfered with some of the findings and their interpretations. For example, one group of investigators concluded that a "source of intersubject variation could be the amount and/or type of physical activity performed by the astronauts during the flight" (104). Furthermore, these researchers concluded that "the testing sequence employed during the spaceflight and bed rest may have served as a resistance training countermeasure to attenuate whole muscle strength loss" (97). In the case of the LMS mission, this complication is particularly unfortunate in that the potential for interference from the exercise testing regimens had been recognized after a parallel bed rest study conducted a year before the space mission (5). As a result of these types of complications, most areas of spaceflight-related physiological investigation must go forward without a clear "gold standard" for comparison with ground-based models.

GROUND-BASED STUDIES OF MUSCLE UNWEIGHTING

The opportunity to study human physiology during and after spaceflight has been and continues to be extremely limited. In addition to the relative infrequency of such opportunities, the sheer cost of spaceflight missions dictates that the flight crews will be tasked to complete a very ambitious schedule to maximize the science return. As a result of this scheduling, the percentage of time available for physiological investigations tends to be severely limited. A number of dedicated space life science missions have been completed. However, factors such as the relatively small number of participating crewmembers, the short flight durations, and the pressure to maximize return via ambitious scheduling have tended to limit the science return from the individual projects involving human muscle physiology. In addition, the above-mentioned limitations have generally precluded the inclusion of basic experimental design elements, such as control subjects and the ability to regulate variables such as diet and exercise.

As a result of these formidable limitations, much of the controlled investigation of physiological responses to spaceflight has been conducted by using ground-based models. In the following sections, we will attempt to evaluate the effectiveness of three ground-based models of spaceflight with specific regard to the adaptations that are thought to occur in skeletal muscle: *1*) bed rest, which involves an essentially complete minimization of weight-bearing activity on all postural body structures and tissues, as well as a significant decrement in activity and energy expenditure; *2*) limb immobilization (IM) via the casting of one lower limb, resulting in extensive restriction of motion for the targeted limb but with maintained ambulatory activity via crutches; *3*) unilateral lower limb suspension (ULLS), which results in the unloading of one lower limb without movement restriction and with the maintenance of ambulatory activity via crutches.

Due to the relative dearth of reliable spaceflight data related to skeletal muscle physiology, we have focused on measurements of skeletal muscle strength and size. It is most important to note that, within these categories, it has been necessary to include a widely heterogeneous set of data that spans a period of many years and methodologies. It should also be noted that a portion of the functional deficit seen after muscle unweighting cannot be accounted for by structural or phenotypic changes in the muscle itself. Thus a significant component of the adaptation of muscle to changes in loading resides outside the effector organ itself most likely in neurological mechanisms. The neural component of muscle function will not be covered in this review. Similarly, we will not address the impact of nutrition on lean body mass during spaceflight. Readers are directed to the excellent reviews by Stein on this subject (88, 89).

In general, the effects of spaceflight on skeletal muscle appear to involve primarily antigravity muscles. The overwhelming majority of the data related to spaceflight effects on skeletal muscle have been focused on lower limb muscles. Accordingly, the data presented and models discussed in this review will be similarly limited to the lower limb muscles. When presenting results from the literature, whenever possible, we have attempted to provide "baseline" data, representing the impact of a given unweighting model in the absence of any countermeasures. Unfortunately, there are few instances in which this same goal can be met with the data from spaceflight. From the inception of spaceflight, most missions of more than a few days duration included the opportunity for crewmembers to perform some type of exercise. In most of the available published reports, the volume of this exercise performed was not detailed. In general, the findings suggest that the majority of the attempts at countering the impacts of spaceflight on muscle have not been particularly successful. However, even in the essentially complete absence of spaceflight baseline data, it is clear that the various countermeasures have had some mitigating effect that was reflected in the magnitude of the various responses reported (e.g., Ref. 35).

BED REST

Bed rest has served as the primary ground-based model concerning microgravity effects on the musculoskeletal system. When combined with head-down tilt, this model appears to reproduce many of the cardiovascular and skeletal muscle-related aspects of the adaptation to microgravity.

Muscle Strength Measurements After Bed Rest

Relatively short durations of bed rest have been found to significantly impact skeletal muscle performance measures. There are reports of KE performance deficits, such as a 15% decrease in torque, are detectable after 14 days (6) and ~20% after 20–35 days of bed rest (25, 26, 31). Similarly, 35 days of bed rest have been found to result in ~25% decrease in performance of the gastrocnemius and soleus muscles (33, 59). Berg et al. (10) found that 6 wk of bed rest caused a ~29% decrease in KE torque. Taken together, the results from the 14-, 20-, 35-, and 42-day studies (6, 10, 25, 26, 31 ,33, 59) suggest that much of the performance deficit may accrue fairly early in the unloading process. In a series of 120-day bed rest studies, Koryak (47, 51, 53) reported that the MVC of the triceps surae group decreases ~45%. In these studies, female subjects generally experienced lesser decrements in performance (33–36%) compared with men (45%) (49, 51). After 120 days of bed rest, P_o was found to be decreased by ~24% in female subjects (49). After a similar period (17 wk) of bed rest, LeBlanc et al. (63) found that peak torque (60°/s) decreased 30 and 18% for the KE and the plantar flexors, respectively. LeBlanc et al. also reported that the impact of bed rest on muscle performance was much less at higher angular velocities.

Muscle performance measures after various durations of unweighting, due to either actual spaceflight or ground-based models, are presented in Fig. 2. In general, the results from bed rest studies appear to compare favorably with those obtained from spaceflight.

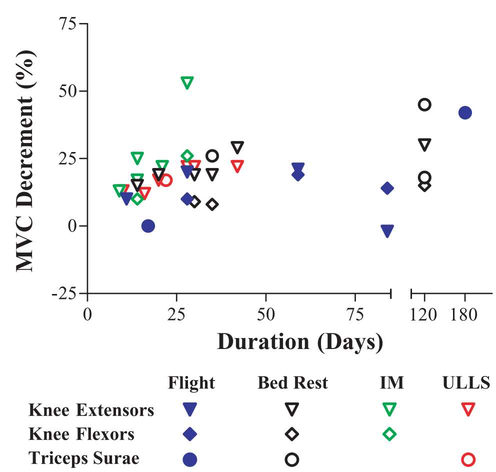

Fig. 2. The decrement in maximum voluntary contraction (MVC) performance induced by spaceflight (blue), bed rest (black), immobilization (IM; green), and ULLS (red). As noted in the text, a variety of methods were used to measure MVC in various studies.

This result is actually somewhat surprising, considering that most spaceflight missions have included varying amounts of exercise designed to counter decrements in performance, whereas the bed rest data presented in Fig. 2 were generated from nonexercising subjects. This could suggest that, in the absence of exercise countermeasures, spaceflight might induce a greater atrophy response than bed rest.

Whole Muscle Size Measurements After Bed Rest

The most common methods used to collect specific measurements of muscle size have been MRI, CT, and ultrasound. The majority of the data regarding muscle size measurements after bed rest have been focused on the lower limb. These data are fairly evenly distributed between reports on the muscles of the thigh and leg. Based on the majority of the literature, it appears that, as a group, the antigravity muscles of the thigh and leg demonstrate a similar sensitivity to bed rest-induced unweighting. As with the change in strength measures, thigh muscle volume decrements appear to occur rapidly after the imposition of bed rest. For example, using MRI, Ferrando et al. (28) reported a significant 3% decrease in thigh muscle volume after just 7 days of bed rest. A number of bed rest studies have reported values for the decrease in thigh or KE muscle size, ranging from ~6 to 11% after 20 days (4, 44, 45, 83, 92) and ~5 to 11% after 30 days (13, 19, 26). After 42 days of bed rest, size decreases of 14–17% have been reported for the KE muscle group (10, 28). LaBlanc et al. (63) found that ~120 days of bed rest resulted in a 15% decrease in thigh muscle volume, suggesting that much of the size decrement may occur at relatively early time points.

At earlier time points, the loss of calf muscle size appears to be similar to that seen in the thigh. For example, the reported range of calf muscle loss is 6–12% after 30–35 days of bed rest (13, 19, 26, 36, 59). A similar range of muscle size decrement, 10–12%, has been found after 17–20 days of bed rest (3, 4, 61). However, in contrast to their report on the thigh muscles, LaBlanc et al. (63) found that the decrease calf muscle was approximately twice that (i.e., 30%) seen in the thigh after ~120 days of bed rest.

Taken together, the results from bed rest studies suggest that much of the loss of lower limb antigravity muscle size occurs in the first few (i.e., 2–3 wk) weeks, most likely with some reduction in the rate of muscle loss thereafter. The pattern of muscle size change seen in bed rest studies appears to be very similar to that found after spaceflight (Fig. 3). Unfortunately, the number of post-spaceflight studies used to create this plot is relatively small. Nevertheless, considering the wide variation in data collection methods, as well as the above-mentioned differences in crew member countermeasure activities, the overall correspondence between spaceflight and bed rest models appears to be similar. One observation that begins to emerge when the spaceflight and bed rest results are compared is that, after longer durations of unloading, the calf muscles appear to demonstrate greater losses in size than the KE.

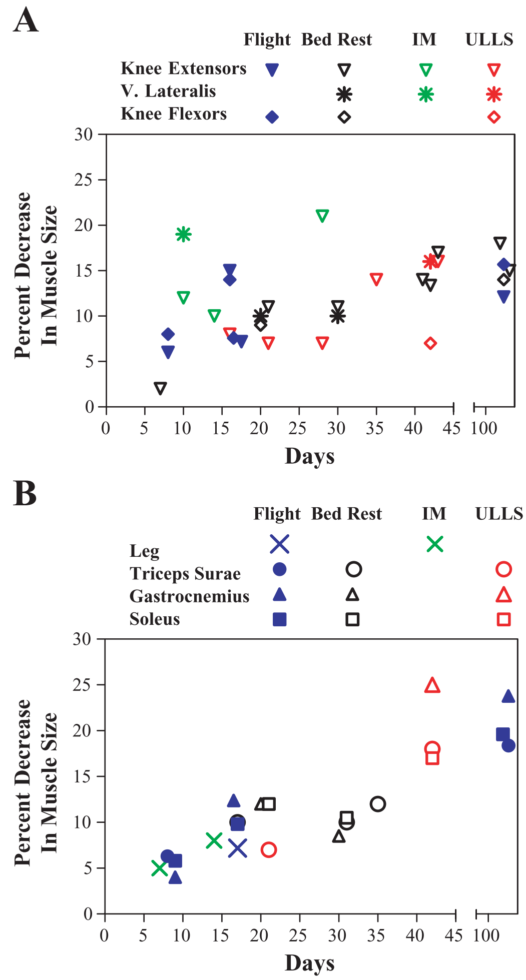

Fig. 3. The decrement in muscle or muscle group size induced by spaceflight (blue), bed rest (black), IM (green), and ULLS (red). The majority of the ground-based studies utilized either computed tomography or MRI scanning technology to determine the cross-sectional area or volume of lower limb muscles. *A*: knee extensors, vastus lateralis, knee flexors. *B*: leg, triceps surae, gastrocnemius, soleus.

Muscle Fiber-size Measurements After Bed Rest

The CSA of slow-twitch (ST) fibers from the soleus of male subjects has been reported to decrease anywhere from 7 to 29% after 60 days of bed rest and from 35 to 48% after 120 days of bed rest (73, 72, 86). Whereas the absolute value of the changes reported had some variability, a common feature of the data from each study was a continuing decrease in soleus ST fiber CSA over the 120-day period. The CSA of fast-twitch (FT) fibers from the soleus was reported to decrease by 34% at 60 days, but no further change was seen at 120 days (86). The change in both ST and FT myofiber size demonstrated a similar pattern in the gastrocnemius muscles of female subjects (86). In women, gastrocnemius ST fibers decreased by 23 and 35% at 60 and 120 days, respectively, of bed rest, whereas the change in FT CSA was 16% at both time points (86). Similar changes in fiber size (FT and ST) of male gastrocnemius muscle have been reported after 60 days of bed rest (55). Compared with the results from the soleus and gastrocnemius, the fiber-type sensitivity and pattern of change in the VL muscle was reversed. For example, in the VL, the ST fiber size was decreased by 16% at both 60 and 120 days. FT fiber CSA decreased by 13% at 60 days and continued to decline such that the decrement was 32% at 120 days (86). Measurements made after 30 days of bed rest suggest that the pattern of greater atrophy in FT vs. ST fibers from the VL may be established at this time point (e.g., FT fiber CSA −17%, ST fiber CSA −12%) (25, 40). However, in general, the results from bed rest studies of shorter duration indicate that, across both muscle and fiber type, the impact of unweighting on fiber size appears to be similar in both ST and FT myofibers (Fig. 4) (6, 10, 25, 40, 99, 103). The findings from the longer term bed rest studies indicate that some divergence in this response may eventually occur and thus establish a pattern of myofiber CSA loss that is specific to the muscle rather than being solely dependent on myofiber type.

There are very little data available for muscle fiber-size changes after spaceflight. However, the pattern of muscle fiber-size changes appears to follow that seen for whole muscle size measurements (e.g., Figs. 3 and 4). In general, the magnitude of the muscle fiber-size decrements appears to be greater than the changes in muscle volume or CSA observed via the various whole muscle imaging techniques. Given the small database, it is not clear whether this is a true difference or a result of methodological issues.

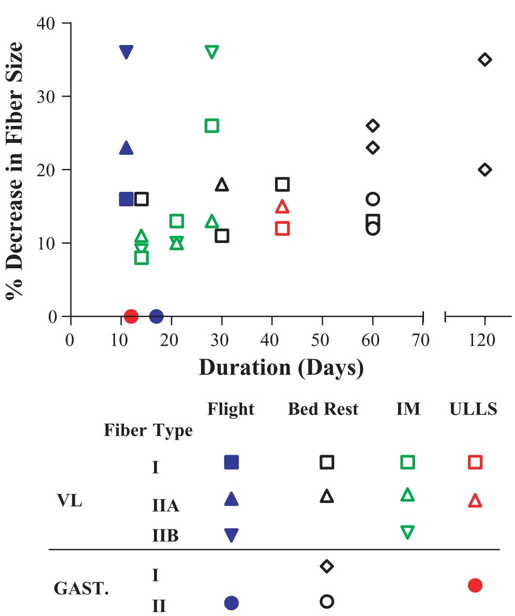

Fig. 4. The decrement in muscle fiber size induced by spaceflight (blue), bed rest (black), IM (green), and ULLS (red). The majority of studies reported histological cross-sectional area. However, several values are from single myofiber analysis. VL, vastus lateralis; Gast, gastrocnemius.

Effects of Bed Rest on V_{max} and Power

Dudley et al. (24) were one of the first groups to provide a good description of the effects of bed rest on the torque-velocity relationships of the KE and flexors. These investigators found that 30 days of bed rest significantly reduced torque production of the KE at all of the angular test velocities (0.52, 1.79, 2.103, and 4.19 rad/s). Interestingly, the greatest losses in KE torque occurred at the slower angular velocities. The torque-velocity relationship of the knee flexors were largely unaffected by 30 days of bed rest.

Subsequently, Berg et al. (10) examined the effects of 37 days of bed rest on the torque-velocity relationship of the KE. Consistent with the findings of Dudley et al. (24), Berg et al. (10) also observed that the greatest losses in torque occurred at the slower angular test velocities. Similar observations have also been made by Funato et al. (31) after 20 days of bed rest.

In contrast to these findings, it should be noted that Trappe et al. (97) recently reported that 17 days of bed rest did not affect the in vivo torque-velocity relationship of the ankle plantar flexors. As noted previously in this review, the LMS study of Trappe et al. was confounded by the testing regime (which may have constituted a countermeasure program) that was employed throughout the 17 days of bed rest. One of the intriguing findings of Trappe et al. and Widrick et al. (101) is that the in vivo torque-velocity relationship was unaffected in the high-velocity range, whereas it was reported in this same cohort of subjects that the maximal unloaded shortening velocity of skinned single fibers was increased. Currently, it is not clear what might account for these discrepancies.

Mechanical power is simply the mathematical product of two factors: force and velocity. When plotting mechanical power as a function of force, it becomes apparent that the power curve has a parabolic shape (see Fig. 1). Importantly, it should be noted that maximal power occurs at ∼30–40% of P_o. From a functional perspective, the speed-specific alterations of the in vivo torque-velocity relationship are quite important because they suggest that maximal power is preserved to a greater extent than what would be predicted by losses in P_o (see Fig. 1*B*). Such findings are consistent with that observed by using rodent animal models. For instance, our laboratory (16) observed that 6 days of microgravity exposure reduced P_o by 33%, whereas maximal power was only reduced by ∼9%. The maintenance of maximal power occurred due to a speeding of the muscle as reflected by an ∼20% increase in V_{max}. Widrick et al. (101) have also made similar observations on skinned single fibers in the soleus muscle of humans after spaceflight.

Is Bed Rest a Good Model For Spaceflight-induced Changes in Skeletal Muscle?

In the context of skeletal muscle physiology, there are a number of important considerations that must be made when evaluating an unweighting model. For example, it has been clearly established that much of the response of muscle to changes in loading state is mediated via mechanisms intrinsic to the muscle itself. This accounts for the specific adaptation seen when individual muscles experience a change in loading state. Conversely, circulating factors, such as cortisol and thyroid hormone, and a number of growth factors may mediate generalized somatic adaptations that will include changes in muscle mass and/or performance. Consistent with the idea that exposure to microgravity results in unweighting-induced atrophy, the results from spaceflight indicate that the adaptation of skeletal muscle is specific to those muscles that experience the loss of loading stimulus, e.g., primarily muscles with antigravity functions (60, 62). There are some reports that the stress of spaceflight is associated with acute changes in circulating factors that might be expected to result in a generalized catabolic response (58, 91). However, the general consensus is that the observed changes do not account for the muscle loss associated with spaceflight (88, 96). The evaluation of ground-based models designed to mimic spaceflight should, therefore, include examination of this local vs. central control processes and responses.

With some exceptions, most reports indicate that the changes in contractile performance and in muscle size seen with bed rest are not uniform (3, 4, 6, 22, 24, 33, 61, 63). For example, LeBlanc et al. (63) found that bed rest had a greater impact on ankle extensor performance and size compared with the changes seen in the ankle flexors. In addition, the impact of bed rest on the lower limb muscles is greater than that of muscles in the arms (63). Such findings indicate that the effects of bed rest on skeletal muscle are probably not mediated primarily via some central or circulating mechanism but rather are a response to the specific alterations in loading on the various muscle groups. In support of this supposition, McCall et al. (67) found that plasma testosterone, cortisol, T_3, and T_4, as well as the bioactive levels of growth hormone, were unchanged during bed rest. These findings suggest that the mechanisms that underlie bed rest-induced alterations in skeletal muscle size and performance are most likely similar to those that mediate the spaceflight response.

Variations and modifications of bed rest. A number of attempts have been made to improve on the bed rest model. One approach has been to use a dry immersion model in which the subjects are supported on fluid with a waterproof fabric. Whereas this model has been reported to result in muscle performance decrements (e.g., Ref. 48), the number of published findings is too low to justify the inclusion of this model in our comparisons. More recently, others have used the pharmacological induction of a hyperthyroid state in an attempt to enhance the impact of bed rest on skeletal muscle properties (65, 106, 107). These studies have resulted in potentially interesting findings involving an increase in circulating levels of myostatin, a potential inhibitor of muscle mass accretion (107). Unfortunately, it is unclear if such an observation represents the response to bed rest or is primarily a function of the pharmacological intervention. Exogenous T_3 can be expected to impact all thyroid-sensitive tissues, including, but not limited to, skeletal muscle. As an example of a potential unintended action, T_3 treatment can induce a dramatic decrease in TSH levels (e.g., Ref. 107). It has become evident that TSH has direct biological activity separate from its actions as part of the thyroid axis (7, 69, 85). For example, it has been suggested that TSH may exert anti-apoptotic activity in human skeletal muscle (69). The nonmuscle-specific effects of T_3 treatment, in conjunction with the finding that spaceflight is not associated with a hyperthyroid state, suggest that the use of this intervention, in combination with the bed rest model, may not be suitable for mimicking the actions of spaceflight on skeletal muscle.

Bed rest summary. Bed rest effectively imposes unweighting on the postural antigravity muscles of subjects, along with a significant decrease in activity and energy expenditure. This model allows investigators to study relatively complete muscle unweighting, as well as potential palliative interventions, such as selective programmed exercise. The bed rest model is particularly appropriate for integrative, multiinvestigator investigations designed to study multisystem effects of reduced activity and unweighting. This is particularly true when the bed rest conditions include head-down tilt for the study of cardiovascular adaptation. In light of the results available to date, it appears that bed rest mimics both the magnitude and possible mechanisms of muscle adaptation seen with spaceflight.

IM

A number of studies have used the limb IM approach via limb casting to study disuse atrophy. When used on the lower limb, common features of this method include the unweighting of one limb, the fixation of the unweighted knee in a flexed position, and the use of crutches enabling ambulatory activity. With the use of this approach, the quadriceps group is fixed in a lengthened position, while a two-joint leg muscle, such as the gastrocnemius, may experience some degree of shortening. In light of the spaceflight-related context of this review, we have limited our examination of this model to studies conducted on healthy subjects.

Strength Measurements After IM

The majority of the performance data from this model have been obtained via measures of knee extension pre- and post-IM. Rozier et al. (82) found that KE isometric MVC was decreased by 13% after just 9 days of IM, suggesting that performance decrements demonstrate a fairly rapid onset. Fourteen days of IM have been reported to cause a 22% decrease in isometric MVC (39), whereas another group that utilized a knee brace for IM found a 17% decline in KE peak torque in a similar time frame (21). Hortobágyi et al. (41) reported a 45% decrease in KE MVC after 21 days of IM. In one of the longer studies involving healthy subjects, 28 days of IM resulted in a 53% decrease in KE torque (60°/s) (98). IM appears to impact the KE to a greater degree than the knee flexors (21, 98). IM also appears to have a significant impact on the performance of the calf muscles, resulting in 22 and 25% decreases in MVC at 21 and 14 days, respectively (20, 100).

Compared with spaceflight and bed rest, IM appears to result in a somewhat more rapid decline in muscle performance (Fig. 2). The slope of a straight line fitting the data from spaceflight in Fig. 2 would be 0.15, whereas the slope of a line fitting the IM data would be ~1.4 (data not shown). However, this relationship could be a function of the lack of IM data points after 28 days.

Whole Muscle Size Measurements After IM

At 10 to 14 days of IM, the CSA of the KE group was decreased ~11% (39, 93). In the 28-day study, Veldhuizen et al. (98) reported that IM resulted in a 21% decrease in KE muscle group CSA. The number of IM studies conducted with healthy subjects that reported muscle size changes is relatively small, and thus conclusions about this measure are necessarily tenuous. However, the data do suggest that, as with the decrements in strength noted above, the pattern of muscle size changes seen with IM indicates the potential for a more rapid response compared with the spaceflight and bed rest data (Fig. 3).

Muscle Fiber-size Measurements After IM

Hespel et al. (39) reported that 14 days of IM resulted in muscle-fiber CSA decrements of 8% for type I, 11% for IIa, and 9% for IIb, indicating that the changes in myofiber size can be detected at this relatively early time point. After 21 days of IM, the CSA of myofibers from the VL was decreased by 13% in type I and by 10% in types IIa and IIx (41). In their 28-day study, Veldhuizen et al. (98) found that the CSA decrements of VL muscle fibers were as follows: type I, 26%; IIa, 13%; and IIb(x), 36%.

The information above represents the only reports of muscle fiber size that were found in the literature for healthy IM subjects (39, 41, 98) (Fig. 4). Therefore, it is not possible to determine whether these data conform with those from other models, such as bed rest. Although not included in Fig. 4, there are results from studies using patients with uncomplicated tibial fractures that might be used to increase this database. For example, Blakemore et al. (15) reported that, after 6 wk of IM for tibial fracture, the CSA of type I and II fibers had decreased by 29 and 36%, respectively. These data appear to be in line with those from the 28-day study in healthy subjects presented in Fig. 4. Assuming that the tibial fracture had relatively little impact on the atrophy process in the VL, these results, when combined with those from healthy subjects, suggest that the rate of myofiber atrophy with IM may be greater than that reported for other models such as bed rest.

Effects of IM on V_{max} and Power

To date, few studies have examined the effects of IM on the in vivo torque-velocity relationship in human skeletal muscle. However, Veldhuizen et al. (98) examined the influence of 4 wk of "long-leg" cast IM on the in vivo torque-velocity relationships of the knee flexors and KE. These investigators reported that the greatest loss of torque occurred at the slowest angular velocities. For instance, at an angular velocity of 0.58 rad/s, Veldhuizen et al. observed a decrease in torque production of ~100 N·m, whereas at 5.21 rad/s the loss in torque was ~40 N·m. These investigators also noted a similar speed-specific phenomenon for the knee flexors, but the absolute and relative losses in torque were much less than that seen for the KE. Importantly, it should be noted that the speed-specific phenomenon observed after IM is similar to that seen for bed rest (see above) and ULLS (see below).

In considering the effects of IM on mechanical power, the speed-specific alterations observed by Veldhuizen et al. (98) demonstrate that the greatest losses in power occur in the high-torque-low-velocity region and that the loss of mechanical power is much less in the moderate-to-high-velocity regions of this relationship.

Is IM a Good Model of Spaceflight-induced Changes in Skeletal Muscle?

As with spaceflight, the results of IM studies clearly indicate that this model induces changes that are limited to muscles in the treatment limb. In this respect, the mechanisms of IM-induced muscle adaptation appear to be similar to those associated with spaceflight (e.g., local vs. central). A potential deviation in this model arises from the fixation of the knee joint, which would not be present in spaceflight profiles. In this respect, it seems possible that the added impact of the fixation might be expected to result in changes that have less fidelity with those resulting from spaceflight than models that do not include this parameter. The data reviewed above suggest that there may be some differences in response between IM, spaceflight, and the other models. However, the majority of the IM data available were clustered at shorter durations and, therefore, render this conclusion open to question. In addition, in contrast to spaceflight and bed rest, IM does not result in cephalad shifts in fluids. For less rigidly defined studies (e.g., no spaceflight context) aimed specifically at understanding the mechanisms that underlie unweighting-induced atrophy of skeletal muscle, IM is clearly a useful model. The usefulness of the IM model for spaceflight-related studies would be enhanced by the addition of data that directly address the impact of joint fixation (in healthy subjects) on the atrophy process and by the collection of data after longer periods of IM.

ULLS

ULLS is another ground-based model for muscle unweighting. It is essentially an offshoot of IM that does not involve the constant fixation of the knee joint. This model historically has involved two approaches: an older version that involves the use of a support strap to suspend one lower limb (e.g., Ref. 8) and thus prevent weight bearing and a more recent version that employs a high-platform shoe on the contralateral limb to prevent weight bearing in the ipsilateral leg (e.g., Ref. 29). In each case, ambulatory activity is performed by using crutches. In the "strap" model, the knee is maintained in flexion during ambulatory activity, whereas, in the platform "shoe" model, the suspended limb is in normal anatomic positions at all times and can move freely. Depending on the amount of time spent in ambulatory activity, it is possible that the changes in muscle length associated with the strap model might induce altered responses similar to those postulated for IM in the previous section.

Muscle Strength Measurements After ULLS

ULLS studies of shorter duration (10–21 days) found KE MVC decreased by ~12–17% (2, 11, 32, 84). Longer ULLS studies of 28, 35, and 42 days have each reported that the MVC of the KE decreased by ~20% (8, 23, 76, 77). Of these longer studies, only one 28-day study (8) employed the strap model. In addition to the data from the thigh, one study also reported that the MVC for the triceps surae group decreased by 17% after 21 days of ULLS (84).

The observed changes in strength after ULLS appear to parallel those reported from both spaceflight and bed rest (Fig. 2). Approximately one-half of the studies included the use of the strap model. From this limited sample, it does not appear that the periods of knee joint fixation imposed by this model resulted in an accelerated loss of strength, such as that suggested by the IM data. For example, the apparent slope for the ULLS data is very similar to that of the spaceflight and bed rest relationship and much lower than that observed in the case of IM (e.g., 0.15 for spaceflight, 0.30 for ULLS, and 1.4 for IM; data not shown).

Whole Muscle Size Measurements After ULLS

The majority of the ULLS studies conducted to date have included MRI- or CT-based measurements of muscle size. The earliest ULLS time point for muscle CSA data was 16 days, with a reported 8% decrease in KE muscle size (2). ULLS of 21- to 28-days' duration resulted in ~7% loss in the thigh and leg (8, 84), whereas a 35-day ULLS protocol is reported to have induced a 14% decrease in the CSA of the KE muscles (76, 77). After 42 days of ULLS, Hather et al. (38) reported that the CSA of the thigh muscles (combined extensors and flexors) decreased by 12%. In that study, it was found that the KE CSA decreased by 16%, whereas the decrement in the KF was 7%. In the study by Hather et al., the antigravity muscles of the leg demonstrated the greatest sensitivity to ULLS with 17 and 25% declines in CSA in the soleus and gastrocnemius muscles, respectively.

In general, the muscle size changes found with the ULLS model appear to be consistent with those reported for spaceflight (Fig. 3). In what appears to be a possible exception to this, the CSA of the gastrocnemius muscle was decreased by ~26% after 42 days of ULLS (Fig. 3). This decrement is substantially greater than the 18% loss in the gastrocnemius reported by LeBlanc et al. (60) after Mir missions of >115 days. However, the 26% reduction after 42 days of ULLS represents unweighting without countermeasures, while most Mir crew conduct some level of countermeasure exercise in addition to normal mission activities. In a similar result, LeBlanc et al. (63) found that the CSA of the calf muscles was decreased ~30% after 120 days of bed rest. Taken together, these results suggest that the antigravity muscles of the leg (e.g., calf muscles) may be more sensitive to unweighting that those of the thigh.

Muscle Fiber-size Measurements After ULLS

Compared with bed rest and IM, the development of the ULLS model is relatively recent. As a result, few studies have included measurement of muscle fiber size after ULLS. Using the strap version of ULLS, Widrick et al. (105) reported that 12 days of unweighting induced a 7% decrease in the diameter of soleus fibers, whereas the diameter of fibers from the gastrocnemius was unchanged. After 42 days of the shoe version of ULLS, the CSA of muscle fibers from the VL was decreased by 12 and 15% for type I and type II fibers, respectively (29). Whereas there does not appear to be a notable deviation in the ULLS fiber-size data compared with the spaceflight or bed rest data, it is clearly not possible to determine whether this characteristic demonstrates a response that is comparable with spaceflight based on these few points (Fig. 4).

Affects of ULLS on V_{max} and Power

As noted above, previous studies have shown that both bed rest and limb IM for varying periods of time appear to result in speed-specific alterations in the in vivo torque-velocity relationship of the KE. Similar observations have been made regarding the effects of ULLS on the in vivo torque-velocity relationship. For instance, Berg et al. (9) tested the KE of subjects at angular velocities of 0.52, 1.57, and 2.62 rad/s after 28 days of ULLS. These investigators found that ULLS resulted in losses of 41, 37, and 26 N·m of torque, respectively, at these test velocities. Subsequently, Dudley et al. (23) (42 days), Adams et al. (2) (14 days), and Schulze et al. (84) (21 days) examined the effects of ULLS and also observed that the greatest loss of KE torque occurred at the slowest angular test velocities. In the study by Schulze et al., it was also noted that the plantar flexors of the ankle also exhibited speed-specific alterations in the in vivo torque-velocity relationship that are similar to those observed for the KE.

As noted above for bed rest and IM, these speed-specific changes in the in vivo torque-velocity relationship indicate that the greatest losses in mechanical power occur in the high-torque-slow-velocity region. In general, the mechanical unloading of skeletal muscle that occurs with all three models (e.g., bed rest, IM, and ULLS) seems to alter the functional capacity of skeletal muscle, such that the largest losses of function occur in the high-force-low-velocity region of the force-velocity relationship. This drastically reduces the production of mechanical power in this region (Fig. 1*B*). Hence any activities that require muscles to work in the high-force-low-velocity region of the force-velocity relationship could be severely impacted. In contrast, mechanical unloading of skeletal muscle is thought to produce shifts resulting in an increase fast myosin isoform expression that functionally protects (or even augments) force and power production at very high shortening velocities.

Is ULLS a Good Model For Spaceflight Effects on Skeletal Muscle?

Although potential alterations in circulating factors have not been addressed in this model, the changes seen after ULLS are clearly limited to muscles in the treatment limb (e.g., Ref. 29). This indicates that ULLS conforms to the criterion of local unweighting-induced effects and not effects induced by systemic phenomena. In general, the ULLS model results in decrements in muscle performance and size that conform to those seen after bed rest and spaceflight (Figs. 2–4). However, in a recent report, Widrick et al. (105) contended that ULLS is not a satisfactory model for spaceflight-induced alterations at the single-fiber level in skeletal muscle. This conclusion has been challenged by one of the authors of this review (1). Widrick et al. (105) questioned the fidelity of the ULLS as a model for spaceflight based on a ULLS study that was compared with the results obtained from the LMS spaceflight and a parallel bed rest study (5, 78, 79, 97, 101–104). These authors concluded that several components of their results, such as a greater reduction in single-fiber peak power measurements (soleus slow fibers) after ULLS (105), compared with results from the LMS mission (101) and its corresponding bed rest study (102), indicate that the ULLS model does not mimic either bed rest or spaceflight. However, to reach these conclusions, comparisons made were between muscle fibers from a ULLS study that did not include any exercise countermeasures (105), whereas the characteristics of muscle fibers from the previously described 17-day bed rest and LMS spaceflight studies involved significant amounts of exercise, including resistance-type exercise as part of the experimental protocol (5, 78–80, 97, 101–105). These exercise testing procedures were acknowledged by the authors to have been sufficient to serve as an effective countermeasure to the unweighting-induced effects. They summarized their LMS results indicating that ". . . no changes in calf muscle strength and morphology were observed after the 17-day spaceflight and bed rest" and that ". . . the testing sequence employed during the spaceflight and bed rest may have served as a resistance training countermeasure to attenuate whole muscle strength loss" (97). In light of these circumstances, it is not at all clear that comparisons made between a ULLS study with no exercise vs. spaceflight and bed rest studies with significant levels of exercise provide a basis for evaluating the validity of the ULLS model.

As indicated in Figs. 2–4, the available data clearly suggest that ULLS is an appropriate model for studies specifically aimed at skeletal muscle unweighting. However, in contrast to spaceflight and bed rest, ULLS does not result in cephalad shifts in fluids and thus would not be appropriate for studies of cardiovascular adaptation.

Practical considerations when selecting a ground-based model of skeletal muscle unweighting. In the context of skeletal muscle adaptation to unweighting, the response to the three models discussed in this review appears to have been quite similar (see Figs. 2–4). In fact, despite the high level of heterogeneity in measurement techniques, analysis of the limited number of data sets, which included measures of both muscle size and strength performance changes, indicates that there is a strong, linear relationship between these two variables (Fig. 5). In addition to the common criteria used for evaluating a given model, each of the three models outlined has unique characteristics that should be considered by investigators interested in studying the effects of unweighting on skeletal muscle (see Table 1).

BED REST. The strength of this model lies in the whole body unloading and relative inactivity that is imposed. With the inclusion of head-down tilt, bed rest is particularly well suited to integrated musculoskeletal and cardiovascular investigations. However, the bed rest model is resource intensive (e.g., specialized bed rest facilities that have showers that accommodate gurneys, scales for weighing recumbent subjects, etc.) requiring 24-h nursing supervision (e.g., Refs. 49, 103). Whereas a number of space agency dedicated bed rest facilities exist in Europe, the only NASA facility in the

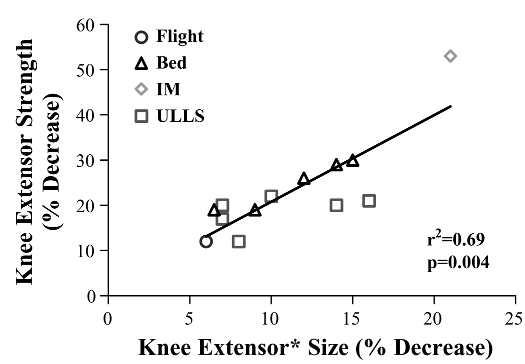

Fig. 5. The relationship between the decrement in muscle size and muscle strength (measures of knee extension) induced by spaceflight (○), bed rest (△), IM (◇), and ULLS (□). The data presented are from those publications that reported both measures from the same study subjects. *Knee extensor size data were both methodologically and qualitatively heterogeneous, including measurements of either the quadriceps group or the whole thigh. Because the cross-sectional area of the knee flexors generally decreases to a lesser extent than that of the knee extensors, thigh cross-sectional area values would tend to underestimate the decrement in muscle area associated with knee extension movements. The single flight data point also had an unequal number of subjects (n = 17 for MVC and n = 2 for quadriceps volume) (35).

Table 1. *Some important factors to be considered when choosing bed rest, IM, or ULLS as the experimental approach used to mimic the unloading of skeletal muscle that occurs in microgravity*

	Bed Rest	ULLS and IM
Multisystem studies	A number of physiological systems can be studied	Studies confined to muscle and possibly bone
Cardiovascular deconditioning	Prolonged bed rest known to produce cardiovascular deconditioning For dedicated muscle studies, it may be inappropriate to expose subjects this potential side effect	Minimal effects
Confinement	Requires the confinement of the subject to a bed Extremely sedentary activity profile	Subjects ambulatory Participation in many daily activities possible
Venous thrombosis	None reported for spaceflight-related studies	Strap-free version of ULLS has not been reported to produce venous thrombosis Some potential with casting
Potential for falls	No risk	Some risk
Costs and demand on resources	Costly (without GCRC support) Specialized facilities 24-h nursing supervision	Low cost No specialized facilities No nursing support

ULLS, unilateral lower limb suspension; IM, immobilization; GCRC, General Clinical Research Center.

US appears to have been "mothballed" by NASA due to budget constraints (http://spaceresearch.nasa.gov/research_projects/facilities.html). Consequently, outside of Europe, bed rest studies pertinent to NASA would have to be conducted in more generic facilities such as clinical research centers.

Research groups interested exclusively in studying the unweighting of one or a few muscle groups should also consider the implications of imposing a whole body treatment, such as bed rest, on subjects. It should be recognized that there are some potential cautionary issues with the bed rest model. For example, markers of increased bone resorption can be detected after relatively short periods of bed rest (e.g., 7 days) (e.g., 14, 66, 87). Whereas a single bout of short-term bed rest may not result in detectable bone loss, this process must be viewed in the context of accumulated cycles of bone loss and gain over an individual's life time. On a more acute level, after a period of unloading, skeletal muscles appear to be more susceptible to injury (43, 77, 81, 95). Because bed rest imposes unweighting on all postural muscles, as opposed to targeting a specific limb, specific muscle group, or muscles, the risks of such injury on return to activity expands from just the target muscles to all of the affected muscles (e.g., all antigravity muscles including those in the back) and should be countered with a comprehensive program of rehabilitation. There is also evidence to suggest that bed rest results in some degree of cardiovascular deconditioning, possibly including cardiac atrophy (64, 74, 75). In addition, the energy expenditure of bed rest subjects is decreased compared with ambulatory periods, whereas that of flight crews is either elevated or unchanged compared with ground-based activities (18, 57, 89, 90). In this context, it might be questioned as to whether a model involving extended sedentary behavior is particularly appropriate for research related to spaceflight. At the very least, some attempt at equalization of activity levels should be included in the experimental design.

IM. Single lower limb IM allows for the study of the targeted muscles in a given limb and, based on the studies reviewed above, may provide a somewhat accelerated response. However, it is not clear that the restricted movement of the limbs in IM mimics spaceflight conditions in which the lower limbs move through a wide range of motion while unloaded.

Whereas the activity levels of IM subjects can be expected to decrease to some extent, continued ambulatory activity will allow for weight bearing in the nontargeted muscles. In addition, because IM allows the subjects to remain relatively active compared with bed rest, it would not be expected to result in the more global effects, such as extensive cardiovascular detraining.

Relative to bed rest, the facilities, equipment, and personnel necessary to conduct IM studies are relatively modest. Whereas the possibility exists that, with one limb casted, a loss of balance may result in a fall, or, in fairly extreme cases, improper use of crutches can result in axillary injuries, the overall risk of this treatment is relatively low.

ULLS. As with IM, the specificity of the ULLS model for the unweighting of the muscles in one limb offers several advantages for studies devoted exclusively to skeletal muscle (Table 1). With the ULLS model, the subjects remain ambulatory, and the majority of the musculoskeletal system remains loaded. In contrast to IM, the current model of ULLS maintains the unweighted limb in normal anatomic positions and allows a full range of motion during ambulatory activity (e.g., Refs. 2, 23, 29, 76, 77). As a result, subjects participating in ULLS studies can continue to conduct most occupational activities without compromising the study. The periods of upright posture and the maintenance of relatively normal activity levels should help to ameliorate the side effects of more sedentary models such as bed rest (e.g., skeletal unloading, cardiovascular deconditioning). The muscle size and performance data shown in Figs. 2–4 indicate that the responses to ULLS may more directly mimic spaceflight than those from the IM model. This could be a function of the range of limb motion allowed by the ULLS model. Even when the support strap version of the ULLS model is employed, the lower limbs would be maintained in "normal" anatomic positions the majority of the time, other than during ambulatory activities (11). The more recent platform shoe version of the ULLS does not involve any joint fixation or range of motion restriction (2, 23, 29, 76, 77). In contrast, cast IM would fix the limb position at all times, including during the sleep period.

Similar to IM, the resources necessary to conduct a ULLS study would be substantially less than those associated with bed rest. Other than those related to data collection, no special facilities are necessary for ULLS studies. Compared with IM, the concern of falling during ambulatory activity is greatly reduced in ULLS because the unweighted limb is available for use if balance is lost. There was one report of an adverse event, development of venous thrombosis in the calf in two subjects in a study using the ULLS model (11). However, this occurred in a strap-version ULLS study. Modification of the ULLS model eliminating the strap and, therefore, the attendant restriction of movement, appears to have eliminated this problem (2, 23, 29, 76, 77).

CONCLUSIONS

A primary aim of this review was to address the validity and appropriateness of ground-based models for skeletal muscle adaptation to microgravity in humans. However, we must acknowledge that the paucity of data for the gold standard, i.e., spaceflight, renders definitive conclusions difficult. As a result of the high costs of conducting in-flight research during the space shuttle era, relatively few missions have had a substantial component devoted to human physiological measurements focused on skeletal muscle. As noted above in the comments on the 17-day LMS mission, in those instances in which skeletal muscle measurements have been made, the observations tended to be compromised. Similar circumstances (e.g., uncontrolled, voluntary exercise) have plagued previous human research projects involving life science missions (35). As a result, the skeletal muscle research community still awaits a well-conceived, experimentally designed spaceflight mission with significant time and resources devoted to human skeletal muscle physiology that includes uncompromised control subjects. Whereas it is recognized that long-term microgravity exposure without some attempt at a countermeasure is unacceptable, shorter duration missions, such as non-ISS-related STS flights, could be used to gather this critical baseline data. With the advent of the ISS, it is hoped that the opportunity to conduct well-designed, long-term basic science and countermeasure-related skeletal muscle physiology studies will be offered. However, as presently configured, the ISS can only accommodate a crew of three. Considering the normal "housekeeping" workload of ISS crews, it does not appear that the gaps in spaceflight-related physiological data will be resolved in the immediate future.

In the final analysis, it seems clear that the primary concern with regard to the impacts of spaceflight on skeletal muscle is the prevention of weight bearing and thus the initiation of locally mediated adaptations that may mimic spaceflight conditions. In this respect, the limited data available do not support the selection of one of the reviewed models over another. Therefore, the selection of bed rest, ULLS, or possibly IM as a model for spaceflight can be made primarily based on the specific aims of a given study and with consideration of the more practical aspects of the models.

DISCLOSURES

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-45594 (to G. R. Adams) and AR-46856 (to V. J. Caiozzo) and National Space Biomedical Research Institute Grant NCC9–58 (to K. M. Baldwin).

REFERENCES

1. **Adams GR.** Human unilateral lower limb suspension as a model for spaceflight effects on skeletal muscle. *J Appl Physiol* 93: 1563–1565, 2002.
2. **Adams GR, Hather BM, and Dudley GA.** Effect of short-term unweighting on human skeletal muscle strength and size. *Aviat Space Environ Med* 65: 1116–1121, 1994.
3. **Akima H, Kubo K, Imai M, Kanehisa H, Suzuki Y, Gunji A, and Fukunaga T.** Inactivity and muscle: effect of resistance training during bed rest on muscle size in the lower limb. *Acta Physiol Scand* 172: 269–278, 2001.
4. **Akima H, Kuno S, Suzuki Y, Gunji A, and Fukunaga T.** Effects of 20 days of bed rest on physiological cross-sectional area of human thigh and leg muscles evaluated by magnetic resonance imaging. *J Gravit Physiol* 4: S15–S21, 1997.
5. **Arnaud SB, Trappe S, and O'Hara DB.** *Life and Microgravity Sciences Spacelab Mission: Human Research Pilot Study*. Moffett Field, CA: NASA Ames Research Center, 1996, p. 1–52. (NASA TM-110395)
6. **Bamman MM, Clarke MS, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA, and Greenisen MC.** Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. *J Appl Physiol* 84: 157–163, 1998.
7. **Bell A, Gagnon A, Grunder L, Parikh SJ, Smith TJ, and Sorisky A.** Functional TSH receptor in human abdominal preadipocytes and orbital fibroblasts. *Am J Physiol Cell Physiol* 279: C335–C340, 2000.
8. **Berg HE, Dudley GA, Haggmark T, Ohlsen H, and Tesch PA.** Effects of lower limb unloading on skeletal muscle mass and function in man. *J Appl Physiol* 70: 1882–1885, 1991.
9. **Berg HE, Dudley GA, Hather B, and Tesch PA.** Work capacity and metabolic and morphologic characteristics of the human quadriceps muscle in response to unloading. *Clin Physiol* 13: 337–347, 1993.
10. **Berg HE, Larsson L, and Tesch PA.** Lower limb skeletal muscle function after 6 wk of bed rest. *J Appl Physiol* 82: 182–188, 1997.
11. **Berg HE and Tesch PA.** Changes in muscle function in response to 10 days of lower limb unloading in humans. *Acta Physiol Scand* 157: 63–70, 1996.
12. **Berry CA.** Summary of medical experience in the Apollo 7 through 11 manned spaceflights. *Aerospace Med* 41: 500–519, 1970.
13. **Berry P, Berry I, and Manelfe C.** Magnetic resonance imaging evaluation of lower limb muscles during bed rest—a microgravity simulation model. *Aviat Space Environ Med* 64: 212–218, 1993.

14. **Bikle DD and Halloran BP.** The response of bone to unloading. *J Bone Miner Metab* 17: 233–244, 1999.
15. **Blakemore SJ, Rickhuss PK, Watt PW, Rennie MJ, and Hundal HS.** Effects of limb immobilization on cytochrome-c oxidase activity and GLUT4 and GLUT5 protein expression in human skeletal muscle. *Clin Sci (Lond)* 91: 591–599, 1996.
16. **Caiozzo VJ, Baker MJ, Herrick RE, Tao M, and Baldwin KM.** Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle. *J Appl Physiol* 76: 1764–1773, 1994.
17. **Caiozzo VJ, Haddad F, Baker MJ, Herrick RE, Prietto N, and Baldwin KM.** Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle. *J Appl Physiol* 81: 123–132, 1996.
18. **Convertino VA.** Physiological adaptations to weightlessness: effects on exercise and work performance. *Exerc Sport Sci Rev* 18: 119–165, 1990.
19. **Convertino VA, Doerr DF, Mathes KL, Stein SL, and Buchanan P.** Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity. *Avait Space Environ Med* 60: 653–658, 1989.
20. **Davies CT, Rutherford IC, and Thomas DO.** Electrically evoked contractions of the triceps surae during and following 21 days of voluntary leg immobilization. *Eur J Appl Physiol* 56: 306–312, 1987.
21. **Deschenes MR, Giles JA, McCoy RW, Volek JS, Gomez AL, and Kraemer WJ.** Neural factors account for strength decrements observed after short-term muscle unloading. *Am J Physiol Regul Integr Comp Physiol* 282: R578–R583, 2002.
22. **Desplanches D, Hoppeler H, Mayet MH, Denis C, Claassen H, and Ferretti G.** Effects of bedrest on deltoideus muscle morphology and enzymes. *Acta Physiol Scand* 162: 135–140, 1998.
23. **Dudley GA, Duvoisin MR, Adams GR, Meyer RA, Belew AH, and Buchanan P.** Adaptation to unilateral lower limb suspension in humans. *Aviat Space Environ Med* 63: 678–683, 1992.
24. **Dudley GA, Duvoisin MR, Convertino MR, and Buchanan P.** Alterations of the in vivo torque-velocity relationship of human skeletal muscle following 30 days exposure to simulated microgravity. *Aviat Space Environ Med* 60: 659–663, 1989.
25. **Dudley GA, Gollnick PD, Convertino VA, and Buchanan P.** Changes of muscle function and size with bed rest. *Physiologist* 32: S65–S66, 1989.
26. **Duvoisin MR, Convertino VA, Buchanan P, Gollnick PD, and Dudley GA.** Characteristics of preliminary observations of the influence of electromyo-stimulation on the size and function of human skeletal muscle during 30 days. *Aviat Space Environ Med* 60: 671–678, 1989.
27. **Edgerton VR, Zhou MY, Ohira Y, Klitgaard H, Jinag B, Bell G, Harris B, Saltin B, Gollnick PD, and Roy RR.** Human fiber size and enzymatic properties after 5 and 11 days of spaceflight. *J Appl Physiol* 78: 1733–1739, 1995.
28. **Ferrando AA, Stuart CA, Brunder DG, and Hillman GR.** Magnetic resonance imaging quantitation of changes in muscle volume during 7 days of strict bed rest. *Aviat Space Environ Med* 66: 976–981, 1995.
29. **Ferretti G, Berg HE, Minetti AE, Moia C, Rampichini S, and Narici MV.** Maximal instantaneous muscular power after prolonged bed rest in humans. *J Appl Physiol* 90: 431–435, 2001.
30. **Fluck M, Ziemiecki A, Billeter R, and Muntener M.** Fibre-type specific concentration of focal adhesion kinase at the sarcolemma: influence of fibre innervation and regeneration. *J Exp Biol* 205: 2337–2348, 2002.
31. **Funato K, Matsuo A, Yata H, Akima H, Suzuki Y, Gunji A, and Fukunaga T.** Changes in force-velocity and power output of upper and lower extremity musculature in young subjects following 20 days bed rest. *J Gravit Physiol* 4: S22–S30, 1997.
32. **Gamrin L, Berg HE, Essén P, Tesch PA, Hultman E, Garlick J, McNurlan MA, and Wernerman J.** The effect of unloading on protein synthesis in human skeletal muscle. *Acta Physiol Scand* 163: 369–377, 1998.
33. **Gogia P, Schneider VS, LeBlanc AD, Krebs J, Kasson C, and Pientok C.** Bed rest effect on extremity muscle torque in healthy men. *Arch Phys Med Rehabil* 69: 1030–1032, 1988.
34. **Greenisen MC and Edgerton VR.** Human capabilities in the space environment. In: *Space Physiology and Medicine*, edited by Nicogssian AE, Huntoon CL, and Pool SL. Philadelphia, PA: Lea & Febiger, 1994, p. 194–210.
35. **Greenisen MC, Hayes JC, Siconolfi SF, and Moore AD.** *Functional Performance Evaluation in Extended Duration Orbiter Medical Project Final, Report 1989–1995*, edited by Sawin C, Taylor G, and Smith W. Houston, TX: NASA, 1999, p. 3–1–3–24. (Publ. SP-1999–534 NASA)
36. **Greenleaf JE.** Intensive exercise training during bed rest attenuates deconditioning. *Med Sci Sports Exerc* 29: 207–215, 1997.
37. **Greenleaf JE, Bulbulian R, Bernauer EM, Haskell WL, and Moore T.** Exercise-training protocols for astronauts in microgravity. *J Appl Physiol* 67: 2191–2204, 1989.
38. **Hather BM, Adams GR, Tesch PA, and Dudley GA.** Skeletal muscle responses to lower limb suspension in humans. *J Appl Physiol* 72: 1493–1498, 1992.
39. **Hespel P, Op't Eijnde B, Van Leemputte M, Urso B, Greenhaff PL, Labarque V, Dymarkowski S, Van Hecke P, and Richter EA.** Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and alters the expression of muscle myogenic factors in humans. *J Physiol* 536: 625–633, 2001.
40. **Hikida RS, Gollnick PD, Dudley GA, Convertino VA, and Buchanan P.** Structural and metabolic characteristics of human skeletal muscle following 30 days of simulated microgravity. *Aviat Space Environ Med* 60: 664–670, 1989.
41. **Hortobágyi T, Dempsey L, Fraser D, Zheng D, Hamilton G, Lambert J, and Dohm L.** Changes in muscle strength, muscle fibre size and myofibrillar gene expression after immobilization and retraining in humans. *J Physiol* 524: 293–304, 2000.
42. **Jaweed MM.** Muscle structure and function In: *Space Physiology and Medicine*, edited by Nicogssian AE, Huntoon CL, and SL Pool. Philadelphia, PA: Lea & Febiger, 1994, p. 317–326.
43. **Kasper CE, White TP, and Maxwell LC.** Running during recovery from hindlimb suspension induces transient muscle injury. *J Appl Physiol* 68: 533–539, 1990.
44. **Kawakami Y, Akima H, Kubo K, Muraoka Y, Hasegawa H, Kouzaki M, Imai M, Suzuki Y, Gunji A, Kanehisa H, and Fukunaga T.** Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. *Eur J Appl Physiol* 84: 7–15, 2001.
45. **Kawakami Y, Muraoka Y, Kubo K, Suzuki Y, and Fukunaga T.** Changes in muscle size and architecture following 20 days of bed rest. *J Gravit Physiol* 7: 53–59, 2000.
46. **Kischel P, Stevens L, Montel V, Picquet F, and Mounier Y.** Plasticity of monkey triceps muscle fibers in microgravity conditions. *J Appl Physiol* 90: 1825–1832, 2001.
47. **Koryak Y.** Contractile properties of the human triceps surae muscle during simulated weightlessness. *Eur J Appl Physiol* 70: 344–350, 1995.
48. **Koryak Y.** Mechanical and electrical changes in human muscle after dry immersion. *Eur J Appl Physiol* 74: 133–140, 1996.
49. **Koryak Y.** Effect of 120 days of bed-rest with and without countermeasures on the mechanical properties of the triceps surae muscle in young women. *Eur J Appl Physiol* 78: 128–135, 1998.
50. **Koryak Y.** Electrical and contractile parameters of muscle in man: effects of 7-day "dry" water immersion. *Aviat Space Environ Med* 70: 459–464, 1999.
51. **Koryak Y.** The effects of long-term simulated microgravity on neuromuscular performance in men and women. *Eur J Appl Physiol* 79: 168–175, 1999.
52. **Koryak Y.** Electrically evoked and voluntary properties of the human triceps surae muscle: effects of long-term spaceflights. *Acta Physiol Pharmacol Bulg* 26: 21–27, 2001.
53. **Koryak YA.** Influence of 120-days 6 degrees head-down tilt bed rest on the functional properties of the neuromuscular system in man. *Aviat Space Environ Med* 69: 766–770, 1998.

54. **Kozlovskaya IB, Kreidich YuV, Oganov VS, and Koserenko OP.** Pathophysiology of motor functions in prolonged manned space flights. *Acta Astronaut* 8: 1059–1072, 1981.
55. **Kuznetsov SL and Stepantsov VV.** Reactions of human skeletal muscle fibers to a 370-day period of hypokinesia with head down tilt combined with physical exercise. *USSR Space Life Sci Digest* 30: 48–52, 1991.
56. **Lambertz D, Perot C, Kaspranski R, and Goubel F.** Effects of long-term spaceflight on mechanical properties of muscles in humans. *J Appl Physiol* 90: 179–188, 2001.
57. **Lane HW, Gretebeck RJ, Schoeller DA, Davis-Street J, Socki RA, and Gibson EK.** Comparison of ground-based and space flight energy expenditure and water turnover in middle-aged healthy male US astronauts. *Am J Clin Nutr* 65: 4–12, 1997.
58. **Leach CS and Altchuler SI.** The endocrine and metabolic responses to space flight. *Med Sci Sports Exerc* 15: 432–440, 1983.
59. **LeBlanc A, Gogia P, Schneider V, Krebs J, Schonfeld E, and Evans H.** Calf muscle area and strength changes after five weeks of horizontal bed rest. *Am J Sports Med* 16: 624–629, 1988.
60. **LeBlanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, and Feeback D.** Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. *J Appl Physiol* 89: 2158–2164, 2000.
61. **LeBlanc A, Rowe R, Evans H, West S, Shackelford L, and Schneider V.** Muscle atrophy during long duration bed rest. *Int J Sports Med* 18: S283–S285, 1997.
62. **LeBlanc A, Rowe R, Schneider V, Evans H, and Hedrick T.** Regional muscle loss after short duration spaceflight. *Aviat Space Environ Med* 66: 1151–1154, 1995.
63. **LeBlanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, and Spector E.** Regional changes in muscle mass following 17 weeks of bed rest. *J Appl Physiol* 73: 2172–2178, 1992.
64. **Levine BD, Zuckerman JH, and Pawelczyk JA.** Cardiac atrophy after bed-rest deconditioning: a nonneural mechanism for orthostatic intolerance. *Circulation* 96: 517–525, 1997.
65. **Lovejoy JC, Smith SR, Zachwieja JJ, Bray GA, Windhauser MM, Wickersham PJ, Veldhuis JD, Tulley R, and delaBretonne JA.** Low-dose T_3 improves the bed rest model of simulated weightlessness in men and women. *Am J Physiol Endocrinol Metab* 277: E370–E379, 1999.
66. **Lueken SA, Arnaud SB, Taylor AK, and Baylink DJ.** Changes in markers of bone formation and resorption in a bed rest model of weightlessness. *Bone Miner* 8: 1433–1438, 1993.
67. **McCall GE, Goulet C, Grindland RE, Hodgson JA, Bigbee AJ, and Edgerton VR.** Bed rest suppresses bioassayable growth hormone release in response to muscle activity. *J Appl Physiol* 83: 2086–2090, 1997.
68. **McCall GE, Goulet C, Roy RR, Grindeland RE, Boorman GI, Bigbee AJ, Hodgson JA, Greenisen MC, and Edgerton VR.** Spaceflight suppresses exercise-induced release of bioassayable growth hormone. *J Appl Physiol* 87: 1207–1212, 1999.
69. **Monici MC, Rodolico C, Toscano A, Messina S, Benvenga S, Messina C, and Vita G.** Apoptosis and apoptosis-related proteins in thyroid myopathies. *Muscle Nerve* 26: 383–388, 2002.
70. **Narici MV, Kayser B, Barattini P, and Cerretelli P.** Changes in electrically evoked skeletal muscle contractions during 17-day spaceflight and bed rest. *Int J Sports Med* 18: S290–S292, 1997.
71. **NASA.** Critical Path Roadmap Baseline Document 000502 [Online]. http://criticalpath.jsc.nasa.gov [2000].
72. **Ohira Y, Yoshinaga T, Nonaka I, Ohara M, Yoshioka T, Yamashita-Goto K, Izumi R, Yasukawa K, Sekiguchi C, Shenkman BS, and Kozzlovskaya IB.** Histochemical responses of human soleus muscle fibers to long-term bedrest with or without countermeasures. *Jpn J Physiol* 50: 41–47, 2000.
73. **Ohira Y, Yoshinaga T, Ohara M, Nonaka I, Yoshioka T, Yamashita-Goto K, Shenkman BS, Kozlovskaya IB, Roy RR, and Edgerton VR.** Myonuclear domain and myosin phenotype in human soleus after bed rest with or without loading. *J Appl Physiol* 87: 1776–1785, 1999.
74. **Pawelczyk JA, Zuckerman JH, Blomqvist CG, and Levine BD.** Regulation of muscle sympathetic nerve activity after bed rest deconditioning. *Am J Physiol Heart Circ Physiol* 280: H2230–H2239, 2001.
75. **Perhonen MA, Franco F, Lane LD, Buckey JC, Blomqvist CG, Zerwekh JE, Peshock RM, Weatherall PT, and Levine BD.** Cardiac atrophy after bed rest and spaceflight. *J Appl Physiol* 91: 645–653, 2001.
76. **Ploutz-Snyder LL, Tesch PA, Crittenden DJ, and Dudley GA.** Effect of unweighting on skeletal muscle use during exercise. *J Appl Physiol* 79: 168–175, 1995.
77. **Ploutz-Snyder LL, Tesch PA, Hather B, and Dudley GA.** Vulnerability to dysfunction and muscle injury after unloading. *Arch Phys Med Rehabil* 77: 773–777, 1996.
78. **Riley DA, Bain JL, Thompson JL, Fitts RH, Widrick JJ, Trappe SW, Trappe TA, and Costill DL.** Disproportionate loss of thin filaments in human soleus muscle after 17-day bed rest. *Muscle Nerve* 21: 1280–1289, 1998.
79. **Riley DA, Bain JL, Thompson JL, Fitts RH, Widrick JJ, Trappe SW, Trappe TA, and Costill DL.** Decreased thin filament density and length in human atrophic soleus muscle fibers after spaceflight. *J Appl Physiol* 88: 567–572, 2000.
80. **Riley DA, Bain JL, Thompson JL, Fitts RH, Widrick JJ, Trappe SW, Trappe TA, and Costill DL.** Thin filament diversity and physiological properties of fast and slow fiber types in astronaut leg muscles. *J Appl Physiol* 92: 817–825, 2002.
81. **Riley DA, Ellis S, Slocum GR, Sedlak FR, Bain JLW, Krippendorf BB, Lehman CT, Macias MY, Thompson JL, Vijayan K, and De Bruin JA.** In-flight and post flight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. *J Appl Physiol* 81: 133–144, 1996.
82. **Rozier CK, Elder JD, and Brown M.** Prevention of atrophy by isometric exercise of a casted leg. *J Sports Med Phys Fitness* 19: 191–194, 1979.
83. **Schaller MD.** Biochemical signals and biological responses elicited by the focal adhesion kinase. *Biochim Biophys Acta* 1540: 1–21, 2001.
84. **Schulze K, Gallagher P, and Trappe S.** Resistance training preserves skeletal muscle function during unloading in humans. *Med Sci Sports Exerc* 34: 303–313, 2002.
85. **Sellitti DF, Dennison D, Akamizu T, Doi SQ, Kohn LD, and Koshiyama H.** Thyrotropin regulation of cyclic adenosine monophosphate production in human coronary artery smooth muscle cells. *Thyroid* 10: 219–225, 2000.
86. **Shenkman B, Belozerova I, Nemirovskaya T, Cheglova I, Yudaitcheva A, Kiseleva E, and Mazin M.** Time-course of human muscle fibre size reduction during head-down tilt bedrest. *J Gravit Physiol* 5: P71–P72, 1998.
87. **Smith SM, Nillen JL, Leblanc A, Lipton A, Demers LM, Lane HW, and Leach CS.** Collagen cross-link excretion during space flight and bed rest. *J Clin Endocrinol Metab* 83: 3584–3591, 1998.
88. **Stein TP.** Nutrition and muscle loss in humans during spaceflight. *Adv Space Biol Med* 7: 49–97, 1999.
89. **Stein TP.** Nutrition in the space station era. *Nutr Res Rev* 14: 87–117, 2001.
90. **Stein TP, Leskiw MJ, Schluter MD, Hoyt RW, Lane HW, and Gretebeck RE.** Energy expenditure and balance during spaceflight on the space shuttle. *Am J Physiol Regul Integr Comp Physiol* 276: R1739–R1748, 1999.
91. **Strollo F.** Adaptation of the human endocrine system to microgravity in the context of integrative physiology and ageing. *Pflügers Arch* 441: R85–R90, 2000.
92. **Tabata I, Suzuki Y, Fukunaga T, Yokozeki T, Akima H, and Funato K.** Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest. *J Appl Physiol* 86: 909–914, 1999.
93. **Thom JM, Thompson MW, Ruell PA, Bryant GJ, Fonda JS, Harmer AR, De Jonge XA, and Hunter SK.** Effect of 10-day cast immobilization on sarcoplasmic reticulum calcium regulation in humans. *Acta Physiol Scand* 172: 141–147, 2001.

94. **Thorton WE and Rummel JA.** Muscular deconditioning and its prevention in space flight. In: *Biomedical Results From Skylab,* edited by Johnson RS and Deitlein LF. Washington, DC: NASA, 1977, p. 191–197.
95. **Tidball JG, Berchenko E, and Frenette J.** Macrophage invasion does not contribute to muscle membrane injury during inflammation. *J Leukoc Biol* 65: 492–498, 1999.
96. **Tipton CM, Greenleaf JE, and Jackson CG.** Neuroendocrine and immune system responses with spaceflights. *Med Sci Sports Exerc* 28: 988–998, 1996.
97. **Trappe SW, Trappe TA, Lee GA, Widrick JJ, Costill DL, and Fitts RH.** Comparison of a space shuttle flight (STS-78) and bed rest on human muscle function. *J Appl Physiol* 91: 57–64, 2001.
98. **Veldhuizen JW, Verstappen FT, Vroemen JP, Kuipers H, and Greep JM.** Functional and morphological adaptations following four weeks of knee immobilization. *Int J Sports Med* 14: 283–287, 1993.
99. **Walker JW, Cowell LL, Greenisen M, and Squires WJ.** *Astronaut Adaptation to 1 G Following Long Duration Space Flight*. Warrendale, PA: SAE International, 1991. [SAE Tech Paper 911463]
100. **White MJ, Davies CT, and Brooksby P.** The effects of short-term voluntary immobilization on the contractile properties of the human triceps surae. *Q J Exp Physiol* 69: 685–691, 1984.
101. **Widrick JJ, Knuth ST, Norenberg KM, Romatowski JG, Bain JL, Riley DA, Karhanek M, Trappe SW, Trappe TA, Costill DL, and Fitts RH.** Effect of a 17 day spaceflight on contractile properties of human soleus muscle fibres. *J Physiol* 516: 915–930, 1999.
102. **Widrick JJ, Norenberg KM, Romatowski JG, Blaser CA, Karhanek M, Sherwood J, Trappe SW, Trappe TA, Costill DL, and Fitts RH.** Force-velocity-power and force-pCa relationships of human soleus fibers after 17 days of bed rest. *J Appl Physiol* 85: 1949–1956, 1998.
103. **Widrick JJ, Romatowski JG, Bain JL, Trappe SW, Trappe TA, Thompson JL, Costill DL, Riley DA, and Fitts RH.** Effect of 17 days of bed rest on peak isometric force and unloaded shortening velocity of human soleus fibers. *Am J Physiol Cell Physiol* 273: C1690–C1699, 1997.
104. **Widrick JJ, Romatowski JG, Norenberg KM, Knuth ST, Bain JL, Riley DA, Trappe SW, Trappe TA, Costill DL, and Fitts RH.** Functional properties of slow and fast gastrocnemius muscle fibers after a 17-day spaceflight. *J Appl Physiol* 90: 2203–2011, 2001.
105. **Widrick JJ, Trappe SW, Romatowski JG, Riley DA, Costill DL, and Fitts RH.** Unilateral lower limb suspension does not mimic bed rest or spaceflight effects on human muscle fiber function. *J Appl Physiol* 93: 354–360, 2002.
106. **Zachwieja JJ, Smith SR, Lovejoy JC, Rood JC, Windhauser MM, and Bray GA.** Testosterone administration preserves protein balance but not muscle strength during 28 days of bed rest. *J Clin Endocrinol Metab* 84: 207–212, 1999.
107. **Zachwieja JJ, Smith SR, Sinha-Hikim I, Gonzalez-Cadavid N, and Bhasin S.** Plasma myostatin-immunoreactive protein is increased after prolonged bed rest with low-dose T3 administration. *J Gravit Physiol* 6: 11–15, 1999.
108. **Zhou MY, Klitgaard H, Saltin B, Roy RR, Edgerton VR, and Gollnick PD.** Myosin heavy chain isoforms of human muscle after short-term spaceflight. *J Appl Physiol* 78: 1740–1744, 1995.