Initiation of Protein Biosynthesis in Skeletal Muscles at Feeding

Akademisk avhandling

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The thesis is based on the following papers:

I Iresjö B-M, Svanberg E, Lundholm K
Reevaluation of amino acid stimulation of protein synthesis in murine- and human-derived skeletal muscle cells assessed by independent techniques.
Am J Physiol Endocrinol Metab. 2005 May;288(5):E1028-37.

II Iresjö B-M, Körner U, Larsson B, Henriksson BÅ, Lundholm K.
Appearance of individual amino acid concentrations in arterial blood during steady-state infusions of different amino acid formulations to ICU patients in support of whole-body protein metabolism.
JPEN J Parenter Enteral Nutr. 2006 Jul-Aug;30(4):277-85.

III Iresjö BM, Körner U, Hyltander A, Ljungman D, Lundholm K.
Initiation factors for translation of proteins in the rectus abdominis muscle from patients on overnight standard parenteral nutrition before surgery.
Clin Sci (Lond). 2008 May;114(9):603-10.

IV Iresjö BM, Svensson J, Ohlsson C, Lundholm K.
Liver derived circulating IGF-I is not critical for activation of skeletal muscle protein synthesis following oral feeding.
Submitted 2010.

V Iresjö BM, Lundholm K.
Induction of myosin heavy chain 2A and α-actin synthesis by amino acids in skeletal muscle.
Manuscript.

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Initiation of Protein Biosynthesis in Skeletal Muscles at Feeding

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Abstract:

Background and aim. Artificial nutrition by intravenous feeding has for decades indicated less than optimal support of whole-body protein metabolism and balance during some treatment conditions. Therefore, the present project was aimed to evaluate the role and effects by amino acid provision to skeletal muscle cells in the light of other known important factors as amino acid infusion kinetics, IGF-I and insulin in support of myofibrillar protein synthesis.

Methods. Murine L6 and human rhabdomyosarcoma cells were cultured at standardized conditions in the presence of various amino acid concentrations. Commercially available amino acid formulations were infused by constant rates to patients scheduled for elective surgery and to ICU patients. Transgenic female mice with selective knockout of the IGF-I gene in hepatocytes were used in refeeding experiments to evaluate the role of circulating IGF-I for muscle protein synthesis, which was estimated by the flooding dose technique ([^{14}C]-phenylalanine). Protein factors for translational control of protein synthesis and cell signaling (4E-BPI, eIF4E, p70s6k, mTOR) were estimated in Western blots. Transcripts of muscle IGF-I, IGF-IR, PI3-kinase, AKT, mTOR, acta 1 (α-actin), mhc2A (myosin) and slc38a2/Snat 2 (amino acid transporter) were quantified by qPCR. Plasma amino acids were measured by HPLC.

Results. Incorporation rate of amino acids into muscle proteins gave incorrect results in a variety of experimental conditions. Methods independent of labeled amino acids (ribosome profiles, initiation factor analyses) indicated that essential amino acids activate initiation of protein translation, while non-essential amino acids had no such effects. Insulin at physiologic concentration (100 μM/ml) did not stimulate global muscle protein synthesis, but did so at supraphysiologic concentrations (3mU/ml). Circulating IGF-I was not critical for activation of muscle protein translation, while tissue produced IGF-I and IGF-IR controlled feeding induced protein synthesis, which involved mTOR signaling in skeletal muscles. In general, provision of exogenous amino acids was related to plasma concentrations and probably to steady state levels of amino acids in peripheral tissues of patients. Amino acids caused activation of translation initiation of muscle proteins as demonstrated for myosin heavy chain and α-actin. These effects by amino acids were in part supported by increased transcription and utilization of amino acid transporters as Snat 2 mRNA in muscles. Microarray analysis indicated up-regulation of genes in the mevalonate-pathway following amino acid exposure, important for steroidogenesis and lipid metabolism, which may imply new and additional mechanisms behind anabolic reactions in muscle cells related to nutrition.

Conclusion. Our results re-emphasize that labeled amino acids should be used with great caution for quantification of muscle protein synthesis. Measurements of regulating factors in the control of initiation of muscle protein synthesis represent alternative and convenient applications in estimation of directional changes in protein synthesis during non steady state conditions as demonstrated by overnight preoperative provision of standard TPN to patients. Our results confirm that muscle cells are sensitive to alterations in extracellular concentrations of amino acids, which signal to activate translation of transcripts for myofibrillar proteins. Such dynamics are highly dependent on the presence of membrane transporters of amino acids.

Key words: protein synthesis, translation initiation, amino acids, Snat 2, MHC2A, IGF-I

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