

**P017** Negative regulation of translation initiation by amino acids in mammary epithelial cells

**Itamar Barash**

*Institute of Animal Science, ARO, The Volcani Canter Bet Dagan. Israel*

In murine mammary epithelial cells cultured under lactogenic conditions, we studied the role of essential amino acids (AA) on protein synthesis via the mTOR pathway. Leu, Ile and Val increased S6K1 phosphorylation compared to that measured in AA-deprived cells. Trp, Phe and Met had no effect. Surprisingly, Lys, His and Thr inhibited S6K1 phosphorylation, with Thr being the most potent inhibitor. In non-deprived cells, there was no observable effect of these AA on S6K1 phosphorylation at concentrations up to five times those in the medium. However, their addition as a mix revealed a synergistic negative effect. Individual supplementation of Lys, His and Thr abrogated mTOR phosphorylation, with no effect on Akt Ser 473—an mTORC2 target. This confirms specific mTORC1 regulation of S6K1 phosphorylation. The individual supplementation of Lys, His and Thr maintained a low level of IRS-1 phosphorylation, which was dose-dependently increased by their combined addition. Thus, in parallel to inhibiting S6K1 activity, these AA may act synergistically to activate an additional kinase, phosphorylating IRS-1 via an S6K1-independent pathway. Under these conditions, cellular protein synthesis decreased by up to 65%, accompanied by a more severe decrease in beta casein synthesis. These findings indicate that both positive and negative signalling from AA to mTOR pathway and modulation of insulin sensitization mediate the rates of synthesis of total and specific proteins in mammary epithelial cells.