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Fiber Type and Subcellular-Specific Analysis of Lipid Droplet Content in Skeletal Muscle

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Abstract

Skeletal muscle lipid infiltration, known as myosteatosis, increases with obesity and ageing. Myosteatosis has also recently been discovered as a negative prognostic factor for several other disorders such as cardiovascular disease and cancer. Excessive lipid infiltration decreases muscle mass and strength. It also results in lipotoxicity and insulin resistance depending on total intramyocellular lipid content, lipid droplet (LD) morphology, and subcellular distribution. Fiber type (oxidative vs glycolytic) is also important, since oxidative fibers have a greater capacity to utilize lipids. Because of their crucial implications in pathophysiology, in-depth studies on LD dynamics and function in a fiber type-specific manner are warranted. Herein, a complete protocol is presented for the quantification of intramyocellular lipid content and analysis of LD morphology and subcellular distribution in a fiber type-specific manner. To this end, serial muscle cryosections were stained with the fluorescent dye Bodipy and antibodies against myosin heavy chain isoforms. This protocol enables the simultaneous processing of different muscles, saving time and avoiding possible artifacts and, thanks to a personalized macro created in Fiji, the automatization of LD analysis is also possible. © 2022 JoVE Journal of Visualized Experiments.

Index Keywords

fat droplet, lipid, myosin heavy chain; chemistry, human, insulin resistance, skeletal muscle, ultrastructure; Humans, Insulin Resistance, Lipid Droplets, Lipids, Muscle, Skeletal, Myosin Heavy Chains

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