

P037 Identification and characterization of serine 357, a new phosphorylation in insulin receptor substrate (IRS)-1
Rizwana S. Wariach, Louise Immel, Miriam Hoene, Cora Weigert, Alexander Beck, Wolfgang Voelter, Erwin.D. Schleicher, Hans-Ulrich Häring, Rainer Lehmann
University Hospital Tübingen, Germany

Background and aims: The function of IRS-1 is modulated by phosphorylation at multiple Ser/Thr residues. The present work was aimed to study the (patho)physiological function and interplay of novel protein kinase C (PKC) Ser/Thr phosphorylation sites in IRS-1. Results: Based on mass spectrometric identification of Ser³⁵⁷ of IRS-1 as a PKC-mediated phosphorylation sites we generated a pSer³⁵⁷ specific antibody. In C2C12 skeletal muscle cells Ser³⁵⁷ is phosphorylated by insulin and phorbol ester stimulation. This **biphasic phosphorylation is mediated, at least partially, by PKC- δ** . Investigating the physiological function of this site in downstream signaling using Ala³⁵⁷ IRS-1 expressing skeletal muscle cells (simulating the unphosphorylated state) we found a more pronounced activation of Akt/PKB. **Additionally, the phosphorylation of Ser³⁵⁷ at the early time points of insulin stimulation (5 and 10 min) is more pronounced if Ser³¹⁸ is in the unphosphorylated state (represented by the Ala³¹⁸ mutation), giving a hint for an interplay of this two PKC phosphorylation sites in IRS-1.** Conclusion: Ser³⁵⁷ is a novel PKC-mediated phosphorylation site of IRS-1, which may exert negative effects on insulin signal transduction.