The Journal of Physiology

PERSPECTIVES

Not all disuse protocols are equal: new insight into the signalling pathways to muscle atrophy

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Muscle is a highly plastic tissue quickly responding with appropriate functional and structural changes to variations in volume and intensity of activity. Disuse, i.e. reduction of load and number of action potentials delivered by motoneurons, is followed by loss of muscle mass and strength. This allows skeletal muscles to adapt to the new requirements of a low performance. Diseases or traumatic injuries are often the cause of a period of muscle disuse. The subsequent muscle adaptation not only makes it difficult to return to previous life habits but also induces metabolic and cardiovascular alterations affecting the whole organism. In spite of the great difference in environmental conditions, similar effects of disuse are experienced also by astronauts who spend a period in microgravity in the space station.

Due to the relevance for translational medicine, experimental protocols have been designed to study the mechanisms responsible for the adaptation of muscle to disuse. The three main and most studied models are (i) bed rest, which implies that participants spend several days or weeks without leaving their bed, free to move but without any load on the lower limbs; (ii) unilateral lower limb suspension (ULLS), in which one leg is kept flexed and suspended above the ground by the use of a shoulder harness; and (iii) unilateral lower limb immobilization (ULLI), in which a cast blocks movement and loading of one limb.

All three protocols are effective and sufficient to induce in a few days the expected adaptations (loss of mass and loss of force) of muscles to disuse. However, available evidence suggests that subtle but relevant differences are present in the response to each specific protocol.

Those differences were first underlined by Widrick *et al.* (2002) who showed that while both bed rest and ULLS induce decrease of muscle fibre thickness and loss of specific tension (P_o), more pronounced in the anti-gravitary muscle soleus, the variations of unloaded shortening velocity (V_o) go in opposite directions. Bed rest as well as microgravity induces after few days an increase in V_o, whereas ULLS induces a decrease. Since mechanical power output, which is the parameter most relevant for physiological muscle function, is the product of tension and velocity, the increase of V_o can partially compensate the decrease of P_o in bed rest but not in ULLS. To explain the difference, Widrick and coworkers observed that in bed rest and spaceflight, unloaded limbs are allowed a free range of motion, while movements of the unloaded limb suspended above the ground are partially restrained in ULLS. They also took into consideration the possible impact of a reduction in blood flow in the unloaded limb. Moving down to the molecular level, the increase of V_o was interpreted as an effect of thin filament degradation and subsequent decrease in number of attached cross bridges (see Widrick *et al.* 2001). Cohen *et al.* (2009) later confirmed that distinct degradation pathways are utilized to remove proteins belonging to thick and thin filaments.

In a paper published in this issue of *The Journal of Physiology* by Brocca *et al.* (2015), the difference between the response to bed rest and the response to ULLS is re-proposed with new insights. The main effects of 3 weeks of ULLS on muscle fibres collected from biopsy of vastus lateralis in young healthy subjects were a marked decrease of fibre thickness (atrophy), a significant decrease of specific tension, and no change of V_o, in full agreement with the results of Widrick *et al.* (2002).

Brocca and her coworkers, however, went further as they analysed the recovery phase after ULLS and rehabilitation based on resistance training: they also, and more importantly, studied the accompanying variations in the protein complement and in the signalling pathways. This allowed a comparison with their recently published results on bed rest of similar duration (Brocca *et al.* 2012). An attempt to summarize and compare the response to bed rest and ULLS is given in Table 1.

The loss in muscle fibre cross-sectional area (23%) and myosin concentration (28%) was very similar in both disuse protocols. However, whereas in bed rest all anti-oxidant defence systems were down-regulated, *NRF2*, a master gene controlling expression of antioxidant proteins, was consistently up-regulated, and protein carbonylation occurred indicating that oxidative stress developed. In ULLS anti-oxidant defence systems were up-regulated, *NRF2* was unchanged and no protein carbonylation occurred indicating an effective cellular response to a possible redox imbalance. Moreover, whereas in bed rest autophagy and proteasome ubiquitination systems were up-regulated, thus contributing to atrophy progression and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) was down-regulated supporting metabolic derangement, in ULLS no autophagy or proteasome activation and no PGC-1α down-regulation occurred. Interestingly, the Akt–mTOR pathway that controls protein synthesis at the translational level was down-regulated in ULLS, but not in bed rest. A possible explanation is that in bed rest a reduced response to insulin occurs, this is followed by increased insulin secretion, and insulin impinges on the Akt signalling pathway. This does not rule out a possible impairment in protein synthesis, as there are indications of reduced protein synthesis in the presence of normal activation of Akt/mTOR in a condition of disuse due to lower limb immobilization (Glover *et al.* 2008).

The complete understanding of such diversity in the signalling pathways needs further investigation. However, since both bed rest and ULLS reproduce conditions of clinical interest, it is clear that the present advancement towards the identification of the mechanisms responsible for the specific adaptations of muscles could be relevant to designing countermeasures or rehabilitation protocols.

DOI: 10.1113/JP271613

Table 1. Summary and comparison of the response to bed rest and ULLS

Response parameters	Bed rest	ULLS
Fibre thickness	$\downarrow^{1,3}$	$\downarrow^{2,4}$
Specific tension	$\downarrow^{1,3}$	$\downarrow^{2,4}$
Maximum shortening velocity	$\uparrow^{1,3}$	$=^{2,4}$
Myosin concentration	$\downarrow^{3}$	$\downarrow^{4}$
Glycolytic enzymes	$\downarrow^{3}$	$\downarrow^{4}$
Mitochondrial enzymes	$\downarrow^{3}$	
Antioxidant defence system (SOD, catalase, CAH)	$\downarrow^{3}$	$\uparrow^{4}$
Protein carbonylation (oxyblot)	$\uparrow^{3}$	$=^{4}$
Mitochondrial biogenesis (PGC-1α)	$\downarrow^{3}$	$=^{4}$
Ubiquitin ligase (MURF, atrogin)	$\uparrow^{3}$	$=^{4}$
Autophagy (beclin, LC3)	$\uparrow^{3}$	$=^{4}$
Akt, mTOR, S6 pathway	$=^{3}$	$\downarrow^{4}$

$\downarrow$, decrease; $\uparrow$, increase; $=$, no change. Sources: 1, Widrick *et al.* (2001) for bed rest; 2, Widrick *et al.* (2002) for ULLS; 3, Brocca *et al.* (2012) for bed rest; 4, Brocca *et al.* (2015) for ULLS. CAH, carbonic anhydrase; LC3, microtubule-associated protein Light Chain 3; MURF, Muscle-specific Ring finger protein; SOD, superoxide dismutase.

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Additional information

Competing interests

None declared.