

P022 Time-course of insulin signal transduction *in vivo*
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We examined the effect of different high-fat diets on the time-course of phosphorylation of insulin signalling pathway intermediates in rat skeletal muscle *in vivo* using experimental conditions similar to those employed to assess insulin action. Using phospho-specific antibodies, components of the metabolic and mitogenic insulin signalling pathways were assessed by immunoblotting, during the initial stages of a physiological elevation in circulating insulin. The phosphorylation profiles of insulin receptor (IR) at Tyr1162/1163, insulin receptor substrate (IRS)-1 at Tyr612, IRS-1 at Tyr895, and protein kinase B (PKB) at Ser473 in control rat muscle revealed that different temporal profiles of phosphorylation are involved in transmission of the insulin signal despite a constant level of insulin stimulation. In muscle from rats fed high n-6 polyunsaturated or saturated fat diets, there was no insulin-stimulated increase in IRS-1 Tyr612 phosphorylation and a temporal difference in PKB Ser473 phosphorylation, despite no difference in IR Tyr1162/1163 phosphorylation, IRS-1 Tyr895 phosphorylation and extracellular signal-regulated kinase phosphorylation. These results demonstrate that under conditions of increased insulin, similar to those used to assess insulin action *in vivo*, chronic high-fat feeding impairs insulin signal transduction related to glucose metabolism at the level of IRS-1 Tyr612 and PKB Ser473 and that these effects are independent of the dietary fat consumed.