# INSULINE LIKE GROWTH FACTOR-1 AND LEUCINE ACTIVATE PIG MYOGENIC SATELLITE CELLS THROUGH MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

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## Introduction

The mammalian target of rapamycin (mTOR) is a large protein with a molecular weight around 290kD. It is involved in sensing of nutritional status in cells and coordinates nutrient status with protein synthesis (Sakamoto et al. 2003). Phosphorytion of eukaryotic initiation factor-4E binding protein 1(4EBP1) by mTOR releases eukaryotic initiation factor-4E (eIF4E) which bind to eIF4G and initiate translation; mTOR directly phosphorylates S6K which then phosphorylates ribosomal protein S6 (Bodine et al., 2001), The phosphorylation of ribosomal protein S6 drives translation of a small family of abundant transcripts that encode primarily ribosomal proteins and components of the translational apparatus. In a word, activation of mTOR up-regulates the translation machinery and promotes protein translation.

It is reported that insulin-like growth factor-1 (IGF-1) and leucine can stimulate skeletal muscle growth, which may link to the enhanced activation of myogenic satellite cells. However, despite numerous studies on the mTOR signalling of skeletal muscle as a whole, few studies were conducted on the alteration of mTOR signalling of myogenic satellite cells due to IGF-1 and leucine treatments. Existing studies on myogenic cells uses C2C12 cells (Park and Chen 2005) and no study was conducted on the primary cultured myogenic satellite cells. The objective of this study is to evaluate mTOR signalling in cultured myogenic satellite cells as affected by IGF-1 and leucine.

## Materials and Methods

Following treatments, Primary myogenic stem cells were obtained from the *longissimus dorsi* muscle of three different 6 month old pigs. Skeletal muscle was minced in PBS buffer (pH 7.4), and then incubated in 1 mg/ml protease (Sigma) at 37°C for 1 hr. The tissue slurry was centrifuged at 1,500 g for 10 min and the pellet was suspended in DMEM medium and centrifuged at 400 g for 5 min. The supernatant was transferred to another tube and centrifuged at 1,500 g for 10 min. The resulting pellet containing myoblasts was used for cell culture (Doumit and Merkel 1992). Cells were cultured in DMEM medium containing 10% heat-inactivated FBS, plus 50 U/ml penicillin and 50 g/ml streptomycin in an incubator under a humidified atmosphere of 5% $CO_2$/95% air at 37°C.

Electrophorisis and immunoblotting cells were lysed in a buffer containing 50 mM HEPES pH 7.4, 137 mM NaCl, 1% NP-40, 10% glycerol, 2 mM $Na_3VO_4$, 100 mM NaF, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2.5 mM EDTA, 1% protease inhibitor cocktail (Sigma). Cell lysates were boiled in Laemmli sample buffer with 5% mercaptoethanol for 5 min. A Hoefer mini-gel system was used for casting gels and running electrophoresis. Following electrophoresis, proteins on the gel were transferred to nitrocellulose membrane in a transfer buffer containing 20 mM Tris-base, 192 mM glycine, 0.1% SDS, and 20% methanol. Membranes were incubated in a blocking solution consisting of 5% nonfat dry milk in TBS/T (0.1% Tween-20, 50 mM Tris-HCl pH 7.6, and 150 mM NaCl) for 1 h followed by overnight incubation at 4 $^oC$ in primary antibodies adequately diluted with TBS/T containing 1% nonfat dry milk. At the end of the primary antibody incubation, the membranes were washed three times for 5 min each with 20 ml of TBS/T. After that, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at appropriate dilution for 1 h in TBS/T with gentle agitation. Following three 10-min washes, membranes were visualized using ECL Western blotting reagents (Amersham Bioscience, Piscataway, NJ) and exposure to film (MR, Kodak, Rochester, NY).

## Results and Discussion

*Activation of mTOR signaling pathway*. After treated with control, IGF-1(250ng/mL), leucine (2mM) and IGF-1 plus leucine for thirty minutes, both IGF-1 and leucine treatment stimulated mTOR signalling (Fig. 1). IGF-1 treatment increased mTOR phosphorylation for 83.82 ± 7.68 % and leucine for 83.42 ± 5.71 %. No synergistic effect of IGF-1 and leucine on mTOR phosphorylation was observed. The phosphorylation of down-stream effector of mTOR, ribosomal protein S6 kinase, was increased for 148.57 ± 6.54 % and 190.95 ± 7.66 % due to IGF-1 and leucince treatment respectively. The phosphorylation of another down-stream effector of

mTOR, 4E-BP1 was also increased due to IGF-1 and leucine treatments (Fig. 1). No synergistic effect of IGF-1 and leucine treatments on mTOR signalling was observed. (Fig. 1).

IGF-1 stimulates skeletal muscle growth mainly through activation of phosphoinositide-3 kinase (PI3K/Akt) signaling pathway (Latres et al. 2005). One of the major targets of PI3K/Akt signaling is mTOR. Accumulating data show the importance of mTOR signaling in the control of skeletal muscle growth. mTOR controls the rate of phosphorylation of key proteins involved in protein synthesis, including 4E-BP1 and ribosomal protein S6 kinase (S6K). Thus, activation of mTOR up-regulates the translational machinery and promotes protein translation. In this study, both IGF-1 and leucine enhanced the phosphorylation of mTOR and its down-stream effectors, S6K and 4E-BP1, showing that mTOR signalling was activated due to IGF-1 and leucine treatments in myogenic satellite cells. The up-regulation of mTOR signalling should be responsible for the enhanced protein synthesis in myogenic cells stimulated by IGF-1 and leucine. In our study, no synergistic effect of IGF-1 and leucine on mTOR signalling was observed. The possible reason could be due to the inhibition of up-stream IGF-1 signalling by AMP-activated protein kinase (AMPK). Activation of AMPK improves the sensitivity of IGF-1 signalling through phosphorylation of insulin receptor substrate-1 (IRS-1) at Ser789, which enhances IRS-1 mediated signalling (Jakobsen et al. 2001). Thus, AMPK activation sensitizes IGF-1 signalling.

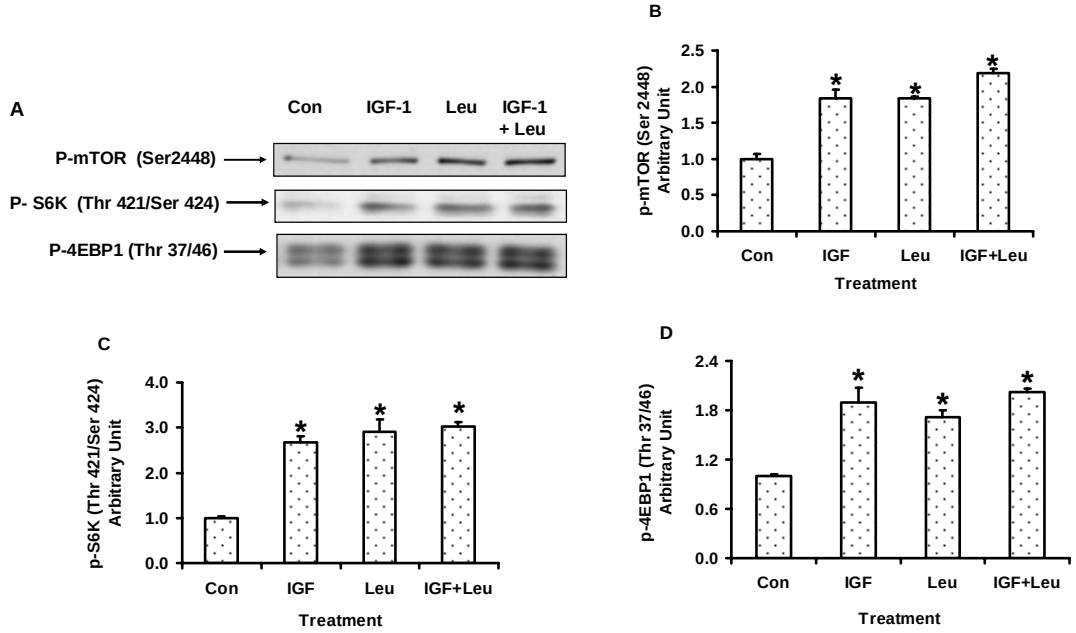


**Fig.1.** Effect of IGF-1 and leucine on mTOR signalling in satellite cells. Cells were incubated in the presence of 250 ng/ml IGF-1 or 2mM leucine. **P* < 0.05 versus Control, n = 3.

## Conclusions

In summary, we demonstrate that IGF-1 and leucine treatments stimulate mTOR signalling in myogenic satellite cells separated from pigs. No synergistic effect of IGF-1 and leucine treatments on mTOR signalling was observed.

## References


1. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol, 2001, 3(11):1014-1019.
2. Doumit ME, Merkel RA. Conditions for isolation and culture of porcine myogenic satellite cells. Tissue Cell, 1992, 24(2):253-262.
3. Jakobsen SN, Hardie DG, Morrice N, Tornqvist HE. 5'-AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. J Biol Chem, 2001, 276(50):46912-46916.
4. Latres E, Amini AR, Amini AA, Griffiths J, Martin FJ, Lin HC, Yancopoulos GD, Glass DJ. Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase /Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway. J Biol Chem, 2005, 280(4):2737-2744.
5. Park IH, Chen J. Mammalian target of rapamycin (mTOR) signaling is required for a late-stage fusion process during skeletal myotube maturation. J Biol Chem, 2005, 280(36):32009-32017.
6. Sakamoto K, Aschenbach WG, Hirshman MF, Goodyear LJ. Akt signaling in skeletal muscle:regulation by exercise and passive stretch. Am J Physiol Endocrinol Metab, 2003, 285(5):E1081-1088.