Lack of Dystrophin Affects Bronchial Epithelium in mdx Mice

GIUSEPPE MORICI,1,2* FRANCESCA RAPPA,1,3,4 FRANCESCO CAPPELLO,1,4 ELISABETTA PACE,2 ANDREA PACE,4,5 GIUSEPPA MUDÒ,1 GRAZIA CRESCIMANNO,2 NATALE BELLUARDO,1 AND MARIA R. BONSIGNORE2,6

1Dipartimento di Biomedicina e Neuroscienze Cliniche (BioNeC), University of Palermo, Palermo, Sicilia, Italy
2Istituto di Biomedicina e Immunologia Molecolare (IBIM), Consiglio Nazionale delle Ricerche (CNR), Palermo, Sicilia, Italy
3Dipartimento di Scienze Giuridiche della Società e dello Sport, University of Palermo, Palermo, Sicilia, Italy
4Istituto Euro-Mediterraneo di Scienza e Tecnologia, Palermo, Sicilia, Italy
5Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), University of Palermo, Palermo, Sicilia, Italy
6Dipartimento Biomedico di Medicina Interna e Specialistica (DiBiMIS), University of Palermo, Palermo, Sicilia, Italy

Mild exercise training may positively affect the course of Duchenne Muscular Dystrophy (DMD). Training causes mild bronchial epithelial injury in both humans and mice, but no study assessed the effects of exercise in mdx mice, a well-known model of DMD. The airway epithelium was examined in mdx (C57BL/10ScSn-Dmdmdx) mice, and in wild type (WT, C57BL/10ScSn) mice under sedentary conditions (mdx-SD, WT-SD) or during mild exercise training (mdx-EX, WT-EX). At baseline, and after 30 and 45 days of training (5 d/wk for 6 weeks), epithelial morphology and markers of regeneration, apoptosis, and cellular stress were assessed. The number of goblet cells in bronchial epithelium was much lower in mdx than in WT mice under all conditions. At 30 days, epithelial regeneration (PCNA positive cells) was higher in EX than SD animals in both groups; however, at 45 days, epithelial regeneration decreased in mdx mice irrespective of training, and the percentage of apoptotic (TUNEL positive) cells was higher in mdx-EX than in WT-EX mice. Epithelial expression of HSP60 (marker of stress) progressively decreased, and inversely correlated with epithelial apoptosis (r = −0.66, P = 0.01) only in mdx mice. Lack of dystrophin in mdx mice appears associated with defective epithelial differentiation, and transient epithelial regeneration during mild exercise training. Hence, lack of dystrophin might impair repair in bronchial epithelium, with potential clinical consequences in DMD patients.


Duchenne muscular dystrophy (DMD) is a lethal X-linked muscle disease affecting 1:3500 newborn boys (Van Putten et al., 2012). DMD is characterized by a defect in the sub-sarcolemmal protein dystrophin, which leads to membrane fragility, muscle necrosis, motor weakness, myofibre death, and replacement of skeletal muscle by fibrous and fatty connective tissue, due to failed regeneration (Matthews et al., 1995). In patients with DMD, chronic respiratory insufficiency is inevitable in the course of disease progression, due to primary loss of respiratory muscle strength. Mechanical ventilation (MV) is the only established clinical strategy to decrease respiratory morbidity and mortality in young adults with DMD (Biggar, 2006).

In the last decade, new strategies have been explored in order to reduce the muscle wasting associated to DMD (Odom et al., 2007; Strimpakos et al., 2014; De Arcangelis et al., 2016), including the implementation of exercise training, but the effects of exercise in patients with DMD have not yet been adequately studied (Grange and Call, 2007). In this context, the mdx mouse model is a useful intermediate step to assess the effects of training on dystrophic muscle (Grounds et al., 2008). Lack of dystrophin in mdx mice leads to cycles of muscle degeneration and regeneration (Radley-Crabb et al., 2014). While excessive or otherwise inappropriate activity may induce muscle damage and increase pathology in mdx mice (Brussee et al., 1997; De Luca et al., 2003; Okano et al., 2005) some protective effects of low-intensity or voluntary exercise (Hayes and Williams, 1996; Call et al., 2010; Baltagalvis et al., 2012; Gordon et al., 2014) have been reported. Exercise training may also affect airway cells in mdx mice. We previously documented increased epithelial thickness, and increased epithelial turnover in small airways of Swiss mice undergoing low-intensity endurance training (Chimenti et al., 2007). Overall, data obtained in mice (Chimenti et al., 2007) and human endurance athletes (Chimenti et al., 2010) suggest that habitual exercise might cause low-level inflammation in the airways secondary to mild epithelial injury. Whether low-intensity endurance training affects the airways of mdx mice is yet unknown.

Conflict of interest: All authors declare that they have no conflict of interest with regard to this paper.

Contract grant sponsor: Euro-Mediterranean Institute of Science and Technology and University of Palermo.
Contract grant sponsor: Italian National Operational Programme for Research and Competitiveness, European Regional Development Fund;
Contract grant number: PONa3_00210.
*Correspondence to: Giuseppe Morici, Dipartimento di Biomedicina e Neuroscienze Cliniche (BioNeC), University of Palermo, Corso Tukory 129, 90134 Palermo, Italy. E-mail: giuseppe.morici@unipa.it
Manuscript Received: 23 January 2016
Manuscript Accepted: 9 February 2016
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 00 Month 2016.
DOI: 10.1002/jcp.25339
Aims of this study were to analyze: (a) the characteristics of airway epithelium in mdx compared to WT mice and (b) the effects of low-intensity endurance exercise training on the airways of mdx mice. In more detail, we assessed whether training induced epithelial remodelling, apoptosis, and changes in the stress marker HSP60 at bronchial and bronchiolar levels.

Materials and Methods

We analyzed lung tissue from the animals previously analyzed for skeletal muscle changes (Frinchi et al., 2014). Briefly, 8-week-old male mdx mice (C57BL/10ScSn-Dmdmdx/J Jackson Laboratories, Bar Harbor, ME), and C57/B1 wild type (WT) mice (C57BL/10ScSn, Harlan, Udine, Italy) were studied. Both mdx and WT mice were randomly assigned to sedentary (S) (MDX-S n = 17; WT-S n = 19) or trained (EX) (MDX-EX n = 14; WT-EX n = 16) groups. Training was performed using a motorized rotating treadmill (Rota-Rod; Ugo Basile, Biological Research Apparatus, Comerio Varese, Italy) in a protected environment and in the same room where the mice were housed, as previously reported (Chimenti et al., 2007). After 2 weeks of acclimatization, mice ran 5 days/week for 6 weeks at progressively increasing loads (Table 1).

Mice undergoing exercise training and sedentary controls in each group (WT and mdx) were sacrificed by an overdose of chloral hydrate anesthesia at time 0 and after 30 and 45 days. The Human Physiology Laboratory of the Department of Experimental Biomedicine and Clinical Neurosciences (BioNeC) of the University of Palermo has been formally authorized by the Italian Ministry of Health to conduct animal studies and gave authorization for this protocol. Procedures involving animals and their care were conducted in conformity with the Italian institutional guidelines (D.L. 116, G.U., suppl. 40, February 18, 1992).

Tissue preparation, histochemistry, and morphological analysis

Samples of lungs were processed for light microscopy examination. Each sample was routinely fixed in formalin and embedded in paraffin. Five-μm-thick sections of lung parenchyma were obtained from paraffin blocks, deparaffinized with xylene for 10 min at 60°C and hydrated with a decreasing ethanol gradient. They were stained with haematoxylin-eosin (Hematoxylin, Merck, Darmstadt, Germany). Samples of lungs were processed for light microscopy examination. Each sample was routinely fixed in formalin and embedded in paraffin. Five-μm-thick sections of lung parenchyma were obtained from paraffin blocks, deparaffinized with xylene for 10 min at 60°C and hydrated with a decreasing ethanol gradient. They were stained with haematoxylin-eosin (Hematoxylin, Merck, Darmstadt, Germany).

From all parafﬁn blocks, 5-μm thick sections were obtained with a cutting microtome. These sections were dewaxed in xylene for 60 min at 37°C and hydrated with a decreasing ethanol gradient. The sections were immersed for 8 min in Sodium Citrate Buffer (pH 6) at 95°C for antigen retrieval and then immersed for 8 min in acetic acid at −20°C. All subsequent reactions were conducted at room temperature. The sections were processed for light microscopy examination. Each sample was routinely fixed in formalin and embedded in paraffin. Five-μm-thick sections of lung parenchyma were obtained from paraffin blocks, deparaffinized with xylene for 10 min at 60°C and hydrated with a decreasing ethanol gradient. They were stained with haematoxylin-eosin (Hematoxylin, Merck, Darmstadt, Germany).

The sections were dehydrated and mounted with a coverslip for histological examination using an automated Leica DM5000 B microscope (Leica, Milan, Italy) connected to a high-resolution camera, Leica DC300 F. Morphological examination was performed on 10 random ﬁelds at 400× magniﬁcation. The goblet cells were quantiﬁed in the bronchi of all samples, and expressed as the percentage of goblet/total cells.

Immunohistochemistry

From all parafﬁn blocks, 5-μm thick sections were obtained with a cutting microtome. These sections were dewaxed in xylene for 10 min and rehydrated by sequential immersion in a descending scale of alcohols and transition in water for 5 min. Subsequently, the sections were immersed for 8 min in Sodium Citrate Buffer (pH 6) at 95°C for antigen retrieval and then immersed for 8 min in acetic acid at −20°C. All subsequent reactions were conducted at room temperature. The reactions were performed by a streptavidin–biotin complex method using Histostain®-Plus 3rd Gen IHC Detection Kit (Invitrogen Corporation, Waltham, MA, 85–8943). The used primary antibodies were anti-HSP60 (HSP 60 (K-19)-Rabbit antibody polyclonal sc-1722-R diluted 1:100) and anti-PCNA (PCNA (F-2) Antibody mouse monoclonal sc-25280 diluted 1:100). Appropriate positive and negative (isotype) controls, were run concurrently. Nuclear counterstaining was carried out using hematoxylin (Hematoxylin aqueous formula, S2020, DAKO). Finally, the slides were prepared for observation with coverslips with an aqueous mounting solution. All the observations were made at a magnification of 400× and the means of triplicate counts were used for statistical analyses. The observation of the sections was performed with an optical microscope (Leica DM 5000 B) connected to a digital camera (Leica DC 300F). Results were expressed as percentage of HSP60- or PCNA-positive cells/total cells.

Detection of apoptosis

Detection of apoptosis was performed using “In Situ Cell Death Detection Kit, AP” (Roche, Basel, Switzerland, 1168409910) on samples of parafﬁn embedded lung tissue. Five-micrometer thick sections were deparaffinized in a 60°C water bath, in two xylene baths and rehydrated in decreasing alcohol baths (absolute, 95, 80, 70%). Then the slides were incubated with proteinase K at 37°C for 10 min. After rinsing with PBS, slides were mounted with glass coverslips and observed using light automated Leica DM5000 B microscope connected to a high-resolution camera, Leica DC300 F. Morphological examination was performed on 10 random ﬁelds at 400× magniﬁcation. The goblet cells were quantiﬁed in the bronchi of all samples, and expressed as the percentage of goblet/total cells.

Statistics

Data are expressed as mean ± SD. Unpaired t-test was used to compare mdx and control mice at different time points according to sedentary or trained status. Time trends in the examined variables were assessed by simple linear regression in each group according to the sedentary or trained status. The relationship between apoptosis and HSP60 expression was assessed by linear regression separately in WT and mdx groups. Unpaired t-test was used to compare mdx and control mice at similar time points according to sedentary or trained status. Signiﬁcance was at

<table>
<thead>
<tr>
<th>Week</th>
<th>Session duration (min)</th>
<th>Rotations min⁻¹</th>
<th>Distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A)</td>
<td>15</td>
<td>16</td>
<td>48</td>
</tr>
<tr>
<td>2 (A)</td>
<td>30</td>
<td>16</td>
<td>96</td>
</tr>
<tr>
<td>3 (T)</td>
<td>30</td>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td>4 (T)</td>
<td>45</td>
<td>20</td>
<td>180</td>
</tr>
<tr>
<td>5 (T)</td>
<td>60</td>
<td>20</td>
<td>240</td>
</tr>
<tr>
<td>6 (T)</td>
<td>60</td>
<td>24</td>
<td>288</td>
</tr>
</tbody>
</table>
Results

Morphology of lung parenchyma and small airways

Figure 1 and supplemental Figures S1–S4 summarize histochemical and immunohistochemical data in sedentary and trained WT and mdx groups, respectively, at the different time points tested. In bronchi, epithelial thickness progressively decreased in WT mice, irrespective of training (linear regression for time trends: WT-SD: \(R^2 = 0.43\), \(r = -0.65\), \(P < 0.0005\); WT-EX: \(R^2 = 0.68\), \(r = -0.82\), \(P < 0.0001\)). No change over time was evident in mdx mice (mdx-SD: \(R^2 = 0.02\), NS; mdx-EX: \(R^2 = 0.09\), NS), and epithelial thickness was significantly higher in mdx compared to WT mice at 45 days in both the sedentary and exercised groups (Fig. 1, part A; Fig. S1). A very modest number of inflammatory cells was found, with a non significant increase in WT-EX mice at 30 days (data not shown).

Few PAS-positive (goblet) cells were found in mdx compared to WT mice at all time points, irrespective of sedentary or trained conditions (Fig. 1, part B; Fig. S2). Moreover, WT mice showed a significant linear increase of PAS-positive cells over time irrespective of sedentary or trained conditions (linear regression for time trends: WT-SD: \(R^2 = 0.23\), \(r = 0.48\), \(P = 0.015\); WT-EX: \(R^2 = 0.36\), \(r = 0.60\), \(P = 0.0015\)). No similar trend was found in mdx mice (not shown).

Apoptosis and regeneration of airway epithelium

Both at bronchial and bronchiolar level, frequency of apoptosis of airway epithelial cells at baseline was higher in WT than in mdx mice, but there was a trend at 30 and 45 days for higher apoptosis in mdx than WT mice irrespective of sedentary or trained status (Fig. 1, part C, and Fig. S3). In mice trained for 45 days, the percentage of TUNEL-positive epithelial cells was significantly higher in mdx than in WT mice. In particular, in bronchi linear regression revealed opposite trends over time in WT (WT-SD: \(R^2 = 0.51\), \(r = -0.72\), \(P < 0.0001\); WT-EX: \(R^2 = 0.29\), \(r = 0.54\), \(P = 0.006\); mdx-SD: \(R^2 = 0.38\), \(r = 0.62\), \(P = 0.00\)). Similar trends were found in bronchioli (WT-SD: \(R^2 = 0.49\), \(r = -0.70\), \(P = 0.04\); WT-EX: \(R^2 = 0.45\), \(r = -0.67\), \(P < 0.0001\); mdx-SD: \(R^2 = 0.58\), \(r = 0.76\), \(P = 0.03\); mdx-EX: \(R^2 = 0.38\), \(r = 0.62\), \(P < 0.0001\)).

Frequency of PCNA-positive cells, that is, cells under active proliferation, at baseline was higher in WT compared to mdx mice in both the bronchial and bronchiolar epithelium. Both strains were similar at 30 days for PCNA positivity, that is, higher proliferation in exercised than sedentary animals in both WT and mdx groups. At 45 days, PCNA positivity was lower in both WT and mdx groups, with no significant difference between WT and mdx groups (Fig. 1, part D).

In summary, the main result was that mdx mice showed a paucity of goblet cells in airway epithelium, scarce progressive thinning of airway epithelium over time, and little evidence of airway inflammation after training.
unchanged in WT mice, but sharply decreased in mdx mice irrespective of training (Fig. 1, part D, and Fig. S4).

In summary, epithelial apoptosis progressively increased in mdx mice over time. Epithelial regeneration sharply decreased in mdx mice at 45 days.

**Chaperonin HSP60**

The level of HSP60, an oxidative stress marker, was higher at baseline in mdx compared to WT mice in both the bronchial (Fig. 2, part A) and bronchiolar (data not shown) epithelium. In particular, HSP60 levels were higher in WT than mdx mice, with the highest values recorded in the WT-EX mice at 30 days. No significant time trend was evident in WT mice (data not shown), while linear regression revealed a progressive decrease in HSP60 levels in mdx mice over time in both bronchial (mdx-SD: \( R^2 = 0.81, r = -0.90, P < 0.002; \) mdx-EX: \( R^2 = 0.78, r = -0.88, P < 0.0015 \)) and bronchiolar epithelium (mdx-SD: \( R^2 = 0.88, r = -0.94, P = 0.0006; \) mdx-EX: \( R^2 = 0.67, r = -0.82, P < 0.0005 \)). The percentage of apoptotic cells in bronchial epithelium was inversely related to HSP60 expression in mdx but not in WT mice (Fig. 2, part B), and a similar relationship was found in bronchioles (\( r = -0.59, P = 0.03 \)).

In summary, HSP60 levels progressively decreased over time, and were inversely correlated to apoptosis, in mdx mice.

**Discussion**

Subtle epithelial abnormalities were found in mdx mice, that is, a low number of goblet cells, and lower PCNA expression at 45 days in mdx compared to WT animals. Moreover, in mdx mice there was a significant trend for increasing apoptosis to be associated with low epithelial expression of HSP60 in airway epithelium. These results suggest that dystrophin might be involved not only at the muscle level, but also in the preservation of bronchial epithelial integrity. In response to low-intensity endurance training, no significant inflammatory infiltrate was observed in the airways of trained WT or mdx mice, at variance with the mild airway epithelial damage and leucocyte influx into and around airways previously documented in a different mouse strain (Chimenti et al., 2007).

The number of goblet cells in the airways of mdx mice was very low in both sedentary and trained animals. To our knowledge, this is a new and intriguing finding. We can only speculate on the potential pathway(s) involved. Recent work has shown that the Notch pathway is central in epithelial homeostasis and remodelling. The Notch pathway is involved not only in airway cell distribution during development (Mori et al., 2015) but also in homeostatic transdifferentiation in the adult lung (Marcet et al., 2011; Tsao et al., 2011; Gomi et al., 2015; Lafkas et al., 2015; Mori et al., 2015). Notch or Jagged inhibition caused an almost complete loss of goblet/club cells in favour of ciliated cells (Marcet et al., 2011; Firth et al., 2014; Lafkas et al., 2015). Our data strongly suggest that low activity of the Notch pathway might be involved in the decreased number of goblet cells found in the airways of mdx mice, as schematically depicted in Figure 3. No data are currently available on the expression and role of dystrophin in airway epithelium.

In patients with Duchenne muscular dystrophy (DMD), mucus elimination from the airways is a major clinical problem.
due to inefficient cough (LoMauro et al., 2014). A reduced number of goblet cells in the airways might actually be favourable in the setting of reduced respiratory muscle function by limiting mucus production. Notch was shown to be downregulated in skeletal muscle of mdx mice, and this finding might be causally related to age-dependent depletion of satellite cells (Jiang et al., 2014). Positive modulation of Notch is a strategy currently explored to rescue the dystrophic muscular, even though increasing Notch expression in animal models of DMD did not improve muscle regeneration (Church et al., 2014). Our data suggest the possibility that rescuing Notch to counteract skeletal muscle damage might exert detrimental consequences at the respiratory level in DMD patients. Further studies are needed to clarify this point.

The reduction in goblet cells in mdx mice might be associated with an increase in ciliated cells, since the airways mucosa is mainly constituted by these two differentiated cytotypes. However, while goblet cells can be easily recognized by PAS staining, there is no histochemical stain available allowing the identification of ciliated cells. Further studies by electron microscopy are needed to confirm the hypothesis that in our model the decrease of goblet cells was associated with an increase in ciliated cells.

At variance with our previous results (Chimenti et al., 2007), we found little epithelial damage associated with exercise training in both mdx and WT mice, despite we used exactly the same training protocol as in our earlier study. Since inflammatory cell infiltration was low in both strains, the lack of an inflammatory response in the airways cannot be attributed to absence of dystrophin, but rather suggests a difference between strains. Such interpretation is supported by other differences between the two studies. For example, in Swiss mice, we found loss of cilia and increased epithelial thickness after training (Chimenti et al., 2007); conversely, no loss of cilia was found in the present study.

Analysis of epithelial apoptosis and regeneration provided some insight into the mechanisms involved in bronchial epithelial integrity in mdx mice. In our previous study, we documented a progressive increase in both PCNA- and TUNEL-positive cells during training, and interpreted this result as indicative of exercise-dependent increase in epithelial turnover (Chimenti et al., 2007). In the present study, the analysis of the PCNA time course showed that at 30 days trained animals showed twofold PCNA expression compared to sedentary animals in both WT and mdx groups. However, later on (i.e., 45 days) WT mice were able to maintain PCNA expression level, whereas mdx mice showed decreased PCNA expression. Moreover, TUNEL positivity at 30 and 45 days tended to be higher in mdx than WT mice, reaching significance in the exercised group at 45 days. A possible interpretation of this complex picture is that mdx mice can effectively increase epithelial regeneration only at an early stage, but such ability shows signs of exhaustion in later phases. Again, this time course is similar to the late depletion of satellite cells in mdx muscle (Jiang et al., 2014), in agreement with a possible role of Notch signalling. The late changes in epithelial thickness in the course of the experiment can be interpreted as an indirect proof of altered homeostasis of airway epithelium. From a morphological point of view, the increased epithelial thickness may indicate a modification in cell differentiation and, in turn, morphology; for example, cells could be taller or cilia longer, or the overall number of cells is increased so that the epithelium results crowded. Again, further studies by transmission electron microscopy are necessary to solve this conundrum. Extension of the training period in future experiments can also help to better understand the relationship between airway epithelial damage and increased epithelial thickness.

HSP60-positive cells progressively decreased over time in mdx mice. Rather than indicating low cellular stress, the low expression of HSP60 at 45 days in mdx mice suggests the progressive exhaustion of a protecting mechanism preserving epithelial integrity, as confirmed by the inverse correlation between HSP60 expression and the percentage of apoptotic cells, as schematically summarized in Figure 3. A similar time course of HSP60 was observed in the diaphragm of mdx mice (Cappello, personal communication). HSP60 is known to exert an anti-apoptotic activity in bronchial epithelial cells by blocking the activation of the caspase cascade (Campanella et al., 2008). Our data extend to the lung previous findings indicating a role of HSP60 in epithelial preservation in the kidney of mdx mice (Loh et al., 2000). The role of HSP60 in bronchial mucosa homeostasis is still under investigation. Low HSP60 levels were found during carcinogenesis of bronchial mucosa in patients with chronic obstructive pulmonary disease (COPD) (Cappello et al., 2005). Moreover, the normal epithelium close to the tumor showed reduced immunopositivity for HSP60 compared to healthy subjects (Cappello et al., 2006). Viceversa, high HSP60 levels were documented in severe/very severe COPD compared to smokers and nonsmoker controls (Cappello et al., 2011). Interestingly, oxidative stress increased the expression and secretion of HSP60 in a human bronchial epithelial cell line (Cappello et al., 2011) while cigarette smoke extracts did not (Corrao et al., 2014). Secreted HSP60 may also act in a paracrine fashion on epithelial cells by downregulating inflammation (Cappello F, personal communication). Therefore, HSP60 appears to exert protective effects in the bronchial epithelium in disease models like cancer or COPD (Loh et al., 2000; Cappello et al., 2005, 2006, 2011; Campanella et al., 2008; Corrao et al., 2014), and the current data extend these observation to the mdx model.

The more relevant implication of our results is that lack of dystrophin affects airway epithelium in addition to the known effects on skeletal muscle. This result is in line with a generalized perturbation of cell metabolism in DMD, which has not been given much consideration until now. Instead, DMD should be considered a systemic disease with major, but not exclusive, involvement of skeletal muscle. From a clinical point of view, it is unknown whether the epithelial changes found in mdx mice also occur in human DMD, and further studies are necessary to test this hypothesis and assess potential pathological effects of exercise training or test specific pharmacologic interventions. In patients with DMD, positive

![Fig. 3. Schematic drawing summarizing the hypothetical changes occurring in mdx mice in the airway epithelium. Differentiation of basal epithelial cells (triangle) into goblet cells (left) or ciliated cells (middle) is modulated by Notch expression. Predominance of ciliated cells in mdx mice might be explained by low Notch expression. A high expression of HSP60 may protect epithelial cells against apoptosis (right) early in the course of the disease.](Image)
effects of inspiratory muscle training have been reported, but data on airway epithelium have not been collected (Wanke et al., 1994).

In conclusion, to our knowledge, this is the first report on airway cells in mdx mice. mdx mice showed a low number of goblet cells, limited epithelial regeneration, and decreased expression of HSP60 in airway epithelium irrespective of sedentary or training conditions. No major inflammatory changes related to mild exercise training were found in WTR or mdx mice. Further studies are needed to assess the potential clinical implications of our findings in patients with DMD.

Acknowledgments

This work was partly supported by the Euro-Mediterranean Institute of Science and Technology (FC, FR, and AP) and the University of Palermo (FC, G Morici, MRB, NB, G Mudo) funds. Part of this work was carried out using instruments provided by the Euro-Mediterranean Institute of Science and Technology (FC, FR, and AP) and the Italian National Operational Programme for Research and Competitiveness 2007–2013 grant (Project code: PONA3_00210, European Regional Development Fund).

Literature Cited


