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Does Insulin and Leucine Stimulate Muscle Protein Synthesis?

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Summary and Implications

Improvement of protein synthesis in muscle will greatly enhance the production of lean pork. This improvement can be traced to changes at the cellular level. The object of this study was to identify the effects of insulin and the branched chain amino acid, leucine on the extent and rate that messenger RNA (mRNA) is translated into protein. Porcine satellite cells were isolated from a 30 lb pig and cultured. The cultured cells were treated with varying levels of insulin and leucine. Increasing levels of insulin and leucine caused an increase in ribosomes, the organelles responsible for synthesis, only after leucine was present in the media in adequate concentrations. With increasing levels of insulin there was an increase in the recruitment of ribosomes into polyribosomes for mRNA translation. However, increasing leucine levels had no effect on polyribosome percentage. In conclusion, insulin stimulates translation of mRNA by increasing both ability and rate. However, adequate levels of amino acid must be available for the stimulation to occur. Increased levels of branched chain amino acid do not create a synergistic effect with insulin to increase polyribosomes for protein synthesis.

Introduction

Pork products are a valuable product of American agriculture and consumers demand lean pork products. Therefore, producers must continue to improve the efficiency of lean meat production. Improved efficiency of lean pork production can be directly related to the rate and efficiency of skeletal muscle protein accretion. Understanding the cellular mechanisms regulating protein accretion in skeletal muscle is an important factor for improving the efficiency of lean-meat production.

Skeletal muscle is the single most valuable component in the pig and is the largest contributor to protein accretion within the animal. Postnatal muscle growth in meat animals is highly dependent on protein accretion. Skeletal muscle growth via protein accretion is highly dependent on the rate and efficiency of both transcription of DNA into messenger RNA (mRNA) and its subsequent translation by ribosomal RNA (rRNA) into protein. This study focused on the translation of mRNA into protein. This was accomplished by measuring the total number of ribosomes and their activity in translating mRNA into protein. The activity was determined by measuring the percent of ribosomes in the polyribosome form.

Insulin and branched chain amino acids — for example, leucine — have been shown to have significant roles in stimulation of muscle protein synthesis. The purpose of this study was to determine if insulin or leucine altered the quantity of ribosomes or their rate of recruitment for protein synthesis. Satellite cells were used in this study because they could be grown in cell culture and more closely represent what would be observed in vivo.

Methods

Primary satellite cells were collected from the *semimembranosus* and *semitendinosus* muscles of a 6-week-old female pig weighing 30 lb. After the gilt was sacrificed, muscles were removed from both the left and right side of the animal. Muscles were cut into 1-cm^3 cubes and satellite cells were liberated from the tissue using an (enzymatic protease) solution. After cells were released from connective tissue, they were separated from the cellular debris by centrifugation. Cells were plated on 75-mm culture flasks Minimum Essential Media-alpha (α-MEM) with 10% FBS and allowed to proliferate. Once they reached 80 percent surface density, they were removed from the plate and frozen (-80° C).

Cells were removed from the freezer, allowed to thaw and plated

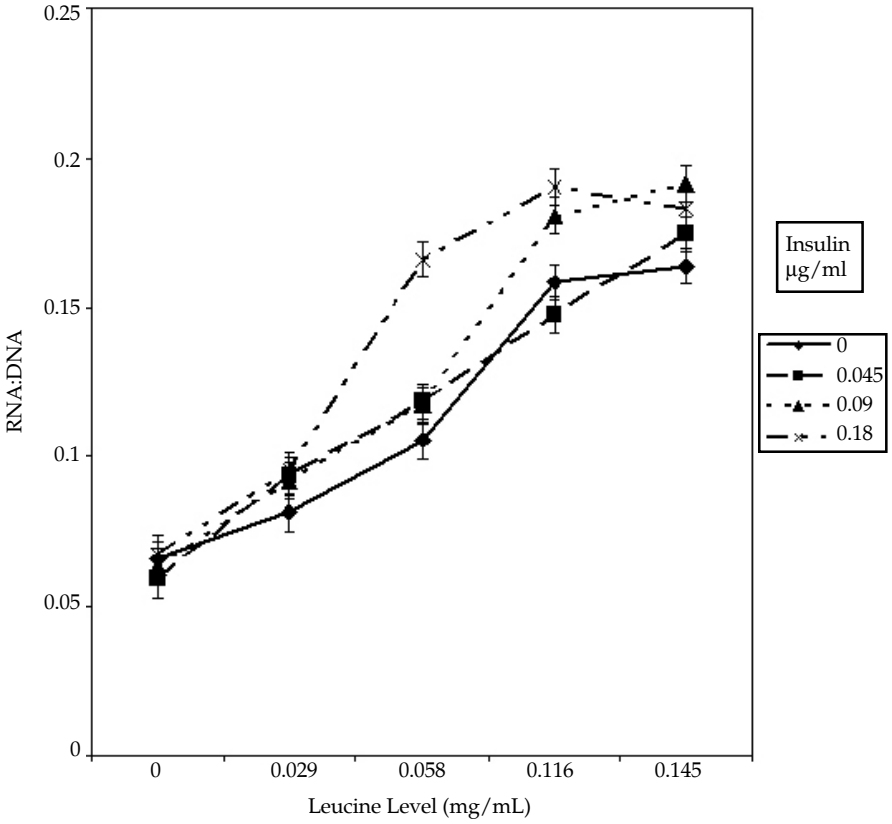

Figure 1. RNA/DNA in proliferating porcine satellite cells treated with serum-free media containing combined levels of insulin and leucine. (n= 6: SEM = .006)

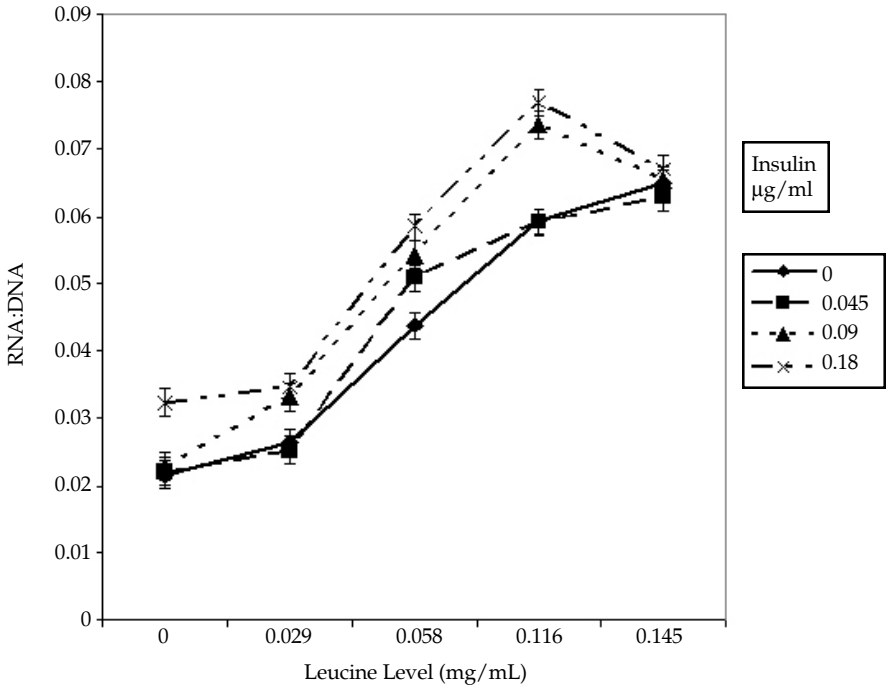

Figure 2. RNA/DNA in fused porcine satellite cells 48 hours after addition of fusion media, and treated with serum-free media containing combined levels of insulin and leucine. (n= 6: SEM= .002))

on 6-well plates with 15 ml of culture media and allowed to proliferate until they reached a 80% confluence. Cells at this stage of development were identified as proliferating satellite cells (PSC) and used as one of the cell types. Additionally, replicate plates containing PSC media were replaced with a fusion media consisting of α-MEM plus two percent horse serum to stimulate cell fusion to form myotubes. Previous research indicated that 48 hours after media replacement was the optimum time for treatment of myotubes. This cell type will be referred to as fused satellite cells (FSC).

Both porcine derived PSC and FSC were used in the study using a serum free media. The study was designed using five concentrations of insulin (0 ,0.0025, 0.045, 0.09, and 0.18 µg/ml) with 0.09 µg/ml representing post feeding physiological concentrations and four concentrations of leucine, 0, 0.029, 0.058, 0.116 mg/ml in serum-free media. Each treatment combination was represented with three wells per treatment and the entire experiment was replicated twice.

Cells were removed from the plate using a buffer to prevent RNA degradation and protein, DNA and RNA were determined. The polyribosomes were isolated and separated using an ultracentrifuge then quantified using a gradient fractionator. The percentage of ribosomes in the polyribosome form was determined and used as a measure of the rate of translation. Total protein, DNA and RNA were quantified.

The statistical design was a randomized complete block design with each plate serving as blocking criteria. The experiment was replicated and analysis was done using Statistical Analysis Systems (SAS). Mean separation was accomplished using the least square means statement. All dif-

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ferences are reported at P < .05 probably.

Results

Concentrations of RNA and protein were adjusted by cell density by presenting data as a ratio to DNA. Differences in total DNA, and therefore cell densities, were corrected for RNA and protein by presenting the data as a ratio to DNA. No differences were observed in protein and DNA within cell types. In both cell types, RNA concentration increased as the level of leucine increased (Figures 1 and 2). Differences (P<.05) in RNA due to insulin were not observed until the media contained levels of leucine comparable to that normally found in the α-MEM media (0.058 mg/ml). Once the leucine requirement was satisfied, RNA concentrations increased (P < .05) with increasing levels of insulin. In the PSC, the high level of insulin responded with a greater increase at lower leucine levels compared to FSC. The increase in polyribosome percentage with high levels of insulin occurred with leucine concentrations at 0.058 mg/ml in PSC compared to 0.116 mg/ml in FSC. This difference may be due to the leucine requirement being higher for FSC versus PSC or it may be related to the responsiveness of the FSC to insulin. Other studies performed in our lab have shown that the response of FSC to insulin, as well as other hormones, is less than PSC. Another observation in FSC was a decline in RNA levels at the highest insulin and leucine levels. This drop-off in RNA levels may be an aberration of data; it was expected for RNA levels to plateau as they did in PSC.

There was no interaction observed between insulin and

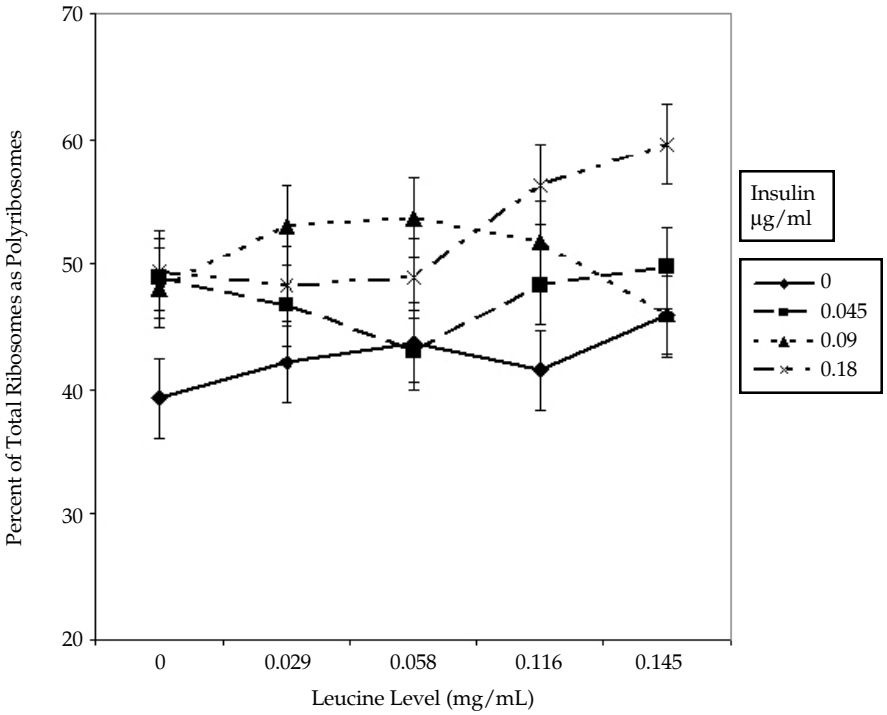

Figure 3. Polyribosome percentages in proliferating satellite cells exposed to serum-free media containing combined levels of insulin and leucine. (n= 6: SEM = 3.2)

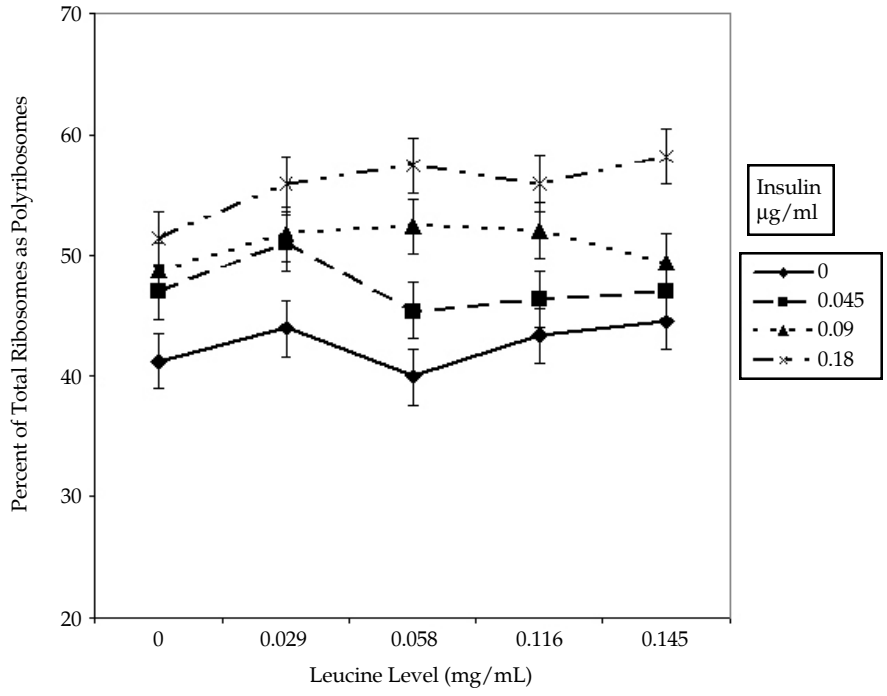

Figure 4. Polyribosome percentages in porcine satellite cells 48 hours after the addition of fusion media, and exposed to serum-free media containing combined levels of insulin and leucine. (n= 6: SEM= 2.29)

leucine with regard to polyribosome percentage within PSC or FSC (Figure 3 and 4). Regardless of leucine levels, polyribosome percentages were higher with increasing levels of insulin for PSC, with main effect estimates of 42.5, 47.4, 50.6, and 52.5 percent for insulin levels 0, 0.045, 0.09, and 0.18 µg/mL treatments, respectively. Similar results were observed in FSC, with insulin effects estimates, regardless of leucine levels, being 42.6, 47.4, 50.9, and 55.7 percent for insulin levels 0, 0.045, 0.09, and 0.18 µg/mL treatments, respectively. In both PSC and FSC increasing levels of leucine had no effect on ribosome recruitment to polyribosomes.

Discussion

Other researchers have observed that both insulin and amino acids have been shown to have significant roles in the stimulation of protein synthesis by increasing the total amount of rRNA; (the machinery responsible for synthesis and the rate of recruitment for protein synthesis in muscle). One group of researchers demonstrated in neonatal pigs that insulin and amino acids increase protein synthesis, but these effects are not additive. Our study demonstrated insulin's powerful effect on protein synthesis by increasing both the number of ribosomes and rate polyribosome formation which functions to synthesize proteins in muscle. Leucine concentrations only impacted the rate of protein synthesis by increasing the amount of rRNA. This response is greater in myoblasts when compared to myotubes. Other studies in our lab have shown fractional protein synthesis rates are higher in the myoblasts compared to myotubes. Only when the amino acids requirements are met can the full anabolic effect of insulin be realized. If essential amino acids are restricted, increased concentrations of insulin will have no anabolic impact on muscle.

The results of this study demonstrate insulin plays a large role in increasing RNA synthesis as well as its activity in the formation of polyribosomes, which are active in both PSC and FSC *in vitro*. This response can be increased if leucine or possibly other branched chain amino acids are supplemented. It was also observed if leucine concentrations decrease below normal media concentrations, the production of RNA, as well as its activity, was significantly decreased. While insulin caused an increase in RNA and its activity, it does not appear to work synergistically with leucine to increase RNA production in either cell type. Leucine only appeared to impact synthesis rates when it was reduced to below baseline levels.

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