MYOTENDINOUS AND NEUROMUSCULAR ADAPTATION TO LONG-TERM SPACEFLIGHT: RESULTS FROM THE SARCOLAB PILOT STUDY

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Background

Exposure to actual and simulated microgravity leads to muscle wasting, with a disproportionate loss of muscle function and motor control leading to a reduction in muscle force per unit of cross-sectional area (F/CSA) [Berg, Larsson et al., 1997;

Narici, Kayser et al., 2003; de Boer, Maganaris et al., 2007; de Boer, Seynnes et al., 2008; Pisot, Narici et al., 2008]. Recent evidence shows that this phenomenon may not only be related to muscle atrophy and alterations in muscle architecture [de Boer, Seynnes et al. 2008] and in neural drive [Edgerton, McCall et al., 2001] but also to tendinous and extracellular matrix changes [Seynnes, de Boer et al., 2007; Seynnes, Maganaris et al., 2008], affecting the mechanical output of the muscle and its ability to transduce mechanical signals into chemical processes driving protein synthesis and catabolism. Disuse-induced alterations in muscle structure and function have also been related to altered expression of structural, contractile, cytosolic, plasma, transport and metabolic proteins [Gelfi, Vigano et al., 2006, Brocca et al., 2015].

Results from both bed rest [de Boer, Seynnes et al., 2008] and lower limb unloading in humans [de Boer, Maganaris et al., 2007], show that muscle atrophy is associated with a decrease in pennation angle. This rapid remodelling of skeletal muscle with unloading seems mediated by alterations in costameric proteins involved in mechanotransduction and the anchoring of sarcomeres to extracellular matrix receptors within the sarcolemma, such as focal adhesion kinase (FAK) [de Boer, Selby et al. 2007, Peter, Cheng et al., 2011]. Both FAK activity and content were found to be depressed by 30% and 20% respectively, after 14 days of unloading. Since FAK is an upstream modulator of the PI(3)K/Akt/mTOR signalling pathway, it seems likely that the mechanisms of these structural alterations involve changes in these mechanosensitive proteins. Notably, mechano-transduction does not only involve costameric proteins but also acts through a network of connective tissue known as the extracellular matrix (ECM). Given the importance of the extracellular matrix (ECM) in force transmission, it cannot be excluded that qualitative and quantitative changes in the ECM may also contribute to the disproportionate force loss induced by chronic unloading. Moreover, muscle atrophy and skeletal muscle remodelling can only explain a portion of the observed force loss since F/CSA is observed to decline both in vitro and in vivo. In vitro, this phenomenon seems due to a decrease in myosin concentration, whereas in vivo, reduced neural drive and a decrease in excitation-contraction coupling may also play a role.

Therefore, the Sarcolab Project was designed to elucidate the various constituents of muscle weakness in space to cast light on the mechanisms of this marked loss of intrinsic muscle strength (F/CSA). We hypothesised that microgravity exposure would lead to a reduction of its plantar flexor muscle's force production capability as well as alterations in its control. Moreover, besides these two major causes of muscle dysfunctions tendon alterations will also contribute to the degradation of muscle function after spaceflight [Reeves et al. 2009].

Method

Here, we report the results from a first pilot study that involved two astronauts. For crew member A and B, respectively, baseline data were collected 79 and 76 days before launch, mission durations were 193 and 185 days. With a custom made plantar flexor dynamometer, the angle torque-relationship, the rate of torque development, activation capacity and fatigue in isometric contraction were assessed pre-flight, and on days R+2/5 and R+15. On the same days, muscle architecture and Achilles tendon mechanical properties were assessed by B-mode ultrasound. Calf muscle volumes were assessed pre-flight, and on days R+2/5 and R+15. Finally, venous blood draws and a soleus muscle tissue sample were obtained pre-flight and on days R+1 and R+16/15. Countermeasure exercise performance data for usage of the advanced resistive exercise device (aRED) and for the T2 Colbert treadmill were obtained by data sharing with NASA.

From the muscle tissue sample, the following analytical variables were obtained: muscle fibre cross-sectional area (CSA), myosin heavy chain (MHC) isoform distribution, expression of focal adhesion kinase (FAK), muscle proteomic analysis, analysis of intracellular signalling pathways, single fibre specific tension, unloaded shortening velocity, and in-vitro motility assay. Different blood circulating markers were investigated including circulating microRNAs.

Results

Single fibre analysis could not be performed due to problems with sample shipment in crew-member B. Otherwise, all procedures and measurements, including harvesting of the muscle biopsy, were performed as foreseen an without complication.

With regards to in-flight countermeasure exercises, both crew members ran for similar amounts of time (approximately 30 minutes per run) on T2 at comparable speed (13 km/hour) and thus covered similar distances (approximately 6 km per run). However, whilst crew member A ran with a load of approximately 50 % of the body weight (BW), crew member B ran with 100 % of BW. Also for heel raise exercises on aRED, loading forces were approximately twice as large for crew member B as compared to A.

Post-flight, isometric plantar flexor strength was changed in crew members A/B, respectively, by -31/-3 %. With regards to muscle volumes, reductions in crew members A/B, respectively amounted to -21/-9 % for the soleus muscle, to -18/-12 % for the medial gastrocnemius muscle's volume, and to -22/-16 % for the lateral gastrocnemius muscle. Soleus muscle pennation angle was changed by -22 %/+4 %, and fascicle length by -8 %/+1 %. No substantial changes were observed in MHC isoform distribution. In crew member A, single fibre specific tension decreased by 23%, but no decrements were observed in the unloaded shortening velocity or in the filaments' sliding velocity. Moreover, the expression of MurF-1 and Atrogin-1, two major atrogenes, and P62 and Belcin-1, two markers of authophagy increased in crew member A. Finally, FAK content was reduced in both crew members by -60/ -44 %. However, FAK activity was maintained in crew member B (+11 %) but not crew member A (-92%). Furthermore, proteomic analyses indicate a statistically significant change in 150 and 140 proteoform species in crew members A and B, respectively. Identified proteins were grouped as structural and contractile, metabolic, stress response and others. Results indicate a differential behavior between the two subjects, particularly in structural/contractile and stress

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response proteins, indicating that further investigation is required to clarify the different outcome to flight exposure. With regards to blood circulating miRs the most interesting changes were observed in inflamma-miRs and myo-miRs being their expression different after landing and recovery time between the two astronauts.

Discussion

The two astronauts tested in this Sarcolab pilot study were comparable in terms of in-flight exercise training volume, but crew member B trained with loads that were substantially greater than those of crew member A, and which in fact were more or less equivalent to those used in Earth-bound training. These differences between the two crew members were paralleled by greater decrements in muscle strength, muscle volume, pennation angle and fascicle length in crew member A compared to B. It therefore seems that training loads, rather than training volume, can explain the difference in phenotypic changes between the two astronauts. This interpretation is strongly supported by the muscle biopsy results. It is known from many ground-based observations, that FAK activitry can be regarded as a skeletal muscle bio-marker accurately reflecting the time-under-tension history [Rahnert & Burkholder, 2013), which is intimately involved in the hypertrophic response. By contrast FAK content is indicative of load bearing fibre recruitment [Flück, Ziemiecki et al 2002]. Moreover, the muscle biopsy data suggest that, at the level of the acto-myosin interaction, a decline in specific tension is a greater concern than velocity of contraction, which confirms the contribution of the reduction in specific tension to the loss of whole muscle F/CSA.

In conclusion, the Sarcolab pilot study has demonstrated 1) that loading force is an important element in exercise countermeasure training, and 2) that information obtained from muscle biopsy samples allows extremely useful insights into the problem of skeletal muscle deconditioning in Space.