

RESEARCH NEWS

Contractile Function

Trouble in store for muscle fibers

Ben Short¹ 

JGP study (Han et al. <https://doi.org/10.1085/jgp.202413729>) reveals that glycerol storage increases the titin-based stiffness of muscle fibers, suggesting that this commonly used method should be avoided by researchers interested in the passive properties of muscle.

Researchers interested in studying muscle physiology and the pathological mechanisms of muscle diseases often have to store precious tissue samples, from either human biopsies or animal models, for extended periods. One common method is to store skinned muscle fibers at -20°C in a solution containing $\sim 50\%$ glycerol, which is thought to stabilize sarcomeric proteins and preserve their contractile function. In this issue of *JGP*, however, Han et al. report that glycerol storage alters the passive stiffness of muscle by modifying the elasticity of the sarcomeric protein titin, indicating that researchers interested in this aspect of muscle physiology should use alternative methods to store their samples (1).

The assumption that glycerol storage has no effect on the contractile properties of muscle is based on just a handful of studies published in the 1940s and 50s (2, 3, 4). Moreover, these studies did not examine whether glycerol storage affected the passive stiffness of muscle fibers, a property that largely depends on the giant filamentous protein titin and its ability to act as a spring that resists the stretching of relaxed muscle (5).

“A few years ago, we became suspicious that glycerol storage impacts the passive stiffness of muscle fibers,” says Henk Granzier, whose group at the University of Arizona studies titin-based stiffness and how it is altered in disease. “We then

stopped using this storage method, but we thought it would be good to really establish the degree to which glycerol storage affects the passive properties of muscle.”

Granzier and colleagues, in particular first author Seong-Won Han, carefully compared the properties of skeletal muscle fibers stored either with or without glycerol (1). While glycerol storage had no effect on the fibers’ contractile properties, it consistently elevated their passive stiffness. This effect could be observed after just 24 h of storage, and after 4 days in glycerol-containing solutions, the passive stress of fibers was increased by over 50%. Cardiomyocytes stored in glycerol also displayed elevated passive stress, indicating that glycerol storage increases the stiffness of both skeletal and cardiac muscle.

Han et al. noticed that the glycerol-induced increases in stiffness were particularly evident in sarcomeres greater than $\sim 2.8\ \mu\text{m}$ in length, a size at which titin’s spring-like function is largely dependent on the extensibility of its PEVK region (6). This domain, which is rich in proline, glutamate, valine, and lysine residues, adopts a flexible, random coil conformation. Though this region can interact with muscle thin filaments, Han et al. found that glycerol storage still increased the passive stress of fibers that had been treated with the actin-severing protein gelsolin, indicating that the mechanism underlying the increased



Seong-Won Han and Henk Granzier.

stiffness does not involve thin filaments and is, instead, intrinsic to titin.

One possibility is that glycerol reacts with and cross-links some of the many lysine residues in titin’s PEVK region, reducing the domain’s extensibility. To test this idea, the researchers treated skeletal muscle fibers with methylglyoxal, a reactive carbonyl and glycation agent known to cross-link lysine residues. Methylglyoxal increased the stiffness of fibers stored in normal, glycerol-free, relaxing solution, but, notably, it had no additional effect on the stiffness of fibers previously stored in glycerol.

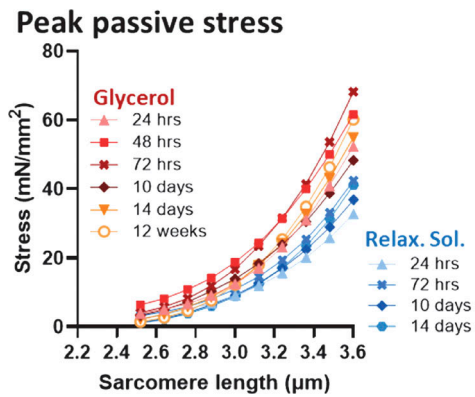
Glycerol may therefore increase the passive stiffness of muscle by cross-linking lysine residues in titin’s PEVK region, thereby inhibiting the protein’s ability to extend when sarcomeres are stretched. “If you want to study passive stiffness, you

¹Science Writer, Rockefeller University Press, New York, NY, USA.

Correspondence to Ben Short: bshort@rockefeller.edu

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Han et al. found that muscle fibers stored in glycerol show increased passive stress, or stiffness, likely due to the cross-linking of lysine residues in titin's PEVK region that limit the sarcomeric protein's extensibility. Researchers interested in the passive properties of skeletal and cardiac muscle should therefore avoid glycerol storage of precious tissue samples and instead use rapid freezing followed by storage at -80°C .

shouldn't use glycerol storage," Granzier and storing them at -80°C has no effect on stiffness, so it may be better to use this method to store samples instead."

Han et al.'s findings may also have important implications for human disease. Methylglyoxal levels are known to be elevated in metabolic disorders, including diabetes and obesity, raising the intriguing possibility that cardiac and skeletal muscle complications associated with these diseases may arise, in part, from an increase in passive stiffness induced by the glycation of titin's PEVK domain.

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