学 位 論 文 要 旨

Studies on the role of leucine and mTOR signaling in skeletal muscle physiology
（骨格筋生理学におけるロイシンと mTOR シグナル伝達の役割に関する研究）

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Skeletal muscle is the largest metabolic organ, consuming about 20 % of the body daily expenditure of energy. Although the main function of skeletal muscle is “contraction” to move body and provide stability, it also works as a protein storage, serving as a source of amino acids to be utilized for energy production during food deprivation. Skeletal muscle tissue is composed by two basic types of fibers (slow and fast) with different metabolic, morphologic and contractile characteristics. Since slow twitch fiber (type I fiber) have a high oxidative capacity and prefer fatty acids as substrate, and are resistant to fatigue, it should be benefit to control muscle fiber type by exercise, drugs and nutrients.

Amino acids are taken in supplement form for a natural way to boost athletic performance or improve health since they play many critical roles in the body. Among these supplemental amino acids, branched chain amino acids (BCAAs) are utilize as an energy for skeletal muscle. Among the BCAAs, leucine is well known to regulate protein metabolism through the activation of mechanistic target of rapamycin (mTOR) signaling. Also, previous reports have demonstrated that mTOR signaling is involved in various cellular events, such as regulation of muscle fiber-type, fiber size, mitochondrial biogenesis and differentiation in skeletal muscle.

However, the role of leucine and mTOR signaling in skeletal muscle physiology has not been fully examined. Thus, in this doctoral thesis, to investigate the effects of leucine and mTOR signaling in skeletal muscle physiology including, fiber-type, myogenic differentiation and metabolic alteration, following studies were performed.

In chapter 2, it was investigated whether the acute oral administration of leucine affect muscle fiber-type and mitochondrial biogenesis in skeletal muscle of rats. Although the gene expression of representative glycolytic enzymes (*Hk2* and *Eno3*) were not altered, leucine administration (135 mg/100 g B.W.) up-regulated the expression of slow-fiber related genes (*Myh7, Myl3* and *Tnni1*) and a mitochondrial biogenesis related gene

(*Ppargc1a*) in the soleus and extensor digitorum longus (EDL) muscles compared with the control. In addition, leucine treatment also up-regulated the slow-fiber genes and mitochondrial gene expression in cultured C2C12 myotubes, while rapamycin inhibited the effects of leucine. The hypothesis was accepted that acute administration of leucine alone can up-regulate mitochondrial genes and slow-fiber related gene expression through mTOR signaling. The results suggested the possibility that leucine can alter fiber-type muscle cells and regulate metabolism in skeletal muscle.

In chapter 3, it was examined whether leucyl-tRNA synthetase (Lars), an intracellular sensor for leucine, is involved in the regulation of mTOR signaling and skeletal muscle physiology, including, myogenic differentiation, hypertrophy and energy metabolism. By using small interfering (si)-RNA, it was shown that knockdown of Lars decreased phosphorylated p70 S6 kinase, a crucial downstream target of mTOR signaling, in C2C12 mouse myoblasts. Lars knockdown inhibited the differentiation of C2C12 myoblasts into myotubes, and this was accompanied with decreased level of Insulin-like growth factor 2 (*Igf2*) mRNA expression from the early stages of differentiation. The results suggested the possibility of an association between the mTOR–IGF2 axis and Lars in myogenic differentiation. However, *Lars* knockdown did not affect the hypertrophy of myotubes and energy metabolism (glycolysis and mitochondrial respiration) of myotubes. The results demonstrated for the first time that Lars is essential for the activation of mTOR signaling in skeletal muscle cells and myogenic differentiation through the induction of *Igf2* expression.

In chapter 4, the role of catabolism of BCAAs in C2C12 myoblasts were investigated. The catabolism of BCAAs is mediated by branched chain aminotransferase 2 (BCAT2) and branched chain alpha ketoacid dehydrogenase (BCKDH) in mitochondria of skeletal muscle. Although BCAAs metabolism is basically assumed to be carried out in differentiated myofibers, it remains unclear whether BCAAs metabolic enzymes are expressed in undifferentiated myoblasts, and the physiological significance of BCAAs metabolism in myoblasts. Since the expression of BCAAs metabolic enzymes (*Bcat2, Bckdha* and *Bckdk*) were confirmed in both undifferentiated myoblasts and differentiated myotubes by qRT-PCR, the catabolism of BCAAs were promoted by the BCKDK inhibitor BT2 in C2C12 myoblasts. The activation of BCAAs catabolism by BT2 impaired C2C12 myoblasts proliferation and differentiation. The results suggested the possibility that increased BCAAs catabolism inhibits myoblasts proliferation and differentiation.

The findings should be important in the fields of nutrition, skeletal muscle physiology and metabolic disease, and contributes to the development of novel functional foods and supplements.