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Gene Doping

Gene therapy for restoring muscle lost to age or disease is poised to enter the clinic, but elite athletes are eyeing it to enhance performance Can it be long before gene doping changes the nature of sport?

By H. Lee Sweeney

Athletes will be going to Athens next month to take part in a tradition begun in Greece more than 2,000 years ago. As the world's finest specimens of fitness test the extreme limits of human strength, speed and agility, some of them will probably also engage in a more recent, less inspiring Olympic tradition: using performance-enhancing drugs. Despite repeated scandals, doping has become irresistible to many athletes, if only to keep pace with competitors who are doing it. Where winning is paramount, athletes will seize any opportunity to gain an extra few split seconds of speed or a small boost in endurance.

Sports authorities fear that a new form of doping will be undetectable and thus much less preventable. Treatments that regenerate muscle, increase its strength, and protect it from degradation will soon be entering human clinical trials for muscle-wasting disorders. Among these are therapies that give patients a synthetic gene, which can last for years, producing high amounts of naturally occurring muscle-building chemicals.

This kind of gene therapy could transform the lives of the elderly and people with muscular dystrophy. Unfortunately, it is also a dream come true for an athlete bent on doping. The chemicals are indistinguishable from their natural counterparts and are only generated locally in the muscle tissue. Nothing enters the bloodstream, so officials will have nothing to detect in a blood or urine test. The World Anti-Doping Agency (WADA) has already asked scientists to help find ways to prevent gene therapy from becoming the newest means of doping. But as these treatments enter clinical trials and, eventually, widespread use, preventing athletes from gaining access to them could become impossible.

Raising IGF-I allows us to break the connection between muscle use and its size.

Is gene therapy going to form the basis of high-tech cheating in athletics? It is certainly possible. Will there be a time when gene therapy becomes so commonplace for disease that manipulating genes to enhance performance will become universally accepted? Perhaps. Either way, the world may be about to watch one of its last Olympic Games without genetically enhanced athletes.

Loss Leads to Gain

Research toward genetically enhancing muscle size and strength did not start out to serve the elite athlete. My own work began with observing members of my family, many of whom lived well into their 80s and 90s. Although they enjoyed generally good health, their quality of life suffered because of the weakness associated with aging. Both muscle strength and mass can decrease by as much as a third between the ages of 30 and 80.

There are actually three types of muscle in the body: smooth muscle, lining internal cavities such as the digestive tract; cardiac muscle in the heart; and skeletal muscle, the type most of us think of when we think of muscle. Skeletal muscle constitutes the largest organ of the body, and it is this type--particularly the strongest so-called fast fibers--that declines with age. With this loss of strength, losing one's balance is more likely and catching oneself before falling becomes more difficult. Once a fall causes a hip fracture or other serious injury, mobility is gone completely.

With gene therapy poised to become a viable medical treatment, gene doping cannot be far behind.

Skeletal muscle loss occurs with age in all mammals and probably results from a cumulative failure to repair damage caused by normal use. Intriguingly, aging-related changes in skeletal muscle resemble the functional and physical changes seen in a suite of diseases collectively known as muscular dystrophy, albeit at a much slower rate.

In the most common and most severe version of MD--Duchenne muscular dystrophy--an inherited gene mutation results in the absence of a protein called dystrophin that protects muscle fibers from injury by the force they exert during regular movement. Muscles are good at repairing themselves, although their normal regenerative mechanisms cannot keep up with the excessive rate of damage in MD. In aging muscles the rate of damage may be normal, but the repair mechanisms become less responsive. As a result, in both aging and Duchenne MD, muscle fibers die and are replaced by infiltrating fibrous tissue and fat.

In contrast, the severe skeletal muscle loss experienced by astronauts in microgravity and by patients immobilized by disability appears to be caused by a total shutdown of muscles' repair and growth mechanism at the same time apoptosis, or programmed cell death, speeds up. This phenomenon, known as disuse atrophy, is still not fully understood but makes sense from an evolutionary perspective. Skeletal muscle is metabolically expensive to maintain, so keeping a tight relation between muscle size and its activity saves energy. Skeletal muscle is exquisitely tuned to changing functional demands. Just as it withers with disuse, it grows in size, or hypertrophies, in response to repeated exertions. The increased load triggers a number of signaling pathways that lead to the addition of new cellular components within individual muscle fibers, changes in fiber type and, in extreme

conditions, addition of new muscle fibers.

To be able to influence muscle growth, scientists are piecing together the molecular details of how muscle is naturally built and lost. Unlike the typical cell whose membrane contains liquid cytoplasm and a single nucleus, muscle cells are actually long cylinders, with multiple nuclei, and cytoplasm consisting of still more long tiny fibers called myofibrils. These myofibrils, in turn, are made of stacks of contractile units called sarcomeres. Collectively, their shortening produces muscle contractions, but the force they generate can damage the muscle fiber unless it is channeled outward. Dystrophin, the protein missing in Duchenne muscular dystrophy patients, conducts this energy across the muscle cell's membrane, protecting the fiber.

Yet even with dystrophin's buffering, muscle fibers are still injured by normal use. In fact, that is believed to be one way that exercise builds muscle mass and strength. microscopic tears in the fibers caused by the exertion set off a chemical alarm that triggers tissue regeneration, which in muscle does not mean production of new muscle fibers but rather repairing the outer membrane of existing fibers and plumping their interior with new myofibrils. Manufacturing this new protein requires activation of the relevant genes within the muscle cell's nuclei, and when the demand for myofibrils is great, additional nuclei are needed to bolster the muscle cell's manufacturing capacity.

Local satellite cells residing outside the muscle fibers answer this call. First these muscle-specific stem cells proliferate by normal cell division, then some of their progeny fuse with the muscle fiber, contributing their nuclei to the cell. Both progrowth and antigrowth factors are involved in regulating this process. Satellite cells respond to insulinlike growth factor I, or IGF-I, by undergoing a greater number of cell divisions, whereas a different growth-regulating factor, myostatin, inhibits their proliferation.

With these mechanisms in mind, about seven years ago my group at the University of Pennsylvania, in collaboration with Nadia Rosenthal and her colleagues at Harvard University, began to assess the possibility of using IGF-I to alter muscle function. We knew that if we injected the IGF-I protein alone, it would dissipate within hours. But once a gene enters a cell, it should keep functioning for the life of that cell, and muscle fibers are very long-lived. A single dose of the IGF-I gene in elderly humans would probably last for the rest of their lives. So we turned our attention to finding a way to deliver the IGF-I gene directly to muscle tissue.

Donning New Genes

Then as now, a major obstacle to successful gene therapy was the difficulty of getting a chosen gene into the desired tissue. Like many other researchers, we selected a virus as our delivery vehicle, or vector, because viruses are skilled at smuggling genes into cells. They survive and propagate by tricking the cells of a host organism into bringing the virus inside, rather like a biological Trojan horse. Once within the nucleus of a host cell, the virus uses the cellular machinery to replicate its genes and produce proteins. Gene therapists capitalize on this ability by loading a synthetic gene into the virus and removing any genes the virus could use to cause disease or to replicate itself. We selected a tiny virus called adeno-associated virus (AAV) as our vector, in part because it infects human muscle readily but does not

cause any known disease.

We modified it with a synthetic gene that would produce IGF-I only in skeletal muscle and began by trying it out in normal mice. After injecting this AAV-IGF-I combination into young mice, we saw that the muscles' overall size and the rate at which they grew were 15 to 30 percent greater than normal, even though the mice were sedentary. Further, when we injected the gene into the muscles of middle-aged mice and then allowed them to reach old age, their muscles did not get any weaker.

To further evaluate this approach and its safety, Rosenthal created mice genetically engineered to overproduce IGF-I throughout their skeletal muscle. Encouragingly, they developed normally except for having skeletal muscles that ranged from 20 to 50 percent larger than those of regular mice. As these transgenic mice aged, their muscles retained a regenerative capacity typical of younger animals. Equally important, their IGF-I levels were elevated only in the muscles, not in the bloodstream, an important distinction because high circulating levels of IGF-I can cause cardiac problems and increase cancer risk. Subsequent experiments showed that IGF-I overproduction hastens muscle repair, even in mice with a severe form of muscular dystrophy.

Raising local IGF-I production allows us to achieve a central goal of gene therapy to combat muscle-wasting diseases: breaking the close connection between muscle use and its size. Simulating the results of muscle exercise in this manner also has obvious appeal to the elite athlete. Indeed, the rate of muscle growth in young sedentary animals suggested that this treatment could also be used to genetically enhance performance of healthy muscle. Recently my laboratory worked with an exercise physiology group headed by Roger P. Farrar of the University of Texas at Austin to test this theory.

We injected AAV-IGF-I into the muscle in just one leg of each of our lab rats and then subjected the animals to an eight-week weight-training protocol. At the end of the training, the AAV-IGF-I-injected muscles had gained nearly twice as much strength as the uninjected legs in the same animals. After training stopped, the injected muscles lost strength much more slowly than the unenhanced muscle. Even in sedentary rats, AAV-IGF-I provided a 15 percent strength increase, similar to what we saw in the earlier mouse experiments.

We plan to continue our studies of IGF-I gene therapy in dogs because the golden retriever breed is susceptible to a particularly severe form of muscular dystrophy. We will also do parallel studies in healthy dogs to further test the effects and safety of inducing IGF-I overproduction. It is a potent growth and signaling factor, to which tumors also respond.

Safety concerns as well as unresolved questions about whether it is better to deliver AAV in humans through the bloodstream or by direct injection into muscle mean that approved gene therapy treatments using AAV-IGF-I could be as much as a decade away. In the shorter term, human trials of gene transfer to replace the dystrophin gene are already in planning stages, and the Muscular Dystrophy Association will soon begin a clinical trial of IGF-I injections to treat myotonic dystrophy, a condition

that causes prolonged muscle contraction and, hence, damage.

A still more immediate approach to driving muscle hypertrophy may come from drugs designed to block myostatin. Precisely how myostatin inhibition builds muscle is still unclear, but myostatin seems to limit muscle growth throughout embryonic development and adult life. It acts as a brake on normal muscle growth and possibly as a promoter of atrophy when functional demands on muscle decrease. Experiments on genetically engineered mice indicate that the absence of this antigrowth factor results in considerably larger muscles because of both muscle fiber hypertrophy and hyperplasia, an excessive number of muscle fibers.

Making Muscle and More

Pharmaceutical and biotechnology companies are working on a variety of myostatin inhibitors. Initially, the possibility of producing meatier food animals piqued commercial interest. Nature has already provided examples of the effects of myostatin blockade in the Belgian Blue and Piedmontese cattle breeds, both of which have an inherited genetic mutation that produces a truncated, ineffective version of myostatin. These cattle are often called double-muscled, and their exaggerated musculature is all the more impressive because an absence of myostatin also interferes with fat deposition, giving the animals a lean, sculpted appearance.

The first myostatin-blocking drugs to have been developed are antibodies against myostatin, one of which may soon undergo clinical testing in muscular dystrophy patients. A different approach mimics the cattle mutation by creating a smaller version of myostatin, which lacks the normal molecule's signaling ability while retaining the structures that dock near satellite cells. This smaller protein, or peptide, essentially caps those docking locations, preventing myostatin from attaching to them. Injecting the peptide into mice produces skeletal muscle hypertrophy, and my colleagues and I will be attempting to create the same effect in our dog models by transferring a synthetic gene for the peptide.

Myostatin-blocking therapies also have obvious appeal to healthy people seeking rapid muscle growth. Although systemic drugs cannot target specific muscles, as gene transfer can, drugs have the benefit of easy delivery, and they can immediately be discontinued if a problem arises. On the other hand, such drugs would be relatively easy for sport regulatory agencies to detect with a blood test.

But what if athletes were to use a gene therapy approach similar to our AAV-IGF-I strategy? The product of the gene would be found just in the muscle, not in the blood or urine, and would be identical to its natural counterpart. Only a muscle biopsy could test for the presence of a particular synthetic gene or of a vector. But in the case of AAV, many people may be naturally infected with this harmless virus, so the test would not be conclusive for doping. Moreover, because most athletes would be unwilling to undergo an invasive biopsy before a competition, this type of genetic enhancement would remain virtually invisible.

And what of the safety of rapidly increasing muscle mass by 20 to 40 percent? Could an athlete sporting genetically inflated musculature exert enough force to snap his or her own bones or tendons? Probably not. We worry more about building muscle in

elderly patients with bones weakened by osteoporosis. In a healthy young person, muscle growth occurring over weeks or months would give supporting skeletal elements time to grow to meet their new demands.

This safety question, however, is just one of the many that need further study in animals before these treatments can even be considered for mere enhancement of healthy humans. Nevertheless, with gene therapy poised to finally become a viable medical treatment, gene doping cannot be far behind, and overall muscle enlargement is but one way that it could be used. In sports such as sprinting, tweaking genes to convert muscle fibers to the fast type might also be desirable. For a marathoner, boosting endurance might be paramount.

Muscle is most likely to be the first tissue subject to genetic enhancement, but others could eventually follow. For example, endurance is also affected by the amount of oxygen reaching muscles. Erythropoietin is a naturally occurring protein that spurs development of oxygen-carrying red blood cells. Its synthetic form, a drug called Epoietin, or simply EPO, was developed to treat anemia but has been widely abused by athletes--most publicly by cyclists in the 1998 Tour de France. An entire team was excluded from that race when their EPO use was uncovered, yet EPO abuse in sports continues.

Gene transfer to raise erythropoietin production has already been tried in animals, with results that illustrate the potential dangers of prematurely attempting such enhancements in humans. In 1997 and 1998 scientists tried transferring synthetic erythropoietin genes into monkeys and baboons. In both experiments, the animals' red blood cell counts nearly doubled within 10 weeks, producing blood so thick that it had to be regularly diluted to keep their hearts from failing.

The technology necessary to abuse gene transfer is certainly not yet within reach of the average athlete. Still, officials in the athletic community fear that just as technically skilled individuals have turned to the manufacture and sale of so-called designer steroids, someday soon a market in genetic enhancement may emerge. Policing such abuse will be much harder than monitoring drug use, because detection will be difficult.

It is also likely, however, that in the decades to come, some of these gene therapies will be proved safe and will become available to the general population. If the time does arrive when genetic enhancement is widely used to improve quality of life, society's ethical stance on manipulating our genes will probably be much different than it is today. Sports authorities already acknowledge that muscle-regenerating therapies may be useful in helping athletes to recover from injuries.

So will we one day be engineering superathletes or simply bettering the health of the entire population with gene transfer? Even in its infancy, this technology clearly has tremendous potential to change both sports and our society. The ethical issues surrounding genetic enhancement are many and complex. But for once, we have time to discuss and debate them before the ability to use this power is upon us.

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