

Exercise-related Fatigue and Injury in NMD Models

Mark A. Wineinger, M.D. (Memorial to Dr. Wineinger at www.nmdinfo.net)

The dystrophic *mdx* mouse has the same genetic defect as the boys with Duchenne muscular dystrophy. However, the mouse does not exhibit the clinical manifestations that are observed in the boys. Although muscles of the boys undergo progressive degeneration and infiltration with fat and connective tissue, this progressive degeneration is seen only in the diaphragm of the mice. Both the mice and the boys lack the large structural protein dystrophin in their muscles. This defect is felt to be responsible for the increased susceptibility of the membrane of the muscle to injury that leads to the degeneration.

A number of reports support the idea that free oxygen radicals can play a role in muscle injury and disease. Free oxygen radicals are normal products of muscle metabolism. There is evidence of lipid peroxidation and increased oxidative damage to proteins in the muscles of boys with DMD. Several studies of *mdx* mice have supported the potential role of peroxidation-related injury in the muscles. Since the lack of dystrophin puts the muscles at a mechanical disadvantage, additional stress on the muscles from exercise may cause increased production of free radicals. The cell membrane is a major source of lipids in the muscle. The oxygen radicals may react with lipids in the cell membrane and contribute to the susceptibility to membrane damage that is generally attributed to the lack of dystrophin.

To simulate the activity of the muscles we subjected muscles from the *mdx* mouse to a fatiguing stimulation *in vitro* and measured the production of indicators of lipid peroxidation in a controlled environment and correlated these levels to the fatigue and weakness measured in these muscles.



The goal of our study was to demonstrate that fatiguing exercise of the *mdx* muscles *in vitro* would increase the production of oxygen radicals and this increase would result in oxidation of lipid in the muscle membrane. The combination of biochemical techniques for measuring the reaction of reactive oxygen with muscle lipids and the *in vitro* physiologic studies of muscle strength and fatigue provided us with a powerful experimental methodology to determine the effects of oxygen radicals on muscle. This system also allowed the testing of the effectiveness of antioxidant compounds in preventing of the production of reactive oxygen and, therefore, the evaluation of their use in prevention of muscle injury and fatigue. Since antioxidant compounds have been proposed to the general public as effective preventive and therapeutic

agents for a variety of conditions and diseases, it is important to define their role in neuromuscular diseases.

Initially, we studied a small leg muscle because in previous studies it had exhibited the greatest reduction in strength and the greatest fatigability. Although the *mdx* muscle was weaker and fatigued much more rapidly than that of the normal mouse, using a variety of fatiguing stimulation protocols we found no difference in the lipid peroxidation in the two. There was also no difference when animals of different ages were studied to see if increasing age affected the lipid peroxidation. In none of these variations did we see the expected increase in lipid peroxidation in the leg muscle.

We then studied the diaphragm of *mdx* and control mice at various ages. There is a progressive deterioration of this muscle in the *mdx* mouse, that is similar to that observed in the muscles of the boys with DMD. The diaphragm of the *mdx* mice was weaker than the diaphragm of the control mice. When the diaphragm was subjected to a fatiguing stimulation protocol, there was no effect of increasing age on the rate of fatigue in the control mice. However, the diaphragm of the *mdx* mice older than 8 months of age had increased fatigue and an increase in lipid peroxidation. Preliminary studies to reduce the observed peroxidation by the addition of the antioxidant n-acetyl cysteine *in vitro* were inconclusive. This is an area where much further work is necessary to determine first, if the peroxidation can be prevented *in vitro* and then, if this intervention is successful, to move to the whole animal to demonstrate the potential of an intervention with antioxidants as a means to delay the deterioration of the muscles in boys with DMD.