Oleic acid supplementation up-regulates transcript expression of oxidative metabolic genes in murine isolated muscle fiber

Yusuke Komiya, Shugo Iseki, Yuto Adachi, Keizo Arihara

Department of Animal Science, School of Veterinary Medicine, Kitasato University, Towada, Japan

Introduction: Two major muscle fiber types exist in mammalian skeletal muscle: type I (slow-twitch oxidative, red muscle) and type II fibers (fast-twitch glycolytic, white muscle). Type I fibers contain more mitochondria, possess a high oxidative capacity, and are resistant to fatigue whereas type II muscle fibers show high rates of glycolytic metabolism and fatigue. Myotubes, differentiated from myogenic cell lines such as L6 and C2C12 or cultures of muscle satellite cells isolated from skeletal muscles of animals, are often used as in vitro models of skeletal muscle fibers however we have reported methods of muscle fiber isolation and culture as a newly in vitro model [1, 2]. Oleic acid, abundant in lard and beef tallow, is a n-9 mono-unsaturated fatty acid. Recently, we found that oleic acid increases the transcript expression of type I fiber marker and genes related lipid metabolism in myotube differentiated from C2C12 myoblast [3]. The present study was aimed to investigate the effects of oleic acid supplementation on muscle fiber type in isolated muscle fibers in vitro.

Materials and methods: The mature muscle fibers were isolated from the flexor digitorum brevis muscle of C57BL/6J mice (8 weeks old, male). Harvested muscle tissues were treated with collagenase solution containing 0.2% collagenase and 10% fetal bovine serum in physiological rodent saline. The isolated fibers were cultured in 100 μM oleic acid supplemented 30% FBS-DMEM for 6 hours, and then total RNA was extracted. The mRNA expression levels of following genes were analyzed by real-time RT-qPCR: type I fiber marker (MyHC-I), lipid metabolism (PDK4, CPT1β, ANGPTL4, CD36), and mitochondria-related factor (PGC1α).

Results: We found that the transcript levels of PDK4, CPT1β, ANGPTL4, and CD36 were significantly upregulated in oleic acid supplemented group. However, the expression of MyHC-I and PGC1α did not increase significantly. This result was not consistent with our previous report which oleic acid increases the transcript expression of both MyHC-I and lipid metabolism genes (PDK4, CPT1β, ANGPTL4, and CD36) in myotube.

Conclusion: The central finding of this study was that oleic acid supplementation increased transcript levels of lipid metabolism genes in isolated muscle fibers however type I fiber marker did not change. We hypothesize that there is a difference in the mechanism of oleic acid-induced type I muscle fiber formation between mature fibers and myotubes. Therefore, further studies are required to clarify the detailed mechanisms underlying the effects of oleic acid on skeletal muscle fiber type.

References:

1. Komiya Y, Anderson JE, Akahoshi M, Nakamura M, Tatsumi R, Ikeuchi Y, Mizunoya W. 2015. Protocol for rat single muscle-fiber isolation and culture. Anal. Biochem. 482: 22-24.
2. Komiya Y, Anderson JE, Akahoshi M, Nakamura M, Tatsumi R, Ikeuchi Y, Mizunoya W. 2015. Data in support of protocol for rat single muscle-fiber isolation and culture. Data in Brief 4: 7-10.
3. Watanabe N, Komiya Y, Sato Y, Watanabe Y, Suzuki T, Arihara K. 2020. Oleic acid up-regulates myosin heavy chain (MyHC) 1 expression and increases mitochondrial mass and maximum respiration in C2C12 myoblasts. Biochem. Biophys. Res. Commun. 525(2): 406-411.