Gene identified that produces benefits of steroids, without the detrimental side effects

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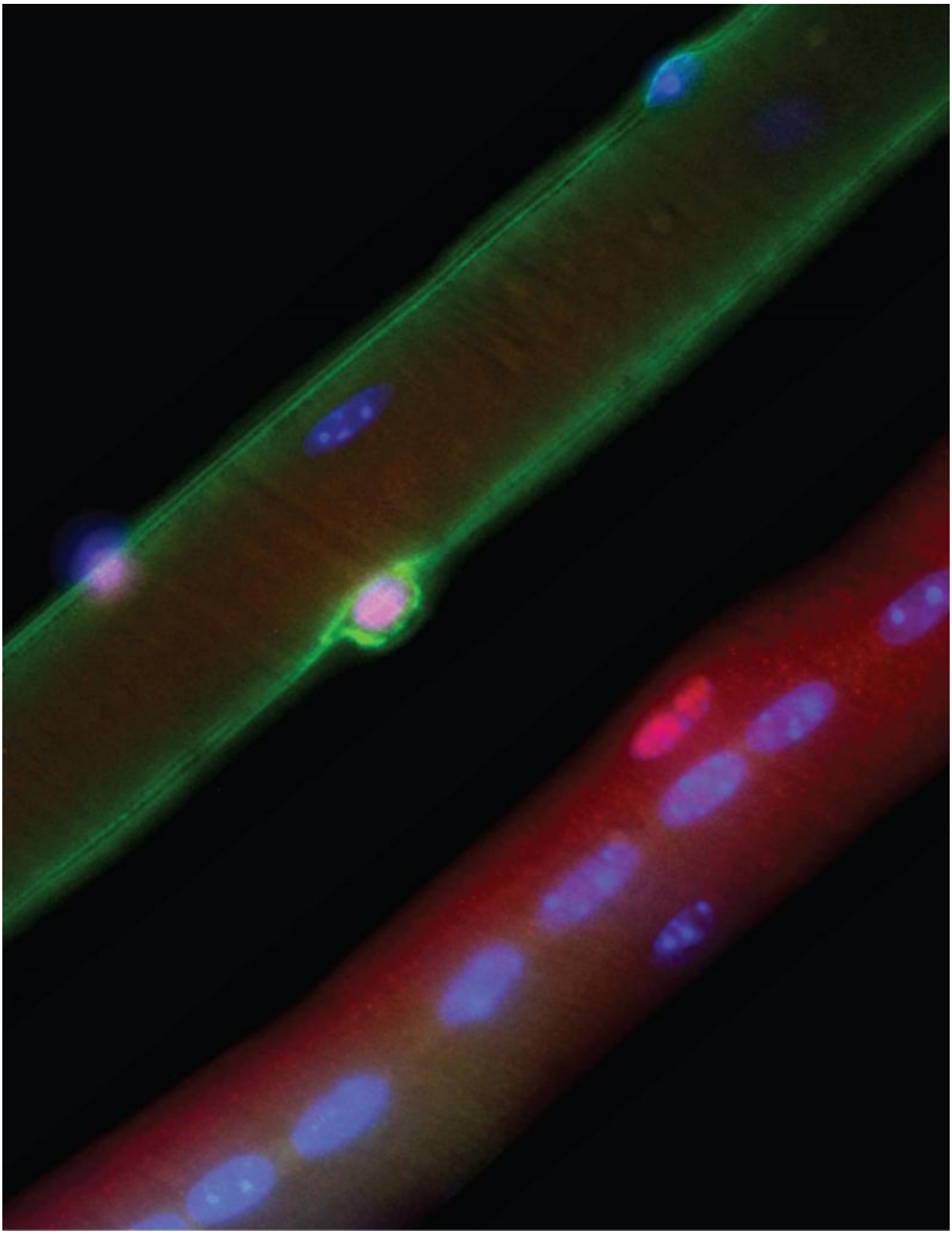

A normal mouse muscle fiber (top left) is contrasted with a muscle fiber from a

mouse model of Duchenne muscular dystrophy (bottom right). In normal mice, stem cells (pink) express dystrophin (green) and are able to easily generate new muscle fibers, but in the disease model, there is no dystrophin and the stem cells lose their sense of direction and have trouble generating new muscle fibers. Reproduced with permission of Will Wang. Credit: Will Wang

Scientists have revealed that glucocorticoids, a class of steroid hormones that are commonly prescribed as drugs, enhance muscle endurance and alleviate muscular dystrophy through activation of the gene KLF15. Critically, this pathway is not involved in muscle wasting or the other major detrimental effects of prolonged steroid use. The discovery, published in *The Proceedings of the National Academy of Sciences*, could lead to the development of new medications that improve muscle function without the negative consequences caused by long-term steroid exposure. This advance is especially important for progressive muscle wasting diseases like Duchenne's muscular dystrophy (DMD).

Glucocorticoids have long been known to boost athletic performance and have been used to treat DMD since the 1980's. However, the way they produce these benefits has remained unknown. Excessive use of glucocorticoids results in muscle wasting (atrophy), bone fragility, cataracts, high blood pressure, fluid retention, and behavioral abnormalities, thereby restricting the viability of these drugs as a long-term treatment option. Therefore, finding ways to harness the positive properties of glucocorticoids while sparing their myriad side effects is an area of intense scientific and clinical interest.

"This study sheds light on the 25-year mystery of precisely how glucocorticoids exert their beneficial effects in DMD," says first author Alexander Morrison-Nozik, PhD, who conducted the research while he was a postdoctoral fellow at Case Western Reserve University. "It was

exciting to discover that KLF15 is both necessary and sufficient to mediate the performance enhancing properties of glucocorticoids, while being completely uninvolved in muscle wasting."

The scientists honed in on KLF15 because the gene is known to be robustly and directly activated by glucocorticoids, and it is involved in key aspects of muscle cell metabolism that are integral to exercise performance.

To test the gene's role in steroid-induced muscle endurance, the researchers first experimentally removed KLF15 from healthy mice. While normal mice could run 50% longer on a treadmill when given a single dose of glucocorticoid drugs, mice deficient in KLF15 did not respond to the endurance enhancing effects of glucocorticoids. However, the KLF15-deficient animals still experienced muscle atrophy after high steroid exposure in a manner equivalent to that experienced by the control mice.

Next, the scientists tested whether boosting KLF15 in skeletal muscle independent of receiving glucocorticoids was sufficient to mimic the performance advantage seen with the drugs. The researchers genetically engineered mice to express KLF15 in skeletal muscle at levels five to six times higher than normal—a degree of KLF15 augmentation that mirrored the increase seen with glucocorticoids. Without administration of any exogenous steroids, these animals had significantly improved endurance exercise capacity and no evidence of muscular atrophy. Thus, KLF15 appears to regulate the important beneficial properties of glucocorticoids, without causing any detrimental side effects.

Applying these findings to disease, the researchers discovered that patients with DMD have dramatically reduced levels of KLF15 in their skeletal muscle. What's more, in a mouse model of DMD, animals with additional depletion of KLF15 had more severe disease symptoms and

were unresponsive to glucocorticoid therapy. This finding led the scientists to conclude that KLF15 is required for the beneficial effects of glucocorticoids in DMD. Furthermore, upregulating KLF15 in the muscles of DMD mice improved strength and endurance, independent of glucocorticoids.

"We now believe DMD is characterized by KLF15 deficiency, and the full therapeutic effect of glucocorticoids requires KLF15," says senior author Saptarsi Haldar, MD, an associate investigator at the Gladstone Institutes and associate professor of medicine at the University of California, San Francisco (UCSF). "Since genetic engineering experiments show that increased KLF15 levels in muscle can improve DMD, the next question is whether we can achieve these same effects by pharmacologically manipulating KLF15 directly, independent of glucocorticoids." Dr. Haldar began the research while he was an associate professor at Case Western Reserve University.

The scientists believe KLF15 achieves its performance-boosting properties by turning on genes involved in the metabolism of amino acids and fatty acids, key nutrients that muscle cells use to fuel their contraction. The researchers are now screening for drugs that will selectively activate KLF15, without going through the glucocorticoid receptor.

More information: Glucocorticoids enhance muscle endurance and ameliorate Duchenne muscular dystrophy through a defined metabolic program, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1512968112

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