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INVITED REVIEW

Unilateral lower limb suspension: integrative physiological knowledge from the past 20 years (1991–2011)

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Abstract In 1991, Hans Berg and colleagues published the first research investigation using unilateral lower limb suspension (ULLS) as a human model to study the influence of unloading on skeletal muscle. ULLS requires a participant to perform all activities with axillary crutches while wearing one thick-soled shoe. The elevated shoe eliminates ground contact with the adjacent foot, thereby unloading the lower limb. Today, ULLS is a well-known ground-based analog for microgravity. The present review will synthesize the physiological findings from investigations using ULLS to study the deleterious effects of unloading. Compromised human performance and the neuromuscular, musculoskeletal and circulatory mechanisms leading to altered function will be a major emphasis of the work. Results from prolonged bed rest will also be included in order for general comparisons to be made between analogs. Finally, the efficacy of exercise to mitigate the negative consequences of unloading is presented.

Keywords Disuse · Inactivity · Space flight analogs · Unweighting

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Introduction and historical perspective

Unilateral lower limb suspension (ULLS) was originally developed in collaboration with Kennedy Space Center in order to study skeletal muscle function in humans after a period of disuse, which is a consequence of musculoskeletal unloading (Berg et al. 1991). Prior to this time bed rest facilities were rare and exceedingly expensive to utilize. Studies using plaster casts to immobilize joint movement were performed (Gibson et al. 1987; Sargeant et al. 1977); however, fixing joints at various angles influenced muscle tone and cell metabolism (Booth 1977; Goldspink et al. 1986). In contrast, human exposure to microgravity during space flight unloaded both muscle and bone while joints were free to move through a wide range of motions. Animals exposed to microgravity during in-flight missions or subjected to ground-based studies (e.g., hind-limb unloading) also provided invaluable information into the mechanistic changes and the chronic adaptations associated with unloading (Musacchia et al. 1988). However, differences in anatomy, growth rate, and an inability to measure voluntary muscle function provided substantial limitations (Booth and Gollnick 1983). Based on these difficulties, the need for a human unloading model that maintained intact joint mobility was warranted, and subsequently, ULLS was developed (Dudley et al. 1992b).

Within the published literature there are two primary versions of ULLS: (1) the original model consisting of a strap to support the leg and (2) a modified strap-free model. In the original model established by Berg and Colleagues, a harness was worn around the waist, and a strap was attached to a modified shoe in order to suspend one lower limb. The strap held the knee in flexion (~90°–120°), and a 50-mm platform shoe was worn on the opposite foot to prevent involuntary weight bearing. All activities were

performed using the assistance of axillary crutches. This method, however, was associated with a greater risk (~2.7%) of deep vein thrombosis (DVT) (Berg and Tesch 1996; Bleeker et al. 2004) and was subsequently modified. In the modified model, the strap was removed, and the platform shoe was raised (~10 cm) to allow the unloaded leg to swing freely (Ploutz-Snyder et al. 1995). To our knowledge, this method has not been associated with the development of DVT, and in more recent studies, investigators further decrease this risk by excluding individuals with family or personal history of blood clotting disorders as well as those using hormone therapy (Lidegaard et al. 2002). Below knee, elastic compression stockings have also been incorporated to prevent blood pooling in the lower leg (Byrne 2001). Today, the majority of studies use the modified ULLS model.

As described by Adams et al. (2003), there are many advantages to ULLS. First, ULLS is a more cost-effective method to study unloading compared to bed rest as there are no specialized facilities or 24 h support staff. In addition, subjects can maintain a relatively normal lifestyle while participating in an investigation. They can sleep in their own home, interact with family members, and maintain employment, while other analogs do not allow for these freedoms. It is acknowledged that in some circumstances these freedoms can threaten internal validity as there can be considerable variability between subjects in everyday activities (stress, diet, and sleep). In addition, in order for outcome measures to have an interpretable meaning, subject compliance is crucial. Besides daily interviews with participants, two of the most common methods for evaluating compliance are surface skin temperature and calf circumference. Typically, skin temperature in the unloaded limb is ~2°C lower than the loaded limb (Adams et al. 1994), while calf circumference is ~2–3 cm greater (when compression stockings are not worn) in the unloaded calf compared to the loaded calf (Adams et al. 1994; Tesch et al. 2004). For a more quantifiable measure of compliance, specially designed plantar accelerometers have been utilized (Cook et al. 2006).

The present review will discuss the knowledge obtained over the past 20 years using ULLS as a model to study the deleterious effects of unloading. Given the immense size of the scientific literature, our comparisons will be delimited to the intact human. Moreover, results from space flight field experiments are always influenced by multiple countermeasures including exercise. Thus, for the purpose of this review, interpretations of unloading via ULLS will be coupled with findings from relatively similar durations of prolonged bed rest. In addition, studies that have used exercise during ULLS to counteract the negative alterations of unloading will be highlighted.

Compromised human performance

At least 24 published manuscripts have reported maximal isometric, isokinetic, and/or isotonic muscle strength before and after ULLS. For comparative purpose, these results are summarized for the knee extensors and plantar flexors in Fig. 1a, b. When evaluated as relatively linear declines, rates of maximal isometric muscle strength loss in the knee extensors and plantar flexors are ~0.72 and ~0.63%, respectively, for each day of ULLS (Bleeker et al. 2005a; Clark et al. 2006a, 2010; de Boer et al. 2007a; Hotta et al. 2010; Schulze et al. 2002; Seynnes et al. 2008b, 2010; Tesch et al. 2004). In the knee extensors, the rate of isometric force loss with ULLS compares very well to what can be interpreted over an equivalent duration of bed rest (~0.73% per day); however, too few studies have examined isometric muscle strength in the plantar flexors following bed rest for general comparisons to be attempted (Narici and de Boer 2011). The decline in maximal force output following ULLS and bed rest is coupled with a decrease in the rate of force development (Bamman et al. 1998; Clark et al. 2006a; Kubo et al. 2000), suggesting not only impaired neuromuscular force production, but also slowed transmission to the skeleton. The rate of force development is dependent on the stiffness of the series elastic component and force–velocity characteristics of the contractile component (Kubo et al. 2000). Hence, an alteration in one or both of these areas may alter explosive or powerful movement capability.

Movement control may be modulated by the steadiness of a muscle contraction. After 28 days of ULLS, force steadiness evaluated at moderate intensities (~25% MVC) declined by ~22 and 12% in the knee extensors and plantar flexors, respectively (Clark et al. 2007b). In this regard, force control during lengthening (eccentric) muscle actions was largely altered, while no changes were reported during shortening muscle actions. Reduced force steadiness at low intensities (~2.5–5% MVC) have been reported after 20 days of prolonged bed rest; however, there was greater impairment in the plantar flexors compared to the knee extensors (Shinohara et al. 2003). These findings suggest that the plantar flexors may be prone to fluctuations in forces steadiness at low force outputs, while control in the knee extensors may only be modulated at higher force outputs (Clark et al. 2007b). Ultimately, impaired forced steadiness following unloading can compromise the performance of tasks where fine motor skills are required.

Muscular endurance has been studied less frequently following unloading compared to measures of strength. In the knee extensors, isokinetic total work capacity was reduced by ~13% after 21 days of ULLS (Schulze et al. 2002), while dynamic endurance was decreased by ~24% by day 30 (Cook et al. 2010). A ~16% decrease in knee

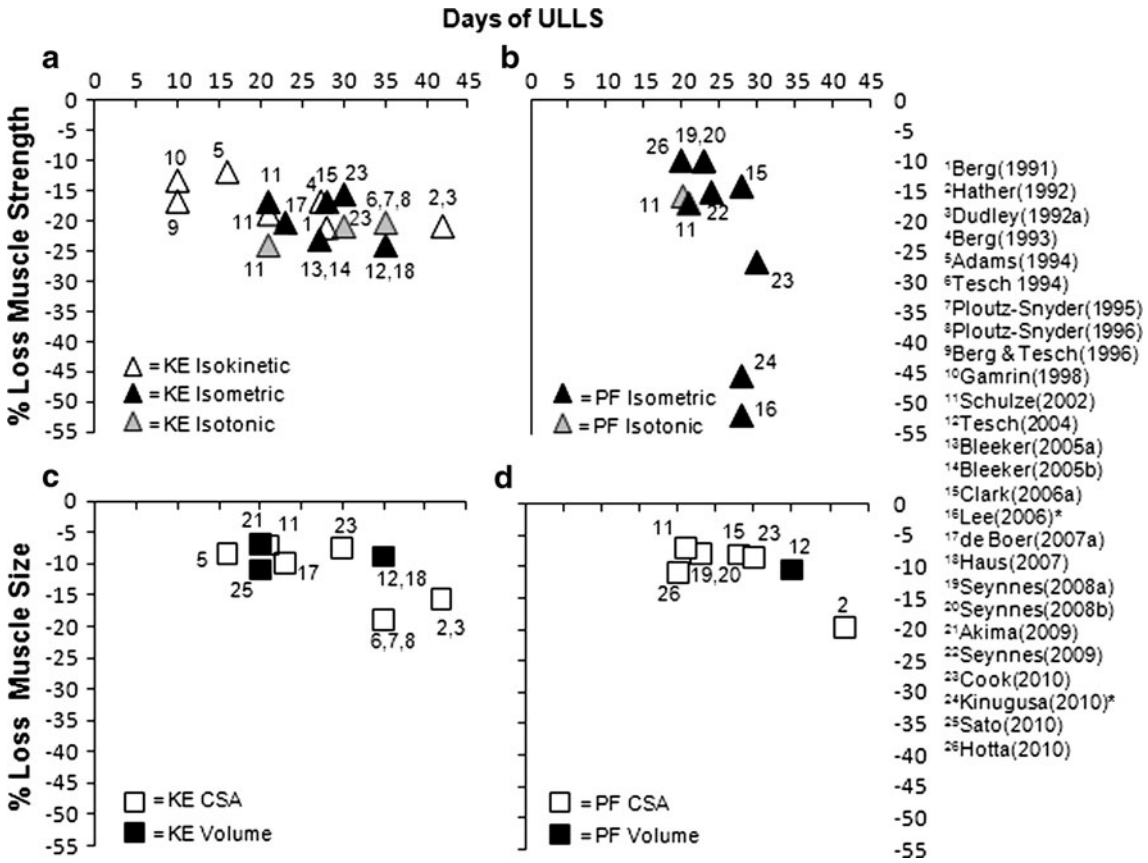

Fig. 1 Percent decline in muscle strength [**a** knee extensor (KE) and **b** plantar flexor (PF)] and muscle size (**c** KE and **d** PF) relative to the duration of ULLS. *Measurement of MVC within MRI

extensor total work capacity was observed following an equal duration of bed rest (Greenleaf et al. 1994). The ability to resist fatigue can be strongly associated with ventilatory threshold and the ability to utilize oxygen (VO_2). Both ventilatory threshold and VO_2 peak are reduced following short duration (10–17 days) bed rest (Jenkins et al. 1998; Sreter 1963). Similarly, Sato et al. (2010) recently showed a 6% reduction in VO_2 peak obtained from single leg cycling after 20 days of ULLS. A reduced asymptote in the fast component of VO_2 at the end of single leg cycling exercise (3 series of 6 min, 60 W, 60 rpm) was also reported over the same duration of unloading (Hotta et al. 2006). The response speed of VO_2 to exercise (fast component) reflects both central and peripheral circulation and oxidative enzyme activity in muscle. Ultimately, Hotta et al. (2006) suggest as follows: unloading the energy supply system becomes more dependent on glycolysis rather than oxidation.

Collectively, lower maximal force output, a decrease in the rate of force development, compromised forces steadiness, reduced muscular endurance, and altered VO_2 kinetics are likely to diminish physical performance. Although ULLS studies have been limited to the study of a single leg, the model generally elicits similar performance-related adaptations compared to a relatively equal duration of prolonged bed rest. Obviously, some subtle differences exist, which limits the use of ULLS for certain outcome variables (e.g., bipedal movement parameters). Presented next are many of the physiological mechanisms that ultimately interact to reduce physical function following prolonged unloading.

Mechanisms

Neuromuscular alterations

Spinal cord and/or Ia afferents

Nervous system impairment following unloading can explain a large proportion of the loss of maximal muscle strength (Clark et al. 2006b) and contribute to muscle fatigability (Allen et al. 2008). The exact sites of modulation within the nervous system have been difficult to determine in vivo. At the level of the spinal cord, the Hoffman (H) reflex can be interpreted as a measure of

spinal excitability, while also reflecting transmission efficiency (presynapatic inhibition) in Ia afferent synapses (Aagaard et al. 2002; Schieppati 1987). H-reflex occurs from submaximal stimulation of sensory nerve fibers that project back and excite the α-motor neurons to create an action potential, thereby bypassing the influence of muscle spindle sensitivity and γ-activation of intrafusal fibers (Clark et al. 2006b). Resting H-reflex has been shown to increase (~18–35%) as measured in the soleus of the unloaded limb following 21–28 days of ULLS (Clark et al. 2006b; Seynnes et al. 2008a, 2010) and following 35 days of bed rest (Duchateau 1995). These results infer presynaptic inhibition and/or homosynaptic post-activation depression; however, the exact origin of dysfunction is not known.

To gain further insight into the adaptations of the spinal cord, an electrophysiological variant of the H-reflex, termed the first volitional (V) wave, has been evaluated in unloaded muscle following ULLS. The V-wave can be assessed using supermaximal electrical stimulation during MVC and is thought to reflect both reflex excitability and neural drive (Aagaard et al. 2002). After 24 days of ULLS the V-wave was unaltered, while H-reflex was increased (Seynnes et al. 2010). These data suggest that increased resting H-reflex may not alter motor out flow in the plantar flexor muscles during maximal contraction. Therefore, it is unclear how changes in resting H-reflex influence task performance, especially during maximal efforts.

Peripheral nerve conduction

Latency responses associated with H-reflex provide information pertaining to peripheral nerve conduction (Clark et al. 2007a). For example, by measuring the time between stimuli onset and the upshot of the muscle action potential (M) wave, M-wave latency can be calculated. This time period has been shown to increase (~8.5–9.4%) following ULLS (24–28 days) in the plantar flexors (Clark et al. 2006b; Seynnes et al. 2010) and following prolonged bed rest (Ruegg et al. 2003). Increased M-wave latency indicates slowed conduction velocity in supply nerves, branching axon terminals, or transmission across the neuromuscular junction (Kim et al. 2005). Additional interpretations have been reported in peripheral nerves using H-wave latency, which is the time interval between the stimulus onset and the upshot of the H-wave. Similarly, H-wave latency is prolonged (~1.5–3.6%) after ULLS in the plantar flexors (Clark et al. 2006b; Seynnes et al. 2010). This change represents the time required for propagation of a signal through the reflex arc, which includes the Ia afferent, synaptic delay at the motor neuron, and smaller (type I) efferent motor units across the neuromuscular junction (Clark et al. 2006b). However, the conduction time through the reflex loop (H-wave − M-wave latencies) was not changed by ULLS, indicating that the slowed signal through the reflex arc is likely reflected by alterations at the neuromuscular junction (Clark et al. 2006a, b).

Motor unit activation

The ability to voluntarily activate all motor units is a key component of force production and is routinely evaluated using the interpolated twitch technique (involving a superimposed supramaximal electrical stimulation during MVC). In this regard, central activation capacity and the central activation ratio have been used to make quantitative comparisons following unloading. Using strict statistical analysis, the majority of the studies show that neither central activation capacity nor central activation ratio are significantly decreased following 20–28 days of ULLS (Clark et al. 2006b; de Boer et al. 2007a; Hotta et al. 2010; Seynnes et al. 2010). However, although voluntary activation capacity did not decline significantly after 28 days of ULLS when analyzed using ANOVA, this property did account for ~50% of the between-person variability in the loss of plantar flexor strength when analyzed using multiple regression (Clark et al. 2006b). Following bed rest, a reduction in central activation explained 33% of the reduction in MVC in the plantar flexors (Duchateau 1995) and was reduced by ~7% in the knee extensors (Kawakami et al. 2001). Overall, it appears likely that the ability to voluntarily activate all motor units following unloading is compromised, which provides some insight into the significant loss of maximal force observed.

Muscle electrical activity

In general, electrical activity evaluated from surface electromyography (EMG) during MVC are reduced compared to before ULLS (Dudley et al. 1992a; Seynnes et al. 2008a, b) and bed rest (Berg et al. 1997; Portero et al. 1996). A decline in surface EMG signal intensity may occur as a result of multiple factors including (1) muscle atrophy, (2) a reduced ability to activate high threshold motor units, (3) increased resting membrane potential, (4) a reduction in maximal firing frequency, and/or (5) a decrease in action potential amplitude (Dudley et al. 1992a). Additionally, surface EMG has been used to examine activation patterns during submaximal tasks following unloading. In these investigations, the EMG signal has been shown to increase following ULLS (Berg and Tesch 1996; Schulze et al. 2002; Tesch et al. 2004) and bed rest (Berg et al. 1997), which indicates that greater neural activation is required to complete the task compared to before the unloading period. Other methods have also been utilized to explore the change in neuromuscular function with unloading. For

instance, T2-weighted magnetic resonance (MR) images from skeletal muscle show exercise-induced contrast enhancement, which is related to exercise intensity (Adams et al. 1992). In two ULLS studies, the unloaded leg displayed elevated T2 enhanced contrast shifts at various exercise loads. As a result, the authors concluded that a greater proportion of muscle mass and/or greater metabolic demand was required to perform the concentric muscle action (Akima et al. 2009; Ploutz-Snyder et al. 1995). Overall, these data suggest that more explosive type IIa or type IIx motor units are recruited to reach the same level of force output following unloading.

Further inferences into the function of skeletal muscle given the neurological alterations previously described have been obtained from electrophysiological interpretations following nerve stimulation. The compound muscle fiber action potential (CMAP) represents the sum of electrical activity following a synchronous depolarization of the muscle fibers innervated by the evoked nerve. After 28 days of ULLS the duration of the soleus CMAP (also referred to as M-max) was slowed (Clark et al. 2006a). Physiologically, this reduction could be attributed to slowing muscle fiber conduction velocity, enhanced temporal dispersions in the response of different motor units, and/or changes in muscle cell membrane properties (Keenan et al. 2006; Kim et al. 2005). Over the same duration of unloading evoked doublet (100 Hz) force decreased (~10%) with an accompanied increase in the twitch to doublet force ratio (Clark et al. 2006a). These results suggest the loss of evoked force at high stimulation frequencies, which could be caused by impaired excitation–contraction coupling (Jones 1996).

Musculoskeletal alterations

Muscle size

A reduction in whole muscle size (via MR imaging or computerized tomography), expressed as anatomical cross-sectional area (CSA) or volume, may be the most frequently reported outcome variable to explain the loss of maximal muscle function. The decline in muscle size is plotted against the duration (up to 42 days) of unloading in the knee extensors and plantar flexors in Fig. 1c, d. Collectively, the observed rates of decline in muscle size for each day of ULLS in knee extensor and plantar flexor size (anatomical CSA or volume) is ~0.40 and ~0.36%, respectively (Adams et al. 1994; Akima et al. 2009; Clark et al. 2006a; Cook et al. 2010; de Boer et al. 2007a; Dudley et al. 1992a; Hather et al. 1992; Hotta et al. 2010; Sato et al. 2010; Schulze et al. 2002; Seynnes et al. 2008b; Tesch et al. 1994, 2004). These data appear to be similar, but slightly less, than the rate of decline observed in the knee extensors (~0.41% per day) and plantar flexors (~0.42% per day) over the same duration of bed rest (Narici and de Boer 2011). Moreover, the rates of individual muscle atrophy following ULLS relative to duration are displayed in Fig. 2. It appears that the vastus lateralis (~0.44% per day of ULLS) may be the most prone to muscle atrophy, followed closely by the gastrocnemius (~0.36% per day of ULLS). It should be noted, however, that as the duration of unloading persists (beyond 50 days) greater atrophy in the plantar flexors compared to the knee extensors is generally reported in the literature (Narici and de Boer 2011).

Muscle architecture

The structural arrangement of muscle fibers can also reflect function (de Boer et al. 2008), and the previously described changes in anatomical CSA or volume ignore any potential alterations in architecture. In this regard, the majority of skeletal muscle fibers insert into an aponeurosis at a pennation angle before this tissue thickens to become tendon (Jones et al. 2004). According to de Boer et al. (2008), reduced fascicle length indicates a decline in sarcomere shortening during contraction, which could influence muscle function depending on where it operates on the length–tension relationship. Alternatively, a reduction in pennation angle is thought to reflect sarcomeres in parallel and packing along the aponeurosis (de Boer et al. 2008). These structural changes are usually interpreted with a calculation of physiological CSA (PCSA), which is determined by dividing muscle volume by fascicle length (assessed in vivo by ultrasonography). After 23 days of ULLS resting fascicle length and pennation angle in the lateral gastrocnemius were reduced by ~4 and 5%, respectively, while PCSA was reduced by ~3% (Seynnes et al. 2008b). Vastus lateralis fascicle length also decreased (~7.7%) over the same duration of unloading (de Boer et al. 2007a), and alterations to muscle architecture in antigravity muscles have been confirmed in several bed rest studies (Akima et al. 2001; Kawakami et al. 2000, 2001). Overall, the realignment of muscle architecture is hypothesized to more accurately reflect functional impairments, especially explosive movements related to muscular power (Seynnes et al. 2008b).

Muscle composition and enzyme activity

Unloading has been associated with a change in energy stores, primarily as a result of altered enzyme activity within skeletal muscle tissue. After 28 days of ULLS, Manini et al. (2007) showed increased intramuscular adipose tissue of ~20 and ~14% in both the calf and thigh, respectively. These changes suggest either an influx of

Fig. 2 Percent decrease in the size of individual muscles per day of ULLS. *VL* vastus lateralis, *VI* vastus intermedius, *VM* vastus medialis, *RF* rectus femoris, *Sol* soleus, *Gast* gastrocnemius, *BF* biceps femoris, *ST* semitendinosus, *SM* semimembranosus, *Sar* sartorius, *Gr* gracilis, *AL* adductor longus, *AdM* adductor magnus

triacylglycerol from the vasculature or decreased fat oxidation (Manini et al. 2007). Studies support impaired fat oxidation as the activity of citrate synthase, a krebs cycle enzyme indicative of oxidative capacity along with beta-hydroxyacyl-CoA dehydrogenase, a key enzyme in fatty acid metabolism both decline with ULLS and bed rest (Berg et al. 1993; Hikida et al. 1989). Based on these results and the interpretation of VO_2 kinetics discussed previously (Hotta et al. 2006), it is likely that there will be greater reliance on carbohydrate for fuel during extended physical activity following an unloading period (Grichko et al. 2000).

Single muscle fiber size, function, and distribution

Skinned and isolated muscle fibers provide insight into cross-bridge mechanics that are independent of processes associated with sarcoplasmic reticulum Ca^{2+} release (Widrick et al. 1997). In these experiments fiber diameter, peak power, maximal unloaded shortening velocity, and force–power characteristics of single muscle fibers are evaluated, while being paired with a representation of myosin heavy chain (MHC) (Trappe et al. 2004). In 2002, there was a considerable debate as to whether single fiber results obtained from ULLS compared favorably to the data obtained following bed rest (Adams 2002; Widrick et al. 2002). Briefly, soleus type I MHC fiber diameter decreased by ~7 and ~5% after 12 days of ULLS and 17 days of bed rest, respectively. Peak Ca^{2+} activated force in these fibers declined to ~18 and ~13% with ULLS and bed rest. Together, these results suggest a greater reduction in specific force (force/diameter) with ULLS (~5%) compared to bed rest (~1%) (Widrick et al. 1997, 2002). In addition, shortening velocity in type I MHC soleus fibers were also reduced by ~10% after ULLS, which was in contrast to the 34% increased observed following bed rest (Widrick et al. 1997). Overall, whether ULLS and bed rest elicit similar single fiber adaptations is likely still open for individual interpretation. In the before-mentioned studies, several factors such as within study exercise testing procedures, unloading limb positioning, and overall unloading duration make definitive conclusions nearly impossible to make (Adams 2002).

Alternatively, fiber type shifting towards faster phenotypes has generally been observed following both ULLS and bed rest. From the ULLS data, it appears that explosive type IIx fibers in the vastus lateralis increase their distribution, but the change in type I and type IIa can be variable depending on the duration of unloading (Table 1). Similarly, after 2 months of bed rest in the soleus, pure type I MHC fibers tended to decline (~8%), and there were de novo appearances of hybrid fibers expressing multiple combinations of MHC (Ohira et al. 1999). Together, the shift away from slow contractile velocities may help maintain functional properties in faster phenotypes and explain why type I MHC fibers are more prone to a loss of single fiber function (Widrick et al. 2002).

Protein composition

Contractile proteins actin and myosin are specifically relevant for force generation in the sarcomere, while connective tissue networks (e.g., collagen) are important for the transfer of force to the skeleton (Haus et al. 2007). Carrithers et al. (2002) examined total cytosolic, total myofibrillar, and the concentrations of MHC and actin in the vastus lateralis and soleus before and after 35 days of ULLS. There were no alterations in any protein concentrations in the vastus lateralis. However, in the soleus MHC and actin remained unchanged, while total protein, cytosolic protein, and myofibrillar protein were reduced significantly (Carrithers et al. 2002). These data were supported in an additional investigation where mixed, sarcoplasmic, and myofibrillar proteins fractions were significantly decreased in the soleus, with no change in force specific proteins (actin, myosin, and collagen) over the same duration of ULLS (Haus et al. 2007). Collectively, it appears that protein fractions in the soleus are augmented to a greater extent than the vastus lateralis. Haus et al. (2007) suggest that titin, nebulin, c-protein,

Table 1 Single fiber size and distribution in the vastus lateralis following ULLS

Variable	Adams et al. (1994)	Berg et al. (1993)	Hather et al. (1992)
Sample size (*N*)	10	6	8
Duration (days)	16	28	42
Type I CSA (% change)	−10	−6	−14
Type IIa CSA (% change)	−0.07	−11	−15
Type IIx CSA (% change)	−18	Ave type IIa/x	−11
Type I distribution (% difference)	−4	+1	−1
Type IIa distribution (% difference)	0	−3	−3
Type IIx distribution (% difference)	+4	+2	+4

m-protein, or other cytoskeletal proteins may be targeted and disproportionally lost following unloading.

Reduced protein synthesis

A decline in the rate of muscle protein synthesis is a mechanism that can lead to a loss of muscle protein. In vivo, there was ~10% decline in the fractional rate of muscle protein synthesis after 10 days of ULLS (Gamrin et al. 1998) and ~50% reduction after 14 days of bed rest (Ferrando et al. 1996). Moreover, de Boer et al. (2007b) suggest that the greatest drop in the rate of muscle protein occurs initially (~10 days) with little change as unloading persists (~21 days). Advanced molecular biology research techniques have allowed for insight into the mechanisms, whereby muscle protein synthesis is reduced. Thirty-five days of ULLS showed decreases in total mRNA (~13%) in the unloaded vastus lateralis (Haddad et al. 2005). The decline in total extractable muscle RNA can be used as an index of muscle protein translational (synthesis) capacity as of 85% of the total RNA pool consists of ribosomal RNA (Haddad et al. 2005). Yet, impairment of a specific intracellular signaling pathway is not provided by this global indicator.

For instance, the protein kinase B (Akt)—mammalian target of rapamycin (mTOR)-p70S6K pathway—is of considerable interest during unloading as hormonal and mechanical phosphorylation is associated with muscle maintenance and growth (Spiering et al. 2008). The mRNA encoding insulin-like-growth-factor-1 (IGF-1) protein, a known activator of Akt when phosphorylated, was unchanged following 35 days of ULLS (Haddad et al. 2005). There was also no change in protein or phosphorylation of components of the Akt-mTOR-p70S6K pathway after 3 days (Gustafsson et al. 2010) or 10 and 21 days of ULLS (de Boer et al. 2007b). Alternatively, Sakuma et al. (2009) report a significant decline in Akt protein after 20 days of ULLS, suggesting a down-regulation of a protein heavily implicated in muscle protein synthesis (Sakuma et al. 2009).

The negative growth regulator myostatin may also explain the reduction in protein synthesis. Myostatin blunts muscle growth by inhibiting the proliferation and differentiation of myoblasts in developing muscle through several downstream mediators (Zhu et al. 2004). In as little as 3 days of ULLS, myostatin mRNA and protein levels were elevated in the soleus and vastus lateralis (Gustafsson et al. 2010), which generally corresponds to data reported by Sakuma et al. (2009) after 20 days of ULLS (Sakuma et al. 2009). In contrast to these data, no changes in myostatin mRNA in the vastus lateralis were reported following 10 or 20 days of ULLS (de Boer et al. 2007b).

Serum response factor (SRF) may also play a significant role in promoting or maintaining muscle size through mechanical transduction via Ras homolog gene family member A (Rhoa)-focal adhesion kinase (FAK) (Carson and Wei 2000). The Rhoa–FAK–SRF pathway can increase differentiation in cultured cells and growth and differentiation of mature cells. These mediator proteins (Rhoa, SRF), however, were unchanged following 20 days, indicating that a down-regulation of the proteins was not related to the ~9% decrease in vastus lateralis CSA observed (Sakuma et al. 2009). Alternatively, phosphorylation of FAK was reduced within the first 10 days of ULLS, facilitating a ~5% reduction in knee extensor muscle CSA by day 14 (de Boer et al. 2007a). Signaling at this protein is hypothesized to lie upstream of SRF, and the authors suggest that the phosphorylation rather than total protein may change signaling capacity (de Boer et al. 2007a).

Increased protein breakdown

In addition to reduced muscle protein synthesis, an increase in the rate of protein breakdown is a mechanism whereby protein can be lost from muscle. Recently, a microdialysis technique was utilized to measure intramuscular 3-methylhistidine (3MH) concentration in order to quantify the breakdown of contractile protein (actin and myosin) (Tesch et al. 2008). Previous measures of 3MH were typically

obtained in the urine as a marker for global protein breakdown. Therefore, by using this new approach, Tesch et al. (2008) suggest that it is possible to circumvent many of the previous limitations of urinary 3MH by allowing insight into the contractile contribution to proteolysis. Concentration of interstitial 3MH was increased by ~44% following only 72 h of ULLS, suggesting significant proteolysis of actin and myosin (Tesch et al. 2008). It should be noted, however, that the results obtained from using this technique have been debated given the difficulty of measuring protein breakdown in vivo (Rennie et al. 2008).

Similar to protein synthesis, gene expression can provide a representation of proteolytic pathways. For instance, the Akt pathway can be implicated in muscle hypertrophy as well as muscle atrophy. Dephosphorylation of Akt removes an inhibitory influence over the forkhead family of transcription factors (FOXO) for E3 proteolytic ligases (i.e., Atrogin-1). After 20 days of ULLS, there was no change in FOXO (1 or 3a) protein or atrogin-1 mRNA (Sakuma et al. 2009). Other ubiquitin–proteosome pathway E3 ligases such as muscle atrophy f-box (MAFbx), muscle ring finger 1 (MuRF-1) and tripeptidyl peptidase II (TPII) were also evaluated following unloading. Overall, MuRF-1 mRNA was elevated after 10 days of ULLS, but decreased along with MAFbx mRNA and TPII by day 21 (de Boer et al. 2007a). If the increase MuRF-1 mRNA represents the proteolytic enzyme capability, there may be evidence of transient elevations in protein breakdown early in the unloading period. However, because MuRF-1 mRNA levels decreased by day 21, it does not appear that proteolysis remained elevated over the entire period of unloading.

Tendon dimensions and properties

The ability to transfer force from muscle contraction to the skeleton is greatly influenced by the size, stiffness, and series-elastic properties of the tendon (de Boer et al. 2007a). Following 10 days of ULLS, the rate of patellar tendon collagen synthesis was reduced by ~50%, which declined to another ~50% by day 21 of unloading. Surprisingly, patellar tendon CSA and resting length were unaltered, suggesting that collagen breakdown must have matched synthesis (de Boer et al. 2007a, b). Another study demonstrated no change in tendon size after 28 days of ULLS (Shin et al. 2008), while Kinugasa et al. (2010) showed the total volume of tendinous tissue (Achilles tendon and distal aponeurosis) increased 6.4% after 28 days of ULLS. It is hypothesized that no change or an increase in tendon size following unloading, in the face of lowered synthetic rate, may occur because of increased water content in extracellular spaces (Kinugasa et al. 2010).

Unloading induces plastic changes in tendon mechanical properties (elongation, stiffness, stress, and strain) as well as material properties (Young's modulus: stiffness normalized to dimensions). Patella tendon elongation was increased (~16.9%) after 23 days ULLS, and subsequently, tendon stiffness decreased by ~29.3% (de Boer et al. 2007a). These studies are consistent with the alterations in tendon stiffness observed following 20 days of bed rest (Kubo et al. 2000, 2004). A greater elongation of a tendon as a result of decreased stiffness would require muscle fibers to shorten more at a defined force level (de Boer et al. 2007a). Subsequently, this change can cause the length–tension relationship to shift, leading to lower force production and delayed execution of force transfer during motor tasks (Proske and Morgan 1987). Moreover, three studies have shown that Young's Modulus is decreased following ULLS (de Boer et al. 2007a; Kinugasa et al. 2010; Shin et al. 2008), which indicates the structure and packing of collagen fibers are altered (Danielsen and Andreassen 1988). Collectively, it is evident that unloading results in dynamic changes to both mechanical and material properties of tendon. These disruptions may have implications for performance and increase injury risk upon reloading (Reeves et al. 2003).

Bone content and properties

The skeleton provides protection and structure for force transfer via the muscle–tendon interaction. During bed rest, bone density declines at average rate of 0.5–1% per month (Pavy-Le Traon et al. 2007). The average duration of ULLS studies are ~30 days; subsequently, little information has been obtained on skeleton using this analog. In one recent investigation, peripheral quantitative computed tomography scans showed that bone mineral content of the suspended tibia epiphysis was significantly reduced (1.1%) after 21 days of ULLS (Rittweger et al. 2006). This change was facilitated by bone mineral loss in the peripheral (55% of the tibia epiphysis) rather than the central portion (45% of the tibia epiphysis) (Rittweger et al. 2006). Moreover, femoral bone mineral density (assessed by a bone densitometer) was decreased by ~4% ($n = 1$) immediately post-ULLS (Ito et al. 2004). Interestingly, systemic markers of bone resorption (bone-specific alkaline phosphatase, osteocalcin) do not appear to be elevated over a period of unloading (Cook et al. 2010, unpublished.). These observations are likely a result of the nature of the ULLS model and may not reflect the dynamic responses occurring in bone in the unloaded leg. Nevertheless, weakness in the skeletal remains a significant risk for health and function following unloading.

Circulatory alterations

Arteries, veins, and capillaries

One limitation of ULLS is the inability to evaluate many cardiovascular variables (Adams et al. 2003); however, some insight into the vasculature of the unloaded leg has been performed. After 28 days of ULLS, the diameter of the common and superficial femoral artery (measured by ultrasound) was reduced by ~12% (Bleeker et al. 2005a). Bleeker et al. (2005a) suggest that a decrease in arterial diameter may represent inward remodeling to reduce exposure to shear stress. Despite the reduction in vessel size, blood flow at the femoral artery (measured by Doppler ultrasound) at rest did not show any significant change between the unloaded and loaded legs. However, calf baseline blood flow (measured by plethysmography) was significantly reduced (~26%), which is consistent with the decline (~26–38%) observed with bed rest (Convertino et al. 1989; Kamiya et al. 2000; Louisy et al. 1997).

Capillary density has been examined in several publications through the use of muscle biopsies. There was no change in capillary density after 28 days of unloading (Berg et al. 1993); yet, 42 days of ULLS resulted in a significant increase (~15%) (Hather et al. 1992). Unfortunately, fatigue resistance does not appear to be elevated given the increase in capillary density as shown with exercise training (Hepple 2000). Instead, the majority of studies show a decline in total work capacity and muscular endurance following unloading (Cook et al. 2010; Schulze et al. 2002). Since the number of capillaries surrounding each fiber did not change, it is likely that fiber atrophy caused the increase in capillary density. The disproportional response indicates that different factors regulate changes in fiber size compared to muscle capillarity (Hather et al. 1992).

Venous capacitance is directly related to the function of the circulatory system and represents the blood volume that can be stored and ultimately returned to the heart (Bleeker et al. 2005a). Following 28 days of ULLS, the venous pressure–volume curve in the calf was shifted downward, suggesting a decrease in venous capacitance. Excess venous pooling can cause orthostatic intolerance upon standing (Bleeker et al. 2005a) and also blunt cardiac output at the start of maximal or submaximal activity (Nobrega et al. 1995). Taken together, these data suggest alterations in the venous system that could contribute significantly to circulatory dysfunction.

Exercise countermeasures

Identifying the most applicable exercise countermeasure and prescription to maintain all components of physical performance is challenging, especially considering the relative incapability of concurrent aerobic and resistance exercise (Hickson 1980). Highlighted outcomes from exercise interventions employed during ULLS are displayed in Table 2. Similar to prolonged bed rest (Akima et al. 2000, 2001; Bamman et al. 1997), high-load resistance exercise has been effective during ULLS although modality (i.e., machine, flywheel) and the exercise prescription are variable. For example, isometric and isotonic knee extension and plantar flexor exercises performed every third day (6 times) over 21 days of ULLS were effective in mitigating the decline in muscle size, strength, and endurance. The exercise prescription consisted of 2 MVCs (5 s duration), one set of 10 isotonic repetitions (~80% of 1RM), and a second set to muscle fatigue (Schulze et al. 2002). Alternatively, four sets of 7–8 maximal concentric and eccentric muscle actions have been efficacious during 35–40 days of ULLS (Caruso et al. 2004; Tesch et al. 2004). Therefore, although exercise prescription is variable, high-load resistance exercise appears to be an effective countermeasure to unloading. In addition, studies are beginning to explore additional countermeasures that may optimize the benefit of exercise. For instance, when inertial flywheel resistance exercise was coupled with Albuterol (maximum dose ~16 mg/day), bone mineral density in the unloaded limb was increased compared to flywheel resistance exercise alone (Caruso et al. 2004). Albuterol is a B_2-agonist that has previously shown to reduce bone loss during hind-limb unloading in animals (Bloomfield et al. 1997). There is also evidence that women may have greater benefits when using this combined intervention compared with men as evidenced by increased total work and power during exercise sessions. The difference has been hypothesized to occur because of enhanced myofibril sensitivity for calcium in women (Caruso et al. 2008). Overall, by coupling this pharmacological agent with resistance exercise, multiple systems are targeted and potentially protected from the negative alterations associated with unloading.

Although high-load resistance exercise training during unloading has been effective, there are several limitations to consider. For example, this type of exercise may be contraindicated for those who have had a previous or existing injury. High-load resistance exercise is also difficult to perform without adequate space, equipment, or the assistance of a spotter. Recently, a novel alternative to high-load resistance exercise has been put forth that incorporates low training loads coupled with a moderate blood flow restriction (Manini and Clark 2009). This type of exercise has recently been shown to be effective for maintaining muscle size, strength, and even increasing endurance during 30 days of ULLS (Cook et al. 2010). The exercise prescription in this investigation consisted of three sets of dynamic contractions

Table 2 ULLS studies with and without exercise countermeasures (2000–2010)

Publication	*N* (gender)	Duration (days)	Countermeasure	Highlighted outcomes
Schulze et al. (2002)	8 (M)	21	None	M.ACSA (CT): ↓7% KE and PF, strength (Isom): ↓17% KE and PF, (Isok): ↓19% KE, (Isot): ↓24% KE, ↓16% PF
	8 (M)		Isom./Isot. Knee Ext. and Plant.Flexion	M.ACSA (CT): ↔ KE and PF, strength (Isok): ↔ KE and PF, (Isot): ↑9% PF
Caruso et al. (2004)	8 (5M, 3W)	40	Placebo + flywheel leg and calf press	M.Mass (DEXA): ↔ kg, ↔ BMC unloaded Leg, ↓BMD pelvis, ↔ Sys/Dia BP
	16 (9M, 7W)		Albuterol + flywheel leg and calf press	M.Mass (DEXA): ↔ kg, ↑ BMC unloaded leg, ↓BMD pelvis, ↔ Sys/Dia BP
Tesch et al. (2004) Haddad et al. (2005)	11 (7M, 4W)	35	None	M.Vol. (MRI):↓9% Quad, strength (Isom): ↓26% KE, ↓total RNA, ↓MHC and actin mRNA, ↔ actin and myosin protein VL
Haus et al. (2007)	10 (7M, 3W)		Flywheel: knee extension	M.ACSA (MRI):↑8% Quad, strength (Isom): ↓8% KE, ↔ total RNA, ↔ MHC and actin mRNA, ↔ actin and myosin protein VL
Clark et al. (2006a)	6 (1M, 5W)	28	None	Strength (Isom): ↓14% PF, ↓17% KE↓, ↓4% KE CAC
Clark et al. (2006b)	6 (3M, 3W)		KE vascular restriction	Strength (Isom): ↓15% PF
	6 (2M, 4W)		PF motor imagery	Strength (Isom): ↓10% PF, strength (Isom): ↓2% KE CAC
Akima et al. (2009)	5 (M)	20	None	M.Vol.(MRI): ↓7% Thigh, ↓11% PF, strength (Isom): ↓19% KE, ↓10% PF, ↓4% CAC PF
Hotta et al. (2010)	6 (M)		Interval cycling	M.Vol. (MRI): ↔ thigh, ↓5% PF, strength (Isom): ↓11% KE, ↔ PF, ↔ CAC PF
Cook et al. (2010)	8 (4M, 4W)	30	None	M.ACSA (MRI): ↓7.5 KE and 8.5% PF, strength (Isom): ↓15.5% KE and ↓26% PF, end (Isot): ↓28% KE, (Isom): ↔ PF
	8 (5M, 3W)		Knee Ext. resistance exercise + BFR	M.ACSA (MRI): ↔ KE and ↓5% PF, strength (Isom): ↓2% KE and ↓20% PF, end (Isot): ↑24%, (Isom) ↔ PF
Sato et al. (2010)	7 (M)	20	None	M.Vol (MRI): ↓11% KE, VO_2 peak: ↓6%
	6 (M)		Interval cycling	M.Vol (MRI): ↔ KE, VO_2 peak: ↔

MRI magnetic resonance imaging, *CT* computerized tomography, *DEXA* dual X-ray absorptiometry, *ACSA* anatomical cross-sectional area, *Vol* volume, *Quad* quadriceps, *VL* vastus lateralis, *KE* knee extensors, *PF* plantar flexors, *Ext* extension, *Isok* isokinetic, *Isom* isometric, *Isot* isotonic, *M* muscle, *M* men, *W* women, *BFR* blood flow restriction, *CAC* central activation capacity

to muscle fatigue with 90 s rest between sets. This resistance protocol was implemented based on a series of studies showing that training with low-loads at a moderate occlusion pressure (~1.3–1.5 systolic) induced large increases in growth hormone (Pierce et al. 2006; Takarada et al. 2000) and elicited large gains in muscle size and strength in ambulatory participants (Abe et al. 2006; Takarada et al. 2002). It appears that the mechanisms for how this exercise facilitates training adaptations are beginning to be uncovered (Manini and Clark 2009); however, optimizing the exercise prescription for each individual based on their unique characteristics (body size, muscle mass, fat mass, and blood pressure) requires further attention.

Finally, although resistance exercise (high-load or low-load) interventions are primarily implemented to combat the loss of muscle size and strength, aerobic exercise interventions are also emphasized for cardiovascular function (Greenleaf 1997). Sato et al. (2010) recently reported that single leg cycling (40–80% VO_2 peak) maintained VO_2 peak following 20 days of ULLS. This type of interval training also protected muscle size and strength over the same duration of unloading in the knee extensors (Akima et al. 2009; Sato et al. 2010), which indicates that intensity may be the most important variable for protecting function. However, muscle size, strength, and voluntary activation capacity were reduced in the plantar flexors (Hotta et al. 2010), which emphasizes the importance of exercise specificity.

Concluding remarks

The ULLS has been utilized as a ground-based analog to study the effect of unloading in humans since 1991. Over

the past 20 years, a wealth of data have been obtained to understand how physical function may be influenced as the duration of unloading is increased. In addition, many of the mechanisms that interact to disrupt nervous, muscular, skeletal, and circulatory systems have been identified. Although exercise countermeasures have been successful in mitigating negative unloading-related adaptations, there is a need for greater integration from areas that could optimize exercise prescription (i.e., nutrition, pharmacology). In the years to come, it is likely that ULLS will continue to be one of the most cost-effective ground-based analogs, where these interactions can be tested.

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