On ground preflight studies on skeletal muscle from mice kept in the Mouse Drawer System (MDS)

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Abstract

The Mouse Drawer System (MDS) has been developed to individually accommodate mice on board the International Space Station. MDS size is 110 x 118 mm (floor area) and 85 mm height, which is several fold smaller than standard laboratory cages, and may affect mouse activity. The study was aimed at establish the effects of 20-day housing in MDS on 2-month-old male mice compared to age-matched controls individually housed in normal cages. After 20 days, body weight of MDS mice (n = 3) decreased by about 12% whereas that of controls (n = 3) increased by about 4.4%. The mean cross sectional area of EDL and soleus muscle fibers of MDS mice was slightly lower than that of controls. SDS-PAGE analysis of myosin heavy chain (MyHC) composition shows that type 2B MyHC was slightly reduced in MDS EDL compared to control. Expression of the activity-dependent gene PGC-1α and of IGF-1 isoforms was up-regulated in MDS soleus, whereas that of chloride channel-1 was unchanged. Expression of ubiquitin-ligases and autophagic genes was only slightly modified. The study shows that short-term housing in the MDS payload produces adaptive changes on hind limb skeletal muscle properties, known to disappear in prolonged housing.

Key Words: space flight, microgravity, muscle atrophy, ubiquitin-proteasome, autophagy, IGF-1 isoforms, chloride channel-1

Basic Applied Myology 19 (2&3): 97-106, 2009

The Italian Space Agency (ASI) recently sponsored an initiative aimed at investigating the effects of microgravity on skeletal muscle properties during a spaceflight mission in the Mouse Drawer System (MDS), a payload developed by Alenia-Space [1], which will host mice on board the International Space Station. The main goal of MDS mission is the identification of countermeasures to prevent osteoporosis by means of transgenic mice over-expressing osteoblast stimulating factor-1 (osf-1) in a 100-day-long spaceflight, headed by R. Cancedda of the University of Genova [3]. In this context, ASI has promoted a tissue sharing program involving investigators of the muscle atrophy group of the OSMA (Osteoporosis and Muscle Atrophy) project. The MDS was designed to individually accommodate 6 mice, 3 osf-1 transgenic mice and 3 wild-types. Each MDS cage has a size of 110 x 118 mm (floor area) and 85 mm height, which is 6.3 fold smaller floor area (more than 11 fold lower volume) than standard laboratory cages, and is expected to affect mouse overall activity. Past evidence demonstrated in fact reduced locomotor activity of 8-10-week-old mice housed in cages with a floor area double that of MDS [21]. Similarly, reduction of locomotor activity was observed in 7/8-week-old rats in a 5-day-long trial in small cages [13]. However, possible consequences of restricted housing on skeletal muscle properties have

Table 1. *Quantitative real-time PCR primers.*

Gene	Forward primer (5' → 3')	Reverse primer (3' → 5')	bp	T°
HK genes				
CycA	AGCATGTGGTCTTTGGGAAGGTG	CTTCTTGCTGGTCTTGCCATTCC	92	57.7
ActB	CAAACATCCCCCAAAGTTCTAC	TGAGGGACTTCCTGTAACCACT	135	57.7
Specific genes				
Murf1	ACCTGCTGGTGGAAAACATC	CTTCGTGTTCCTTGCACATC	96	55
atrogin-1	GCAAACACTGCCACATTCTCTC	CTTGAGGGGAAAGTGAGACG	93	55
LC3β	CACTGCTCTGTCTTGTGTAGGTTG	TCGTTGTGCCTTTATTAGTGCATC	171	57.7
PGC-1α	GGAATGCACCGTAAATCTGC	TTCTCAAGAGCAGCGAAAGC	88	55

not been reported. Therefore, preliminary on-ground experiments have been planned to establish the consequences of stringent MDS conditions.

The OSMA muscle atrophy group, within ASI Tissue Sharing program, has multiple objectives, related to the effects of microgravity exposition on skeletal muscle properties and function. Skeletal muscle disuse in the microgravity environment is associated to a rapid muscle wasting process [8;9;16], presumably resulting from the activation of common programs that lead to the progressive degradation of muscle [10]. Similar muscle changes could be observed in on ground simulations of microgravity, such as bed rest [5;20;22;23] and hindlimb unloading [6;7;18;28]. In atrophying muscles, the ubiquitin-proteasome and the autophagic-lysosomal pathways regulate the accelerated degradation of myofibrillar proteins and muscle organelles. Both pathways are controlled by FoxO transcription factor and comprise the muscle-specific ligases atrogin-1 and MURF-1, and the autophagy-lysosome member LC3β [12;24;30]. The kinase Akt, and its downstream signaling pathways GSK-3β, mTOR and FoxO, plays a key role in the regulation of muscle mass [2;17]. Moreover, IGF-1 is an important factor regulating muscle mass through the specific activation of Akt [14;15].

In addition to the dramatic consequences on muscle mass, disuse and microgravity also causes a slow-to-fast transformation to muscle fibers [7;8]. The fiber type profile of individual muscles is under the control of specific signaling pathways dependent on the overall muscle activity [26]. The continuous activity of antigravitary soleus muscle is a key determinant of its metabolic and contractile specialization, so that the exposition to unloading is expected to have major consequences on soleus. Calcineurin and PGC-1α are activity-dependent genes involved in the slow-type muscle specialization [25;26], making them ideal markers to investigated muscle atrophy. Recent evidence revealed that hindlimb unloading induces a slow-to-fast fiber type conversion [7] also associated to an increase of the chloride conductance [19], a critical function controlling muscle excitability. Interestingly, the increase of chloride conductance, which is supported by the voltage-gated chloride channel ClC-1, was demonstrated to precede the transitions of MyHC isoforms [7;18].

Therefore, the preliminary objective of the Tissue Sharing program was to investigate the effects of restrained MDS housing both on skeletal muscle properties and in the expression of different genes possibly affected by microgravity conditions. In particular, the short-term study was devoted to explore both the morphology and the expression of selected genes involved in the regulation of muscle mass, such as the ubiquitin ligases atrogin-1, MURF-1, the autophagy LC3β, and the growth factor IGF-1, as well as genes controlling muscle specialization, such as MyHC isoforms, the chloride channel ClC-1, and the activity-dependent gene PGC-1α.

Table 2 *Body weight and muscle fiber cross-sectional area (CSA) of MDS mice*

Mouse	Housing	Body weight at T_0	Body weight at T_{20}	Δ (g)	Δ (%)	Mean CSA of EDL fibers	Mean CSA of Sol fibers
# 0	control	25.6	28.5	+ 2.9	+ 11.3	1190 ± 29	1503 ± 21
# 1	control	22.6	28.5	+ 5.9	+ 26.1	1029 ± 37	1134 ± 22
# 6	control	23.8	28.3	+ 4.5	+ 18.9	1461 ± 27	1296 ± 19
#3	MDS	27.1	25.52	- 1.58	- 5.8	1211 ± 52	1319 ± 18
#4	MDS	24.7	18.4	- 6.3	- 25.4	852 ± 23	1018 ± 19
# 13	MDS	23.6	22.35	- 1.25	-5.3	916 ± 16	1023 ± 11

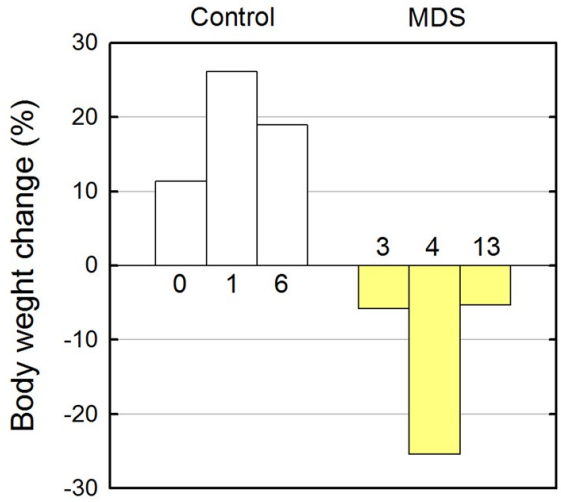

Fig. 1 *Body weight changes after twenty days inside MDS. Three C57BL/6J mice were housed into normal sized cages (mice #0, 1, and 6) and three in the restricted MDS cages (mice #3, 4, and 13) for twenty days. Body weight of mice was measured immediately before and after housing in the respective cages (see Table 2 for raw data).*

The results show that the 20-day spatial restriction in the small MDS cages causes some change in hind limb skeletal muscle properties and in the expression of selected genes. Based on the preliminary observations in long-term MDS test [11], these modifications are probably due to the response of animals in the initial adaptation to the restricted MDS space, estimated to be countered in the prolonged housing. To lessen any possible early change, the utilization in future spaceflight missions of MDS pre-trained mice is recommended.

Materials and Methods

Animal use

The protocol utilized in the study has been authorized by the Public Veterinary Health Department of the Italian Ministry of Health. The experiments were carried out on C57BL/10J male mice (Charles River). All experiments were performed on 6/8-week old male mice.

Housing in MDS cages

The on-ground experiment was carried out at the Vivarium of the Advanced Biotechnology Center in Genova (R. Cancedda). Three experimental mice were individually housed for 20 days into the MDS cages, each one of 110 x 118 mm (floor area) and 85 mm height. Water was delivered to each cage ad libitum by means of water through, whereas food was delivered ad libitum by means of a single food bar. The three control mice were individually housed in a standard laboratory cage of 370 x 220 mm (floor area) and 150 mm height. All animals were exposed to 12:12 hours light-dark cycles [11]. Mice over-expressing the osf-1

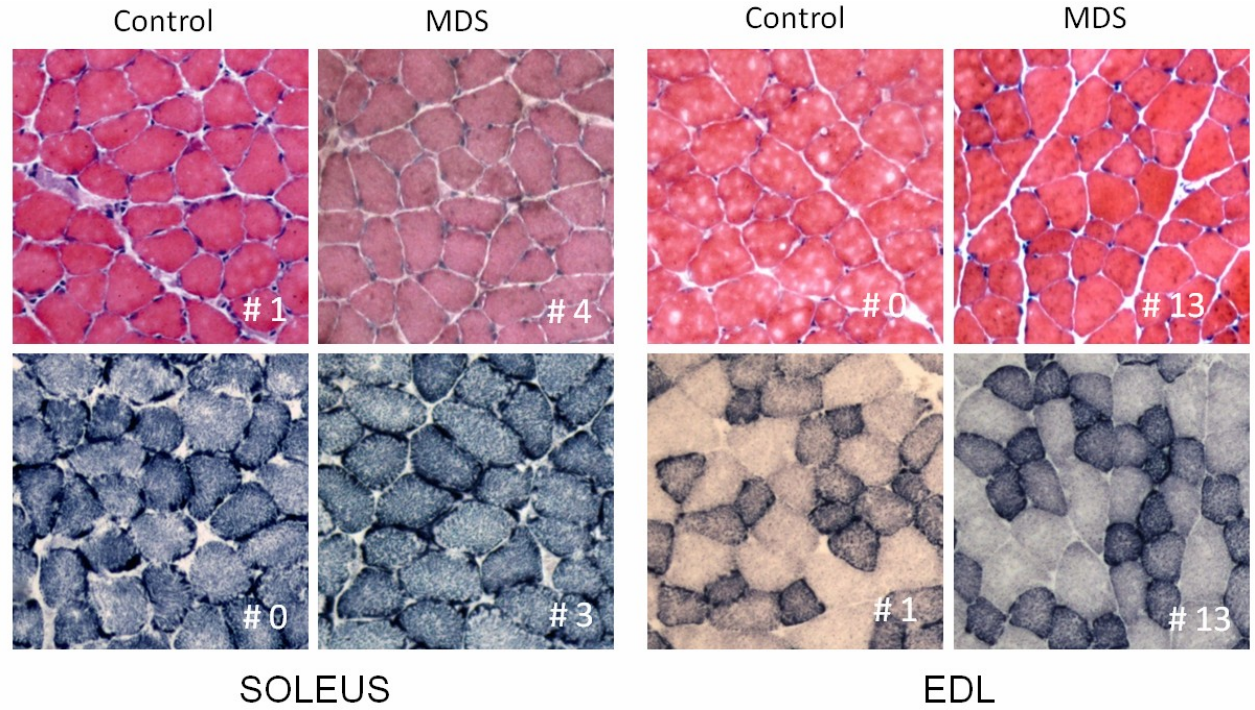

Fig. 2 *Hematoxylin-eosin and succinate dehydrogenase representative staining of muscle fibers from soleus and EDL muscles of mice housed for twenty days either in a normal cage or inside MDS.*

Table 3. *MyHC composition of soleus and EDL muscles as determined by SDS-PAGE*

		1	2A	2X	Neo	2B
Control	Soleus # 0	47.0	36.3	5.8	6.6	4.3
	Soleus # 1	61.9	38.1	-	-	-
	Soleus # 6	58.6	41.4	-	-	-
MDS	Soleus # 3	46.4	36.7	6.5	3.5	6.9
	Soleus # 4	45.3	37.1	9.8	-	7.8
	Soleus # 13	56.1	31.4	12.5	-	-
Control	EDL # 0	-	-	14.9	-	85.1
	EDL # 1	-	3.8	24.5	-	71.7
	EDL # 6	3.3	5.0	12.1	-	79.6
MDS	EDL # 3	4.9	6.8	21.9	-	66.4
	EDL # 4	4.4	5.8	28.2	-	61.6
	EDL # 13	-	5.4	25.7	-	68.9

gene were not included in this study because preliminary analyses determined the presence of osf-1-specific consequences on muscle properties.

Histological and histochemical analysis

Muscles were frozen in liquid nitrogen in a slightly stretched position. Serial cross sections (8-μm thick) were cut in a cryostat microtome set at -24 ± 2 °C (Slee Pearson, UK). For the histological analysis, hematoxylin-eosin staining was performed on muscle sections to examine the general morphology and to determine the cross-sectional area (CSA) of individual fibers. Muscle cryostat section were also stained for the succinate dehydrogenase (SDH) activity. Digital photographs were taken of each muscle section and analyzed by the ImageJ NIH imaging software [4;29]

RNA extraction, reverse transcription and Quantitative Real Time PCR

Total RNA was extracted by using the Qiagen RNeasy Micro Kit. The extracted RNA was eluted in 14 μl RNase-free water, analyzed by capillary electrophoresis (RNA 6000 Pico LabChip, Agilent Technologies) and stored at -80°C until used. Quantification was performed in a 96-well IQ5 Thermal Cycler (Bio-Rad). The following genes were considered: atrogin-1, MURF1, LC3β, PGC-1α, IGF-1 isoforms, and ClC-1. As internal control, the housekeeping genes cyclophilin A (CycA), β-actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used. Specific PCR primers (Table 1) were from Eurofins MWG Operon. The reaction mix consists of 10 μl of 2x iQ SYBR Green Supermix (Bio-Rad), 0.3 pmol/μl primers, 2 ng of cDNA and DNase/RNase-free water up to 20 μl. The PCR parameters were initial denaturation at 95 °C for 30 s followed by 40 cycles of 10 s at 95 °C and 30 s at the corresponding annealing temperature (55-59 °C) for acquisition of fluorescence signal. A melting curve was generated by the iQ5 software following the end of the final cycle for each sample, by continuous monitoring the SYBR Green fluorescence throughout the temperature ramp from 65 °C to 99 °C in 0.5 s increments. The mRNA expression of the genes of interest in each experimental condition was normalized to the housekeeping genes. Transcripts for IGF-1 isoforms and ClC-1 were determined by TaqMan, utilizing specific probes from and under conditions recommended by Applied Biosystems.

SDS-PAGE of muscle extracts

Analysis of MyHC isoforms was performed as previously described [4]. Small muscle fragments from control and MDS muscles were weighed, ground with a ceramic pestle in liquid nitrogen, and extracted at 2 mg/ml in SDS-PAGE sample buffer (62.5 mM Tris, pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol, 10% glycerol). Forty μg of muscle sample was run on 8% SDS-PAGE slab gels in SDS. MyHC protein bands from whole muscles were revealed by Coomassie brilliant blue staining. MyHC isoform percentage composition was determined by densitometry of gels by using a Bio-Rad Imaging Densitometer (GS-670).

Results

Body weight

After 20 days, the mean body weight of MDS mice housed in standard cages. Body weight of MDS mice (#3, 4, and 13) decreased in fact by 18.8 ± 4.3% whereas that of controls (mice #0, 1, and 6) increased by 12.2 ± 6.6% (Table 2). There was a large variability in the body weight values of individual MDS mice, with MDS mouse #4 showing a dramatic loss (about 25%) of body mass (Table 2 and Figure 1). Besides the potential effects of a restrained condition, the MDS mouse #4 experienced a transient water/food delivery

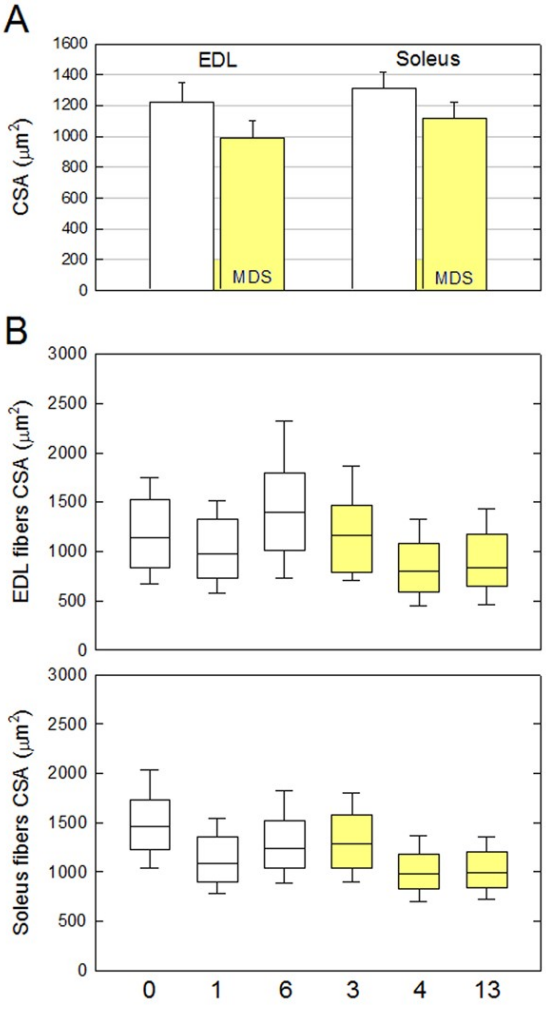

***Fig. 3** - Cross sectional area (CSA) of muscle fibers from EDL and soleus muscles of mice housed for twenty days inside MDS. Three C57BL/6J mice were housed into normal cages (#0, 1, and 6) and three in the restricted MDS cages (#3, 4, and 13) for twenty days. CSA was measured in 200-300 fibers in each muscle. A, mean CSA of soleus and EDL muscle fibers. B, Box plots of fiber CSA from individual muscles describing the median (the horizontal bar in the box) and the lower and upper quartiles (bottom and top bars of the box).*

system malfunctioning, causing a greater body weight loss compared to the other two MDS mice (Table 2).

Histology and histochemistry

A Hematoxylin and eosin staining of EDL and soleus muscle sections from mice housed in MDS and in standard cages demonstrated no evident pathological signs (Figure 2). Intensity of succinate dehydrogenase staining (proportional to mitochondria content) did not obviously differ between EDL and soleus muscle of mice housed in MDS and in standard laboratory cages.

Cross sectional area of muscle fibers was also analyzed to evaluate possible changes of muscle mass. MDS mice showed a reduced mean CSA of both EDL and soleus muscle fibers compared to controls (Figure 3A). Notably, EDL and soleus muscles of MDS mouse #4 showed the lowest CSA values (Table 2). Box plots of CSA values variability confirm that MDS muscle fibers have a lower size than controls (Figure 3B).

Myosin heavy chain isoforms composition

SDS-PAGE analysis of EDL and soleus muscles demonstrates that the 20-day permanence in the restricted MDS cage causes a moderate shift of MyHC isoform profile compared to the muscles of mice in standard cages (Figure 4). EDL muscles from MDS mice show a significant decrease of type 2B MyHC compared to controls, apparently compensated by the increase of type 2X isoform, indicating a slow-to-fast transformation. A slight reduction of type 1 and 2A isoforms is instead perceptible in MDS soleus muscles with a comparable increase of type 2X and 2B isoforms, however these changes were not significant.

It is worth reporting that soleus muscles from both control (#0) and MDS (#3) mice express also the unusual neonatal isoform of MyHC (Table 3 and Figure 4). This isoform is usually found in regenerating muscles, however the histological analysis of the muscles does not evidence signs of degeneration-regeneration (Figure 2). As shown in Table 3, soleus muscles, independently of housing, also express type 2B MyHC, an observation that however is not infrequent in mice.

Gene expression

The study was also devoted to explore the expression level of selected genes involved in the regulation of muscle mass. In particular, a pilot analysis was carried out on genes controlling muscle atrophy, comprising atrogin-1, MURF-1, and LC3β, muscle hypertrophy, including IGF-1 isoforms, and muscle specialization, such as MyHC isoforms, the chloride channel ClC-1, and the activity-dependent gene PGC-1α.

Real time PCR analysis demonstrated that the mean expression level of the ubiquitin ligases atrogin-1 and Murf-1 was not changed in MDS muscles compared to controls, whereas the expression of Murf-1 was significantly lower in control soleus compared to EDL (Table 4). The analysis of individual values shows that the muscles of MDS mouse #4, in agreement with the presence of a mild atrophy, demonstrated an apparent up-regulation of the two atrophy-related genes, more evident in EDL (Table 5). The level of the autophagic gene LC3β was not different in soleus MDS and control, whereas it was significantly down-regulated in MDS EDL. Surprisingly, expression of LC3β was apparently up-regulated in MDS mouse #4.

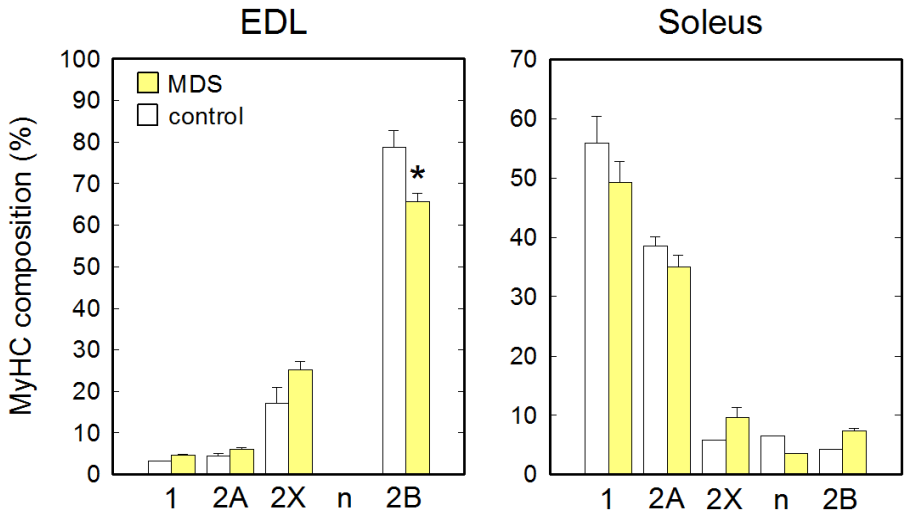

Fig. 4 *MyHC composition of soleus and EDL muscles housed either in normal cages or in MDS. MyHC composition was determined by SDS-PAGE of muscle lysates, as previously shown [4]. 1, 2A, 2X, n, and 2B, refers to type 1, 2A, 2X, neonatal and 2B MyHC isoforms, respectively. *, P < 0.05 compared to control.*

As expected from its muscle phenotype-dependent modulation [18], the ClC-1 gene expression in control mice was about two-fold higher in the fast-twitch EDL muscle with respect to the slow-twitch soleus muscle. Expression of ClC-1 in both muscles was not affected by the permanence in the MDS environment. In contrast, the expression of the activity-dependent gene PGC-1α was significantly up-regulated in MDS soleus compared to control, whereas it was not changed in EDL. Similarly, the analysis of the different isoforms of IGF-1 did not reveal significant differences in EDL muscle between control and MDS wild type mice.

In contrast, real time PCR analysis performed on the soleus muscle revealed a significant increase in the expression of all isoforms (Class1, Ea, and Eb) (Table 4).

Discussion

The Italian Space Agency has promoted a Tissue Sharing program involving investigators of the OSMA muscle atrophy group to investigate the effects of microgravity exposition on skeletal muscle properties. The program takes advantage of the MDS mission sponsored by ASI. The preliminary experiment was dedicated to establish the effects of short-term housing in MDS payload. Further experiments are underway to verify the effects of long-term MDS housing, considering that the spaceflight mission, scheduled for Summer 2009, is expected to last for about 100 days. The results show that 20-day in spatial restriction in MDS cages causes slight decrease in body weight and some changes in hind limb skeletal muscle properties and in the expression of selected genes. Based on preliminary observations in mice housed for 100 days in MDS, early modifications are probably due to the

Table 4 *Gene expression of EDL and soleus muscles from MDS mice*

Gene	EDL		Soleus	
	Control	MDS	Control	MDS
Atrogin-1	1.75 ± 0.23	1.16 ± 0.53	1.13 ± 0.13	0.65 ± 0.17
MURF-1	0.91 ± 0.01	2.60 ± 1.89	0.65 ± 0.06 [a]	0.77 ± 0.13
LC3β	0.21 ± 0.01	0.06 ± 0.01*	0.09 ± 0.01 [a]	0.05 ± 0.03
PGC-1α	0.06 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.20 ± 0.05*
ClC-1	0.90 ± 0.10	0.74 ± 0.34	0.30 ± 0.04 [a]	0.50 ± 0.08
IGF-1, C1	0.44 ± 0.21	0.59 ± 0.22	0.83 ± 0.10	18.83 ± 4.96*
IGF-1, Ea	0.56 ± 0.18	0.34 ± 0.07	0.64 ± 0.11	11.43 ± 3.73*
IGF-1, Eb	0.80 ± 0.15	0.78 ± 0.24	0.74 ± 0.13	8.43 ± 2.06*

*Gene expression was determined by Real Time PCR. Data are the mean ± SEM of three separate experiments. *, P < 0.05 with respect to muscles of control animals; [a], P < 0.05, with respect to EDL.*

Table 5 *Gene expression of individual EDL and soleus muscles from MDS mice*

Mouse	atrogin-1		MURF-1		LC3β		PGC-1α		ClC-1	
	EDL	Soleus	EDL	Soleus	EDL	Soleus	EDL	Soleus	EDL	Soleus
control #0	1.50	1.33	0.93	0.66	0.20	0.10	0.05	0.05	0.99	0.34
control #1	2.21	1.16	0.89	0.75	0.22	0.09	0.06	0.05	1.01	0.34
control #6	1.53	0.90	0.92	0.54	0.22	0.07	0.07	0.03	0.71	0.21
MDS #3	0.42	0.45	0.49	0.64	0.04	0.01	0.08	0.18	0.78	0.36
MDS #4	2.20	0.99	6.38	1.03	0.07	0.10	0.06	0.12	0.38	0.65
MDS #13	0.87	0.51	0.94	0.63	0.08	0.04	0.11	0.29	1.05	0.50

Gene expression was determined by Real Time PCR as described in Materials and Methods.

stress suffered by animals in the initial adaptation to the restricted MDS space since weight changes disappear in the prolonged housing [11].

The major consequence observed in our short-term experiment in MDS cages was the reduction of body weight experienced by all mice. Similar results have been reported for 3/4-month-old mice housed for three weeks in a MDS-like cage (same size as MDS but with standard supply system for water and food) [27]. Interestingly, mice locomotor activity of mice housed in MDS-like cages was reported to be greater than that of control animals in normal cages [27]. Hyperactivity did not caused anxiety to MDS mice but determined a superior drinking/feeding activity. In contrast, it has been reported that housing in cages with a floor area double that of MDS determined a reduction of locomotor activity in 8-10-week-old mice [21] and in 7/8-week-old rats in a 5-day-long trial [13]. In spite of these contradictory observations, all reported altered circadian cycles, particularly higher night-activity over daylight-resting [13;21;27]. None of these works however evaluated whether the reduction of body weight was associated to a parallel decrease of skeletal muscle mass. Here, we report that muscle mass of EDL and soleus muscle of MDS mice, as estimated by the mean CSA of fibers, was slightly reduced compared to controls. As reported recently, body and muscle mass reduction is probably a transient response of mice due to the adaptation to the restricted MDS space, that needs more than 20 days to be completed [11]. The hyperactivity and altered circadian cycles reported in mice in the early time of housing in MDS-like cages [27] could contribute to the temporary deficit noted in our 20-day test.

Our research group, within the ASI Tissue Sharing program, aimed to investigate the effects of restrained MDS housing on the expression of different genes possibly deregulated under microgravity conditions. It is well known that skeletal muscle disuse due to exposition to microgravity causes the onset of a rapid muscle wasting process [8;9;16], as a consequence of activation of specific atrophy programs leading to the increased protein degradation [10]. Similar muscle changes could be observed in on ground simulations of microgravity, such as bed rest [5;20;22;23] and hindlimb unloading [6;7;18;20]. Muscle mass is under the control of the kinase Akt and its downstream signaling pathways GSK-3β, mTOR and FoxO [2;17]. IGF-1 is key factor regulating muscle mass through the specific activation of Akt and calcineurin [14;15]. In atrophying muscles, the reduced activity of Akt determines activation of FoxO, that regulates both the ubiquitin-proteasomal and the autophagic-lysosomal pathways. The muscle-specific ligases atrogin-1 and MURF-1, and the autophagy-lysosome member LC3β then operate the degradation of myofibrillar proteins and muscle organelles [12;24;30]. The analysis of these atrophy elements reveals that the mean expression of atrogin-1 and Murf-1 was not influenced by the permanence in the MDS environment. In contrast, both factors were considerably augmented in EDL muscle of MDS mouse #4 that showed greater muscle atrophy (Table 5). Surprisingly, the expression of atrogin-1 and MURF-1 were not up-regulated in soleus muscle of MDS mouse #4, showing that a typical fast-twitch muscle is more susceptible to the MDS restricted ambient than the slow-twitch, anti-gravitary, soleus. Similarly, the autophagic gene LC3β level was unchanged in MDS soleus, whereas it was significantly down-regulated in MDS EDL. These responses of EDL compared to soleus muscle shows that short-term housing in MDS determined muscle type-specific effects on atrophy genes that deserve additional study.

In addition to the remarkable consequences on muscle mass, disuse and microgravity also cause a slow-to-fast transformation of muscle fibers [7;8]. The fiber type profile of individual muscles is under the control of specific signaling pathways dependent on the

overall activity of the muscle [26]. The continuous activity of antigravitary soleus muscle is a key determinant of its metabolic and contractile specialization, so that the exposition to unloading is expected to have major consequences on soleus. Calcineurin and PGC-1α are activity-dependent genes involved in the slow-type muscle specialization [25;26], and PGC-1α has been shown to prevent muscle atrophy [25]. Our results show that the expression of the activity-dependent gene PGC-1α in EDL muscle was not influenced by the permanence in the MDS cage. By contrast, this factor was significantly up-regulated in MDS soleus. Similarly, the expression of all IGF-1 isoforms considered in our study were also markedly up-regulated in MDS soleus but did not changed in EDL. Up-regulation of PGC-1α and IGF-1 isoforms in soleus muscle of MDS mice suggests that different muscles might be differently influenced by MDS environment. In addition this result can be also justified by the published evidenced that MDS housed mice showed a strong up-regulation of the activity period and a concomitant decrease in inactivity [27]. Nevertheless, the molecular mechanisms that links MDS-conditioning locomotor behavior and PGC-1α and IGF-1 expression is not defined yet. Further studies will clarify this interesting aspect of the biology of skeletal muscle.

Recent evidence revealed that hindlimb unloading induces a slow-to-fast fiber type conversion [7] also associated to an increase of the chloride conductance [18], a critical function controlling muscle excitability. The increase of chloride conductance, which is supported by the voltage-gated chloride channel CLC-1, was demonstrated to precede the transitions of MyHC isoforms [7;18]. Hindlimb unloading also causes slow-to-fast changes of the calcium-activated potassium channel properties [28]. As confirmed in the present work, the expression of the ClC-1 gene is higher in the fast-twitch muscle EDL compared to the slow-twitch soleus [18], expression levels that were not influenced by the permanence in the MDS environment. By contrast, the analysis of MyHC composition evidences a moderate fast-to-slow shift in MDS EDL muscle but not in soleus muscle.

In conclusion, the present work shows that short-term housing in the restricted MDS environment causes a slight reduction in mice body mass with limited muscle atrophy. Importantly, preliminary observations in long-term experiments (100 days) however demonstrate that the body weight of MDS mice increases like that of mice housed in standard laboratory cages [11]. As a consequence, the body mass loss and partial muscle atrophy observed in 20-day MDS housing most probably represent an initial stress response to the restrained environment. In addition, it is possible that hyper-excitability and deregulated circadian cycles demonstrated in short-term MDS housing [27], could be responsible for the changes in the expression of some of the genes investigated in our study. It is possible that these transients responses of MDS mice could be eliminated with an adaptive preliminary experience in the restricted MDS space. This training should be considered in the future ASI and European Space Agency spaceflight missions with the MDS payload.

Acknowledgements

The present work was supported by grants from ASI (project OSMA) and by C.N.R. institutional funds. We thank Chiara Tenconi and Giancarlo Falcetti (Alcatel Alenia Space, Italy) and Yi Liu and Ranieri Cancedda (University of Genova, Italy) for making available the MDS payload and mice used in this study.

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