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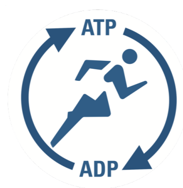

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A translational model of muscle protein synthetic bioactivity in vitro, ex vivo and in vivo

Brian Carson,Robert Davies,Joseph Bass,Catherine Norton,Bijal Patel,Miryam Amigo-Benavent,Sylvia Murphy,Patrick Kiely,Philip Jakeman
University of Limerick

Objective The aim of this research was the development and validation of a translational model for the evaluation of exercise and nutrient stimulated muscle protein synthesis (MPS). To achieve this overall aim, three primary objectives had to be realised: (i) Development of an *in vitro* skeletal muscle cell bioassay to measure muscle growth and MPS; (ii) Development of an *ex vivo* model to evaluate the humoral effect on MPS in response to nutrient feeding and exercise; (iii) Use of a stable isotope technique to evaluate MPS in response to nutrient feeding and exercise *in vivo.*

Methods To develop a novel *in vitro* skeletal muscle cell bioassay to measure muscle growth and MPS, C2C12 myoblasts were proliferated and subsequently differentiated to myotubes over 8 days in DMEM (2% HS). Changes in cell behavior and adhesion properties were monitored by measuring impedance via interdigitated microelectrodes using the xCELLigence system. MPS was measured by puromycin incorporation using the SUnSET technique, intracellular signalling measured by western blot, and myotube thickness by microscopy. To demonstrate the capability to monitor nutrient regulation of muscle growth, media was conditioned with a known potent regulator of MPS (leucine) in a dose response experiment (0.20 - 2.0 mM). To establish the ability of the bioassay to measure the humoral effect of MPS in response to feeding and exercise, media was conditioned by *ex vivo* human serum from fasted, rested, fed (protein and isonitrogenous non-essential amino acid (NEAA) control) and post-exercise conditions. To evaluate MPS in response to nutrient feeding and exercise *in vivo,* acute MPS (5 h) was assessed by measuring stable isotope deuterium oxide (D_2O) incorporation into m. vastus lateralis skeletal muscle following consumption of either a Whey Protein (WP) or an isonitrogenous NEAA control combined with resistance exercise in resistance trained males.

Results *In vitro* experiments observed a dose-response effect with a 32 % increase in cell index and a 27 % increase in cell thickness after 2 h in the presence of 2.0 mM leucine when compared with control myotubes. *Ex vivo* serum following ingestion of NEAA had no effect on protein signalling or MPS whereas WP fed serum significantly increased mTOR, P70S6K and 4E-BP1 phosphorylation ($p<0.01$, $p<0.05$) compared to fasted serum. Furthermore, the effect of WP fed serum on protein signalling and MPS was significantly increased ($p<0.01$, $p<0.05$) compared to NEAA fed serum. *Ex vivo* human serum following resistance exercise was also increased MPS (29 %) and phosphorylation of mTOR (6 %), p70S6K (12 %) and 4EBP1 (7 %), compared with resting serum. These *ex vivo/in vitro* findings translated to the *in vivo* model as myofibrillar fractional synthetic rates (myoFSR) (Basal 0.068±0.002%h^{-1} vs. WP 0.084±0.006 %h^{-1}, p=0.033) and absolute synthetic rates (ASR) (Basal 10.34±1.01 vs. WP 13.18±0.71 g.day^{-1}, p=0.026) were increased from basal levels only when resistance exercise was combined with WP ingestion and not the NEAA control (NEAA MPS 0.072±0.004%h^{-1}, NEAA ASR 10.23±0.80 g.day^{-1}). Thus, ingestion of WP in combination with resistance training augments acute MPS responses in resistance trained young men.

Conclusions We have developed a translational model of muscle protein synthetic bioactivity using *in vitro, ex vivo* and *in vivo* methodologies. We have shown that we can impact MPS *in vitro* using *ex vivo* human serum to condition media, that MPS *in vitro* is differentially regulated by *ex vivo* serum containing bioactive WP compared to a non-bioactive NEAA control, and that this tranlates for resistance exercise combined with WP in humans when MyoFSR is measured using stable isotope

technology. These experiments demonstrate that *ex vivo/in vitro* experiments translate to the *in vivo* model and these methods can be used to inform both exercise and nutrient human interventions.