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Muscular Dystrophy: Misplaced Enzyme Is to Blame for Quick Fatigue After Mild Exercise

Howard Hughes Medical Institute scientists have uncovered a molecular explanation for the profound fatigue brought on by mild exercise in some people with muscular dystrophy.

In studies with genetically engineered mice that showed this form of fatigue after mild exercise, the researchers found that an enzyme called neuronal nitric oxide synthase (nNOS) is not present at its normal location in the membrane surrounding muscle cells. This means the blood vessels that supply active muscles do not relax normally and the animals experience fatigue after very mild exercise.

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Howard Hughes Medical Institute researcher Kevin P. Campbell led a research team from the University of Iowa that reported its findings in the November 27, 2008, issue of the journal *Nature*.

In identifying the mechanism for this specific form of fatigue, the researchers found that the fatigue can be alleviated pharmacologically. When the scientists administered Viagra-like drugs to the mice with muscular dystrophy, they noticed an increase in their ability to move, as well as a dramatic increase in their activity after mild exercise. The treated mice were two to four times more active than untreated mice with muscular dystrophy. Prior to treatment, the same mice would become virtually inert after a short burst of low-intensity activity.

Nitric oxide signaling stimulates the generation of cGMP, a phosphodiester, which leads to a cascade of effects that culminates in the dilation of blood vessels. A phosphodiesterase (PDE) breaks down cGMP, limiting its duration to signal the vessels to dilate. Viagra enhances nitric oxide signaling by inhibiting the PDE from breaking down cGMP, allowing for prolonged vasodilation upon nitric oxide signaling. Further research involving longer-acting versions of PDE inhibitors could lead to the first therapies to improve the physical endurance of patients with muscular dystrophy and improve their quality of life, said Campbell. "Even with patients who have milder dystrophies, when they visit our lab and walk around for a short time become fatigued," he said.

The provocative results might have implications for treatment of other conditions, such as multiple sclerosis, chronic fatigue syndrome, and even the aging-related muscle weakness that can lead to dangerous falls, Campbell speculated.

The exaggerated fatigue response to physical exertion affects people with Duchenne muscular dystrophy and a number of variant forms of the disease, some of which are not as severe as Duchenne. This fatigue can be severely disabling, and there is no treatment. Campbell said that the fatigue response does not appear to stem directly from the atrophy and weakening of muscles that characterizes muscular dystrophies, and researchers have had a difficult time determining its exact cause.

Campbell's group reported in *Nature* that it has traced the phenomenon to a disruption of nitric oxide signaling in muscle cells to blood vessels. Nitric oxide is a key messenger molecule involved in many physiologic processes. Campbell's group showed in their new study that nNOS at the muscle cell membrane plays a crucial role in the fatigue response by dilating blood vessels and increasing blood flow to active muscles. These changes help supply oxygen and nutrients to muscle fibers when stressed by even light exertion. If nNOS signaling is absent or weak and fails to boost blood circulation during muscle activity, the muscles quickly become fatigued.

Campbell's research team, which has long been interested in the molecular pathogenesis of muscular dystrophy, developed a test to measure the physical activity of normal mice and mice afflicted with neuromuscular disorders. After the mice exercise, they are placed in activity chambers equipped with lasers to measure their mobility, which might include foraging, standing on their hind legs, or grooming themselves.

Campbell said that when he showed colleagues at a scientific conference a video of dystrophic mice lying inert following mild exercise, a physician in the audience commented that the listless mice reminded him of some of his patients with Becker muscular dystrophy - a much milder form of the disease than Duchenne.

Researchers knew that patients with Becker muscular dystrophy have a defect in nNOS signaling. That observation prompted Campbell to look at muscle nNOS in other forms of muscular dystrophy. The specific defect in most cases was not an absence of the signal, but a misplacement of the nNOS enzyme. The enzyme is supposed to be in the plasma membrane surrounding muscle cells (the sarcolemma). In dystrophic cells, however, nNOS is not in its proper location, diluting its ability to relax blood vessels during muscle activity.

Moreover, Campbell observed, the nNOS defect and the resulting easy fatigability of muscles occurs long before the muscle shows any signs of the wasting and weakness that occur as muscular dystrophy progresses, confirming that the two phenomena are separate. In fact, mice engineered to lack the nNOS enzyme exhibit the exaggerated fatigue after mild exercise but have no muscle damage or weakness at all, he said.

Subsequently, the researchers treated dystrophic (*mdx*) mice with several chemical agents that cause blood vessels to dilate, and looked for improvements in the rodents' exercise endurance and post-exercise activity. An inhibitor of the enzyme phosphodiesterase (PDE) was the only compound that markedly alleviated the fatigue. When they administered the PDE inhibitor to the mice, the scientists observed increased blood flow in the muscles and found that the treated mice were two to four times more active than untreated mice after mild exercise.

PDE inhibitors such as Viagra are widely used to treat erectile dysfunction, but in themselves are not good candidates for testing in patients with neuromuscular disorders because their effects wear off quickly. Campbell said that pharmaceutical companies have also developed longer-acting PDE inhibitors that could be considered for clinical trials.

Even if such drugs turn out to be effective for alleviating exercise-induced fatigue in patients with muscular dystrophy, he cautioned that it is too early to know what the long-term effects of those drugs might be in this group of people. "The drugs might be helpful because they would increase physical activity and improve quality of life," said Campbell. "But there is concern that excessive muscle contraction may accelerate damage to the muscles and cause the disease to progress faster. That is a question we will need to answer."