The effects of oleic acid on matured muscle fibers isolated from mice: fiber type-specific analysis

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Objectives: Skeletal muscle fibers are composed of four different fiber types in mammals (type 1, 2A, 2X, and 2B), and type 1 fibers are rich in mitochondria and myoglobin, have a high oxidative capacity, and are resistant to fatigue. The composition of muscle fi- ber type is thought to affect the color, pH, water-holding capacity, tenderness, and nutritional value of meat [1, 2], and we previ- ously reported a significant positive correlation between the composition of type 1 fibers and umami taste of beef samples in taste sensor analysis [3]. The final goal of our study is to produce livestock whose muscle is rich in type 1 fibers by feeding control, which can contribute to improving meat quality. Our previous study has demonstrated that treatment of myotubes differentiated from C2C12 myoblast with oleic acid increased mRNA expression of type 1 fiber marker (Myh7) and factors relating to lipid me- tabolism and mitochondria [4]. Myotubes are often used as representative in vitro models of muscle; however, we have reported the difference in contractile properties between myotubes and matured muscle fibers isolated from muscle tissue [5, 6], and we then hypothesized that the response to bioactive substances is different in the two muscle cells. Therefore, the present study aimed to clarify the effects of oleic acid on the muscle properties of matured muscle fibers using muscle fibers isolated from murine muscle. **Materials and Methods:** The flexor digitorum brevis muscle (FDB), soleus muscle, and extensor digitorum longus muscle (EDL) were harvested from male C57BL/6J mice, GFP-Myh7 mice, and Kusabira Orange-Myh1 mice, respectively. Harvested muscle tis- sues were incubated in 0.5% collagenase solution in DMEM and separated into single fibers. Under a stereoscopic microscope,type 1 fibers with GFP fluorescence and type 2A fibers without GFP were collected from soleus muscles, and type 2X fibers with RFP fluorescence and type 2B fibers without RFP were collected from EDL muscles. The isolated muscle fibers were incubated in 30% FBS-DMEM with 100 μM oleic acid for 6 hours, and total RNA was extracted to analyze mRNA expression by RT-PCR.

Results and Discussion: Supplementation of oleic acid increased the expression levels of Pdk4, Cpt1b, Angptl4, and Cd36 in ma- tured muscle fibers isolated from FDB. Thus, oleic acid promotes lipid metabolism in matured muscle fibers too. In contrast, there was no change in the mRNA expression level of Myh7, a marker of type1 fiber, which was not consistent with the result of our pre- vious study using myotubes [4]. Matured fibers, used in this study, isolated from FDB muscle express only type 2A or type 2X iso- forms. Therefore, it is unclear whether supplementation of oleic acid commonly affects all muscle fiber types of matured fibers. To solve this problem, we isolated muscle fibers from GFP-Myh7 and Kusabira orange-Myh1 transgenic mice for fiber type-specific analysis in matured muscle fibers. Data analysis is in progress.

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