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Genetically Engineered "Marathon Mouse" Keeps on Running

By enhancing the function of a single protein, Howard Hughes Medical Institute researchers have produced a "marathon mouse" with altered muscle composition and enough physical endurance to run twice as far as normal mice. Mice with the enhanced protein also showed an innate resistance to weight gain, even when fed a high-fat diet that caused normal mice to become obese.

According to the researchers, their finding will offer important insights into the machinery that powers muscle development and the physiological changes produced by exercise. The finding also suggests a route to designing drugs that enhance muscle development and mimic all the benefits of exercise. The researchers said such drugs could aid patients whose debilitating diseases prevent them from exercising. The researchers recognize that such drugs - already being tested by pharmaceutical companies - could be abused as a way to enhance athletic performance.

Led by Howard Hughes Medical Institute investigator Ronald M. Evans at The Salk Institute, the researchers published their findings [online](#) August 24, 2004, in the journal *Public Library of Science Biology*. Other co-authors are from Seoul National University in Korea.

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In their studies, the researchers were exploring the effects of altering the gene for a protein called PPAR-delta—a master regulator of numerous genes—to enhance that protein's activity. According to Evans, they had not expected the profound and far-reaching physiological effects of this single genetic alteration.

"In previous work, we had shown that in various tissues, particularly adipose tissues, activating PPAR-delta increased fat burning and, as a result, decreased fat-tissue mass," said Evans. "Going into this experiment, the possibility of an effect on muscle fibers was not on our radar screen."

However, when the researchers produced mice with enhanced PPAR-delta activity, they saw a major transformation in skeletal muscle fibers. The mice showed a major enhancement of so-called "slow-twitch" muscle fibers and a decrease in "fast-twitch" muscle fibers.

Slow-twitch fibers are by far the more fatigue resistant of the muscle fibers. This is due to their large numbers of mitochondria - the cell's metabolic power plants, with which they convert fat to energy. By contrast, fast-twitch muscles have far fewer mitochondria, and must derive their energy from glucose instead of fat. As a result, these muscle fibers fatigue quickly. Until the production of the genetically altered mouse, the only known way to increase endurance was through physical training.

"We thought that the enhanced PPAR-delta would just enable the muscle to burn more fat, but we didn't expect it would do so by increasing the population of slow twitch fibers," said Evans.

To confirm that the increase in PPAR-delta activity caused the muscle transformation, the researchers gave normal mice an experimental drug, called GW501516, that activates the protein. The drug is being developed by GlaxoSmithKline to treat people with lipid metabolism disorders. Evans and his colleagues found that treatment with the drug produced muscle and metabolic benefits similar to what they saw in the transgenic mice.

The researchers also found that the genetically altered mice were resistant to weight gain when placed on a high fat, high calorie diet. "These 'marathon mice' are resistant to that weight gain even though they eat the same amount of food as the normal mice and have the same level of activity," said Evans. "So, their resistance to weight gain is not simply due to increased exercise.

"Significantly, the increased number of fat-burning muscle fibers appears by itself to be protective against a high fat diet," he said. "And that's important because it indicates that the presence of more mitochondria in the muscles of these animals probably results in more burning of fat and the release of some energy as heat."

"This leads me to believe that long distance runners, for example, possess a level of protection against weight gain even when they are not exercising. So, in a sense, they have built a kind of metabolic "shield" that keeps them from gaining weight," said Evans.

Also, when normal mice received the PPAR-delta-enhancing drug, they showed a virtually identical protection against weight gain, said Evans. The

genetically altered mice also had lower levels of intramuscular triglycerides, he said, which are often associated with insulin resistance and diabetes in obese people.

The marathon mice lived up to their nickname when Evans and his colleagues tested the animals' endurance on a treadmill. "When we placed these mice, which had never been run before, on a treadmill, the astonishing result was that the mice could run almost twice the distance that a normal mouse can run," said Evans.

Normal mice can run about 900 meters before exhaustion, while the PPAR-delta-enhanced mice could run 1800 meters, or more than a mile, before running out of steam, said Evans. In addition, the marathon mouse runs an hour longer than the 90 minutes that a normal mouse runs, he said.

It was especially surprising that altering a single gene produced such wide-ranging physiological changes in the mice. According to Evans, most physiologists believe that enhancing performance by training is a complicated process. Athletic training not only changes muscle fiber content, but also improves circulation and the motor neuron innervation into those muscles. Training also reengineers the heart to allow it to pump a greater volume of blood.

"So, it was not at all obvious that changing one gene in muscle would lead to coordinated changes throughout the body, from the nervous system to the cardiovascular system to the muscle itself. But the remarkable thing about this experiment is that this one change seems to rewire the entire system.

"That's exciting to us because it says that this complicated system can be coordinately changed by changing just one part. It also shows that it can be changed genetically, without exercise itself. This means that activation of this pathway might be very helpful to patients who are otherwise unable to exercise because of their weight or other complicating problems." Drugs that activate the PPAR-delta pathway could enhance muscle strength, combat obesity, and protect against diabetes, said Evans.

"One reason this approach to combating obesity might be important is that most weight-loss drugs aim at reducing appetite," said Evans. "That's the hardest thing to change in people because their appetite is genetically programmed. Drugs that enhance the PPAR-delta pathway would let people, like our mice, eat the same amount, but would increase their metabolism to burn more energy." Evans noted that such drugs would also have the potential for abuse among athletes, who could take them to enhance performance.

Further studies, said Evans, will aim at understanding the effects of PPAR-delta-enhancing drugs on the performance of normal animals. The researchers also plan to explore how such treatments affect physiology and

longevity in normal animals. And, they will explore the mechanism of action of the protein on the multitude of genes involved in the physiological changes they observed.