

# Duchenne Muscular Dystrophy (DMD): Should it be Considered a Systemic Disease?

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## Abstract

Duchenne muscular dystrophy (DMD) is an X-linked muscle disease characterized by progressive skeletal muscle loss and development of respiratory failure due to involvement of respiratory muscles. Similar to human DMD, the mdx mouse model lacks dystrophin but is characterized by relatively mild muscle injury, allowing testing the effects of mild endurance exercise training on dystrophic skeletal muscle. We were interested to study the effects of exercise training on airway cells in trained mdx mice by applying the same protocol previously tested in Swiss mice. We found that mdx mice showed little airway inflammation associated with training, but developed increasing apoptosis of airway cells over time, irrespective of the trained or sedentary status. These findings suggested subclinical progressive exhaustion of protective mechanisms in airway epithelium of the mdx mouse, possibly involving chaperonin Hsp60. Moreover, a paucity of goblet cells was shown in the airways of mdx mice at all-time points of the study, independently of the sedentary or trained condition. We speculate that a disturbance of the Notch pathway, which has already been described in dystrophic skeletal muscles, might be involved in the almost absent secretory cell phenotype found in the airways of mdx mice. Overall, our findings suggest that dystrophin might affect other tissues beyond skeletal muscles, and exert physiologic effects, which at present are poorly defined, in non-muscular tissues.

**Keywords:** Dystrophin; Airway epithelium; Goblet cells; Chaperonin Hsp60; Notch pathway

## Commentary

Duchenne muscular dystrophy (DMD) is an X-linked muscle disease affecting 1:3500 newborn boys [1], and characterized by a defect in the sub-sarcolemmal protein dystrophin which leads to membrane fragility, muscle necrosis, motor weakness, myofiber death and replacement of skeletal muscle by fibrous and fatty connective tissue [2]. In the course of DMD, chronic respiratory insufficiency inevitably develops due to primary loss of inspiratory and expiratory muscle strength [3].

The muscular lesion of DMD is very complex, and may involve, among other mechanisms, the Notch pathway, a recognized key player in development and repair. Canonical activation of Notch requires cell-to-cell communication, and Notch genetic disturbances are responsible for several syndromes involving defects in brain development and the cardiovascular system [4]. In skeletal muscles, Notch activity is necessary for muscle regeneration and repair during development and postnatal age, respectively [5,6]. Data regarding Notch activity in the dystrophic skeletal muscle are variable, possibly in relation to the animal model employed, differences between animal models and DMD patients, and sampling at variable time points in the course of the disease [7,8]. Church and associates have confirmed altered expression of Notch signalling components in dystrophin deficiency, and major differences between animal models and DMD patients. In addition, they tested the hypothesis that manipulation of genes

involved in Notch might help muscle regeneration, with negative results [9]. Conversely, Vieira and coworkers recently reported that overexpression of Jagged 1, a canonical Notch ligand, occurred in dystrophin-deficient Golden Retriever dogs which did not show any clinical sign of muscle disease [10]. Overall, the different studies agree on a decreased activity of components of the Notch pathway associated with lack of dystrophin.

Research on animal models of DMD has mostly examined skeletal muscle pathology, but some data suggest that dystrophin is important in other tissues as well. Lack of dystrophin does not appear to cause gross pathological abnormalities in non-muscle tissues up to 10 weeks of age in the dko mouse model of DMD lacking both dystrophin and utrophin [11]. However, dystrophin is widely distributed in non-muscle tissues, i.e. blood-brain barrier, choroid plexus, retina, and kidney [12]. In the kidney, dystrophin distribution varies along the nephron suggesting a physiological role, even though redundancy of protective mechanisms might account for lack of overt renal dysfunction in DMD patients [13]. Several dystrophin isoforms are expressed in non-muscle tissues, including the lung, in particular full-length dystrophin (Dp427) and the shorter isoform Dp71 are ubiquitously expressed [14], suggesting a physiological role of dystrophin beyond its relevance in skeletal muscle. Little attention to date has been paid to the biology of lung cells in the absence of dystrophin. This is a new area of research, and might turn to be important also in patients' care. For example, reduced airway smooth muscle function has been shown in animal models of DMD [15] suggesting that DMD patients might be relatively resistant to develop bronchoconstriction.

Over the years, our research group has studied exercise training as a model to assess the effects of a physiological stress stimulus on airway epithelium. In both mice [16] and humans [17-19], prolonged endurance exercise caused mild epithelial injury, possibly as a result of insufficient conditioning of inspired air. Such injury was associated with release of interleukin-8 by bronchial epithelial cells [19] and influx of neutrophils into the large airways [17,18], but appeared self-limiting since no significant increase in concentration of inflammatory mediators in the airways could be shown. Normal mice undergoing 45-day endurance training at progressively increasing loads showed increased epithelial turnover, with evidence of both increased apoptosis and regeneration of bronchial epithelial cells [16].

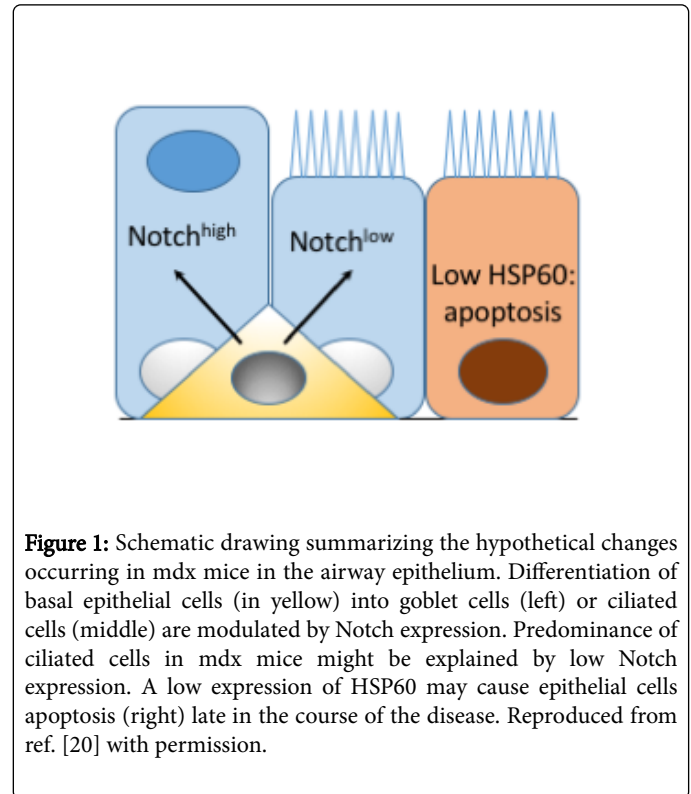
The mdx mouse model of DMD is characterized by lack of dystrophin, but muscle function is relatively preserved compared to the human disease. We therefore repeated the exercise training protocol in mdx and wild-type (WT) mice, but found no major morphological differences in bronchial epithelium between sedentary vs trained, or mdx vs WT groups. At 30 days, regeneration of bronchial epithelium was higher in trained than sedentary animals in both groups; however, at 45 days, epithelial regeneration decreased in mdx mice irrespective of training, and the percentage of apoptotic cells was higher in mdx-trained than in WT-trained mice [20]. Differently from our previous studies in the Swiss mouse strain, no airway inflammation developed during training in either mdx or WT mice.

The chaperonin Hsp60 is known to exert protective effects against oxidative stress in lung epithelial cells [21]. In our study, we measured epithelial expression of Hsp60 and found that it progressively decreased, and inversely correlated with epithelial apoptosis ( $r=-0.66$ ,  $p=0.01$ ) in mdx mice, whereas WT mice did not show any change [20]. Rather than indicating a low level of cellular stress, the low expression of Hsp60 at in mdx mice could indicate the progressive exhaustion of a protecting mechanism preserving epithelial integrity. Our data extended to the lung previous findings indicating a role of Hsp60 in epithelial preservation in the kidney of mdx mice [22]. Therefore, subtle functional abnormalities in airway epithelium may occur after a physiological stressor like exercise, suggesting the opportunity to use this model to further study epithelial function in mdx mice.

However, in our opinion the major result of our study was that mdx mice showed very few goblet cells in the airways irrespective of the trained/sedentary condition. This result is apparently in contradiction with the excessive mucus production which is a common problem in neuromuscular patients. A reduced number of goblet cells in mdx mice is an intriguing result, and if confirmed in human DMD, could actually protect against excessive mucus production in DMD patients.

We speculate that involvement of the Notch pathway could explain the lack of goblet cells in mdx mice, since the Notch pathway was shown to play a major role in the transdifferentiation of goblet-ciliated cells in the airways. The Notch pathway is involved not only in airway cell distribution during development [23] but also in homeostatic transdifferentiation in the adult lung [24-27]. Conversely, Notch or Jagged inhibition caused an almost complete loss of goblet/club cells with large predominance of ciliated cells [24,27,28]. Notch-3 appeared essential to maintain basal progenitor cells in the airway epithelium, and the balance between undifferentiated and committed progenitors [23]. In human primary bronchial epithelial culture, both Notch-1 and Notch-3 have been identified as crucial for secretory vs. ciliated cell differentiation [26]. Instead, in the study by Lafkas and coworkers, Notch-2 appeared as the most important receptor for stimulation by Jagged-2, with a secondary role of Notch-1 [27]. However, the goal of

the majority of studies on bronchial epithelial cells was to study ways to prevent goblet cell metaplasia occurring in asthma [27] or chronic obstructive pulmonary disease (COPD) patients [26,29]. In this regard, DMD and the mdx mouse model may represent a “natural experiment” of negative modulation of goblet cells in the lung. Figure 1 summarizes our hypothesis regarding the changes occurring in airway epithelium in mdx mice.



**Figure 1:** Schematic drawing summarizing the hypothetical changes occurring in mdx mice in the airway epithelium. Differentiation of basal epithelial cells (in yellow) into goblet cells (left) or ciliated cells (middle) are modulated by Notch expression. Predominance of ciliated cells in mdx mice might be explained by low Notch expression. A low expression of HSP60 may cause epithelial cells apoptosis (right) late in the course of the disease. Reproduced from ref. [20] with permission.

Further studies are needed to ascertain whether the low number of goblet cells in mdx mice depends on a low activity of the Notch pathway. Should this be the case, therapeutic strategies aiming at restoring muscle function through Notch potentiation might have detrimental consequences in the respiratory tract, i.e. excessive mucus production. In conclusion, animal models of DMD like the mdx mouse can be used to better understand the physiologic role of dystrophin, and the potential clinical implications of lack of dystrophin, in non-muscular tissues.

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