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## Researchers Identify Drugs that Enhance Exercise Endurance

Researchers have identified two drugs that mimic many of the physiological effects of exercise. The drugs increase the ability of cells to burn fat and are the first compounds that have been shown to enhance exercise endurance.

Both drugs can be given orally and work by genetically reprogramming muscle fibers so they use energy better and contract repeatedly without fatigue. In laboratory experiments, mice taking the drugs ran faster and longer than normal mice on treadmill tests. Animals that were given AICAR, one of the two drugs, ran 44 percent longer than untreated animals. The second compound, GW1516, had a more dramatic impact on endurance, but only when combined with exercise.

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— Ronald M. Evans

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Ronald M. Evans, the Howard Hughes Medical Institute investigator who led the study, said drugs that mimic exercise could offer potent protection against obesity and related metabolic disorders. They could also help counter the effects of devastating muscle-wasting diseases like muscular dystrophy. Evans and his colleagues, who are at the Salk Institute for Biological Studies, published their findings on July 31, 2008, in an advance online publication in the journal *Cell*.

Concerned about the potential for abuse of the two performance-enhancing drugs, Evans has also developed a test to detect the substances in the blood and urine of athletes who may be looking for way to gain an edge on the competition.

In 2004, Evans and his colleagues genetically engineered mice that had altered muscle composition and enough physical endurance to run twice as far as normal mice. These marathon mice had an innate resistance to weight gain, even when fed a high-fat diet. We made these mice and they had low blood sugar, they resisted weight gain, they had low fats in their blood. They were much healthier animals, Evans said. And when we put them on a

treadmill, the engineered mice ran twice as far than normal mice - they transformed into remarkable runners.

The scientists achieved these effects by modifying a gene called PPAR-delta, a master regulator of numerous genes. Evans and his colleagues showed that by enhancing PPAR-delta's activity, they had shifted the genetic network in muscle cells to favor burning fat over sugar as their energy source. But the effects seen in the marathon mice were caused by a genetic manipulation that was present in their bodies as their muscles were developing. Evans's group began to wonder whether they could duplicate these effects by turning on PPAR-delta in adult mice.

We had shown that we could pre-program muscle using genetic engineering. If you express this gene while the muscle is being formed, you can increase the amount of non-fatiguing muscle fibers, Evans says. But what about reprogramming in an adult? When all the muscles are in place, can you give a drug that washes over the muscle for a few hours at a time and reprograms existing muscle fibers? That's a very different question.

PPAR-delta has long been an attractive drug target because of its central role in metabolism, so Evans and his colleagues had no shortage of chemical compounds available to test. They began by testing a compound called GW1516. They treated young adult mice with the drug for five weeks. We measured gene changes and the muscles looked like they were responding, so we knew the drug was working.

Thus, while fully expecting the drug to dramatically increase endurance - Evans says, There was no change at all in running performance. Nothing — not even a percent.

Surprised by this spectacular failure, Evans and his colleagues decided to try a different approach, based on real-life experience. If you're out of shape - and most of us are - and you want to change, you have to do some exercise. The way we reprogram muscle in adults is by training.

So the scientists subjected two groups of mice — one that received the drug and one that did not — to interval training. The mice ran for 30 minutes on a slow treadmill five days a week for a total of four weeks. At the end of the training period, all of the mice - regardless of whether they had received GW1516 - had improved their performance. Those that had received GW1516, however, ran 68 percent longer than those that had only done the exercise training. The dramatic effect of the drug was stunning, Evans said.

The scientists were intrigued by this synergistic interaction and wanted to know how exercise allowed the drug to work. One possibility was an enzyme called AMP kinase (AMPK). During exercise, cells burn ATP as their primary source of energy. In the process, they create a by-product called AMP. When cells sense the presence of AMP, they activate AMPK. Activation of AMPK creates more ATP for the cell to burn. AMPK also triggers changes that lower blood sugar, sensitize cells to insulin, enable cells to burn more fat, suppress inflammation, and otherwise influence metabolic

pathways. This is one reason that exercise is so beneficial.

Evans's team found that in addition to replenishing the cell's energy stores, AMPK also assists PPAR-delta in activating its gene targets. It hops onto PPAR-delta in the nucleus and turbo-charges its transcriptional activity, Evans explained. We think AMPK activity is the secret to allowing PPAR-delta drugs to work.

The critical question was whether chemical activation of AMPK is sufficient to trick the muscle into thinking it has been exercised. The second drug, called AICAR, enabled them to answer that question. AICAR mimics AMP, Evans said, so muscle thinks it's burning fat. The researchers were encouraged when they found that when they gave the drug to mice, they activated many of the genes in muscle that are turned on by exercise.

After four weeks of treatment with AICAR, Evans and his colleagues once again challenged sedentary mice to run on the treadmill. They found that mice that had received AICAR were able to run 44 percent longer than untreated mice. This is a drug that is like pharmacological exercise, Evans says. After four weeks of receiving the drug, the mice were behaving as if they'd been exercised. In fact, he says, those that got the drug actually ran longer and further than animals that received exercise training.

The animals receiving AICAR improved their running performance and their ability to burn fat. None of these effects, however, were as strong as they were in the animals that received both exercise and activation of PPAR-delta via GW1516.

Evans said this indicates that the benefits are likely due to collaboration between cells' AMPK and PPAR-delta signaling pathways. The team's genetic analyses supported this hypothesis; they found that the drugs alone activated a subset of exercise-induced genes, but activating both pathways (by combining GW1516 with exercise) activated a larger group of genes. Many of those genes regulate metabolism and muscle remodeling. Evans and his colleagues called this the endurance gene signature.

Like exercise, the two drugs trigger a variety of changes that contribute to muscles cells' improved endurance and ability to burn fat. These changes include an increase in mitochondria, the structures responsible for producing energy; a shift in metabolism that takes advantage of lipids as an energy source; and an increase in blood flow, which enables the steady delivery of fat to burn. While the scientists only examined the drugs' effects on muscle cells in this study, Evans says it is likely that they confer benefits on other systems impacted by exercise, such as the heart and lungs.

Based on his group's findings, Evans is optimistic about using small molecules that mimic exercise to treat and prevent a variety of common conditions. For example, the way in which the drugs transformed the muscle fibers of mice suggests they might help reverse the muscle frailty associated with aging or diseases like muscular dystrophy. We have now created the potential for a really simple intervention in an area of major health problems

for which there is no intervention, he says.

More broadly, the drugs could offer the benefits of exercise to people who do not get enough. Almost no one gets the recommended 40 minutes to an hour per day of exercise, he says. For this group of people, if there was a way to mimic exercise, it would make the quality of exercise that they do much more efficient. This might be enough to move people out of the 'danger zone' toward a lower risk, healthier set point. By intervening early, you may forestall the emergence of more serious problems.

Evans expects these types of drugs will be attractive to a variety of individuals. If you like exercise, you like the idea of getting more bang for your buck, he says of GW1516. If you don't like exercise, you love the idea of getting the benefits from a pill, as with AICAR. So, while Evans sees tremendous opportunities for health benefits from drugs that mimic exercise, he also sees serious potential for abuse.

Drugs that improve health are not only going to be used by people who have medical problems. They may also be used by people who are healthy - or by athletes who want an edge, said Evans. He noted that the sports world has long been aware of his lab's work demonstrating a link between PPAR-delta and endurance. What's more, GW1516 has a relatively simple chemical structure and can be synthesized easily. Evans anticipates that athletes will seek their own sources of the drug - if they haven't already.

Concerned about the potential for abuse, Evans thought it was important to develop a test that could detect whether the drug was being used as a performance-enhancing substance. With HHMI support, his group has created a highly sensitive test that uses mass spectrometry to detect the two drugs and their metabolic by-products in the blood or urine. While the test is very reliable in mice, Evans says that further analyses are needed to ensure that it is accurate in humans. Evans, HHMI and the World Anti-Doping Agency are now working to certify the detection system and make it available in time to retroactively test athletes who compete in the 2008 Olympics.