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Oleic acid up-regulates mRNA expression of both genes related fat utilization and accumulation and a part of them via PPARd activation in C2C12 myoblasts (#217)

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Introduction

Metabolic characteristics of skeletal muscle are closely related to meat quality. For example, a higher composition of glycolytic fibers in pork meat is positively correlated with meat lightness and drip loss. In sensory evaluation studies, the ratio of glycolytic to oxidative fiber is negatively correlated with juiciness and flavor of Berkshire pigs. Skeletal muscle metabolism is regulated by several transcription factors, one of the most important is peroxisome proliferator-activated receptor (PPAR)d. Activation of PPARd promotes oxidative metabolism and mitochondrial biogenesis in skeletal muscle. Several unsaturated fatty acids have the PPARd agonistic activity. Recently, we found that olive oil (rich in unsaturated fatty acid)-fed mice increased mRNA expression of enzymes related fat metabolism and accumulated intramyocellular lipid droplets in skeletal muscle. Then, we hypothesized that intake of olive oil causes promoting both fat utilization and accumulation via PPARd activation by binding PUFA derived from olive oil in skeletal muscle.

Methods

Experiment 1: PPARd agonistic activity of various fatty acids was analyzed with cell-free co-activator binding assay. We used 50 mM fatty acids (palmitic, linoleic and oleic acid).

Experiment 2: To elucidate whether oleic acid (abundant in olive oil and having PPARd agonistic activity) promotes both fat utilization and accumulation via PPARd activation in skeletal muscle, we analyzed the mRNA expression of genes related them in PPARd -knockdown cultures with oleic acid. Firstly, we create PPARd -knockdown C2C12 myoblast by siRNA lipofection. Cultures were transfected with 20 nM siRNAs (Stealths 604, 1075 and 1234 for PPARd) for 72-hour. Then, PPARd expression was quantified by real-time RT-qPCR and western blotting. Next, 100 mM oleic acid was supplemented in PPARd-knockdown C2C12 culture medium. After 5-hour, the mRNA expression of genes related fat utilization and accumulation was analyzed by real-time RT-qPCR.

Results

In experiment 1, oleic acid had higher agonistic activity to PPARd compared with other fatty acids (Fig. 1).

In experiment 2, Stealth 1075 showed significant decrease of PPARd expression in both transcript and protein level (Fig. 2), then we used Stelath 1075 in experiment of oleic acid supplementation. Supplementation of oleic acid up-regulated mRNA expression of genes related fat utilization (Cpt1b, Angptl4, Pgc1a, Ppara and Cd36) and accumulation (Cd36, Acaca and Acsl1) in normal C2C12. In PPARd -knockdown C2C12, the up-regulation of Cpt1b and Cd36 was suppressed (Fig. 3).

Conclusion

Oleic acid, abundant unsaturated fatty acid in olive oil, up-regulated mRNA expression of both genes related fat anabolism and catabolism in C2C12 myoblast, and a part of up-regulation was induced via PPARd activation.

Notes

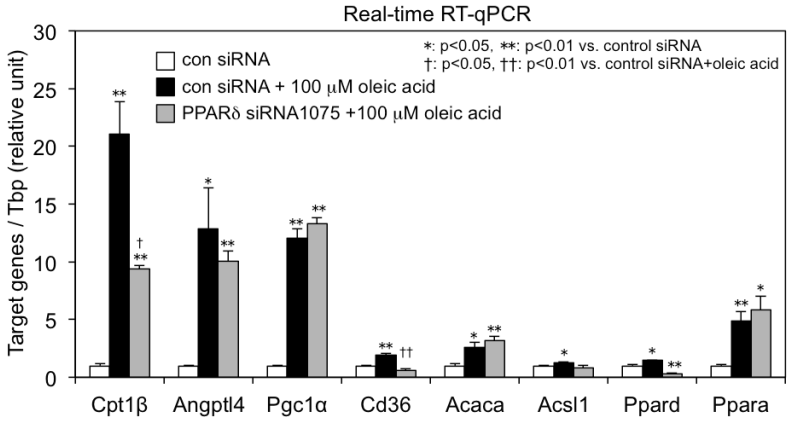

Fig. 3
Messenger RNA expression of genes related fat utilization and accumulation

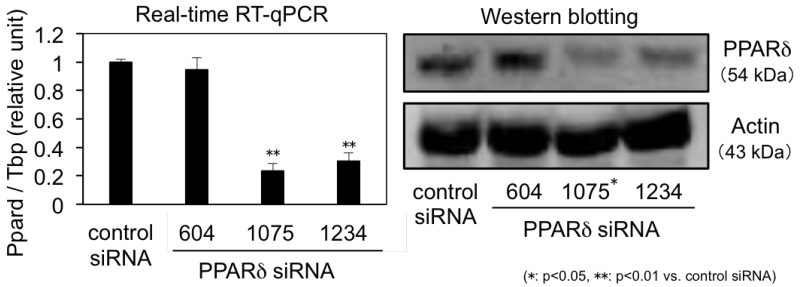

Fig. 2
PPARd expression in C2C12 myoblasts

Notes