# SUPPLEMENT

## INSULIN INDUCES SWELLING-DEPENDENT ACTIVATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR IN RAT LIVER

**Roland Reinehr, Annika Sommerfeld, and Dieter Häussinger**

From the Clinic for Gastroenterology, Hepatology and Infectiology;
Heinrich-Heine-University Düsseldorf; D-40225 Düsseldorf; Germany

Running head: Insulin and EGFR activation

## SUPPLEMENTAL FIGURES

**Supplemental Figure 1: EGF-induced EGFR-phosphorylation in perfused rat liver**

Rat livers were perfused as described in the Methods section with either EGF (50 ng/mL), insulin (35 nmol/L, 5 min) or hypoosmotic medium (225 mosmol/L, 5 min). Liver samples were taken at the time points indicated and phosphorylation of EGFR-$Y^{845}$, $Y^{1045}$ and $Y^{1173}$ was analyzed by use of phospho-specific antibodies. Total EGFR served as loading control. Representative Western blots of three independent perfusion experiments are shown.

EGF induced within 5 min a phosphorylation of EGFR-tyrosine residues $Y^{845}$, $Y^{1045}$ and $Y^{1173}$, whereas insulin or hypoosmolarity led to a phosphorylation of EGFR- $Y^{845}$ and $Y^{1173}$ only (*compare Figure 1 and 4*).

**Supplemental Figure 2: swelling-induced activation of Src family-kinases in perfused rat liver**

Rat livers were perfused as described in the Methods section with either insulin (35 nmol/L) or hypoosmotic medium (225 mosmol/L). Liver samples were taken at the time points indicated and phosphorylation of FAK-$Y^{397}$ and c-Src-$Y^{418}$ was analyzed by use of phospho-specific antibodies. Western blots were then analyzed densitometrically. Phosphorylation level at t=0 min was arbitrarily set to 1. Activating phosphorylation of Yes-$Y^{418}$ and Fyn-$Y^{418}$ was detected by Yes- and Fyn-immunoprecipitation, respectively, and subsequent Western blotting using an anti-phospho-Src familiy-$Y^{418}$ specific antibody. Total FAK, c-Src, Yes and Fyn served as loading controls. Representative Western blots of three independent perfusion experiments are shown.

In line with the literature (15,16), insulin ($^{\#}$, $p<0.05$) and hypoosmolarity (*, $p<0.05$) induced a significant activation of FAK and c-Src, while no activation of either Yes or Fyn occurred under these conditions.

**Supplemental Figure 3: Insulin- and hypoosmolarity do not induced EGFR/Yes- or EGFR/Fyn-association in perfused rat liver**

Rat livers were perfused as described in the Methods section with either insulin (35 nmol/L) or hypoosmolarity (225 mosmol/L) and EGFR was immunoprecipated as described in the Methods section. Samples were then analyzed for EGFR/Yes- or EGFR/Fyn-association by detection of Yes or Fyn, respectively. Total EGFR served as a loading control.

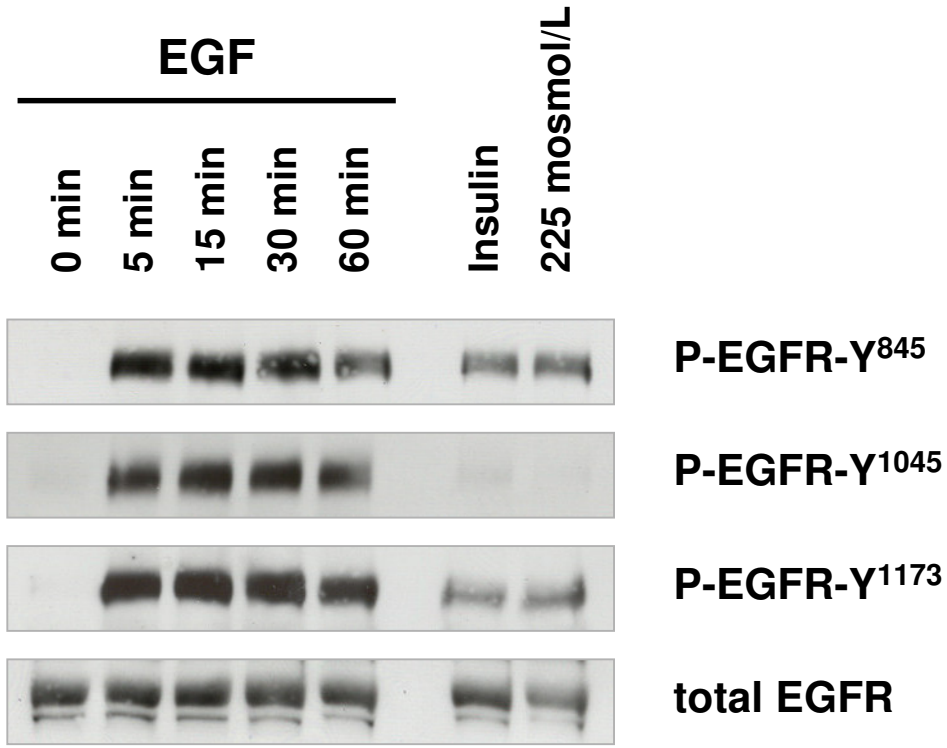
Both, insulin and hypoosmolarity within 60 min did not induce an EGFR/Yes- or EGFR/Fyn-association, respectively.

**Supplemental Figure 4: Insulin-induced IRS-1-phosphorylation in perfused rat liver**

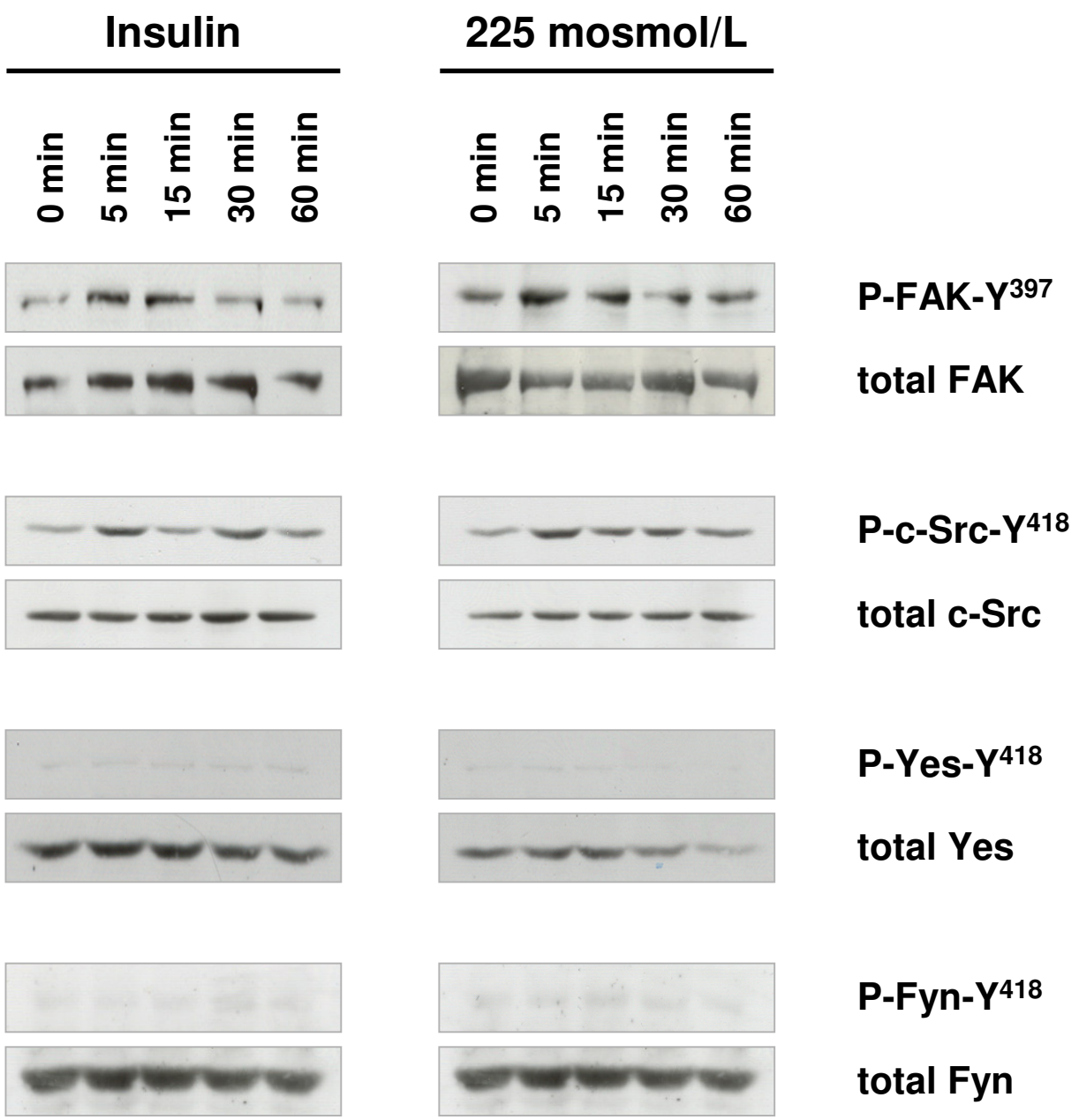
Rat livers were perfused as described in the Methods section. When indicated, RGD peptide (10 µmol/L), PP-2 (250 nmol/L) or AG1478 (1 µmol/L) were added 30 min prior to insulin (35 nmol/L) to the perfusate. Liver samples were taken at the time points indicated and phosphorylation of IRS-1-$Y^{612}$ was analyzed by use of phospho-specific antibodies. Total IRS-1 served as loading control. Western blots were then analyzed densitometrically. Phosphorylation level at t=0 min was arbitrarily set as 1. Representative blots and statistics of three independent perfusion experiments are shown.

Insulin-induced a significant phosphorylation of IRS-1 ($^{\#}$, $p<0.05$) which was not affected by an RGD peptide, PP-2 or AG1478 ($p>0.05$; n.s.=not significant), indicating that these inhibitors do not interfere with upstream events of insulin receptor-mediated signaling.

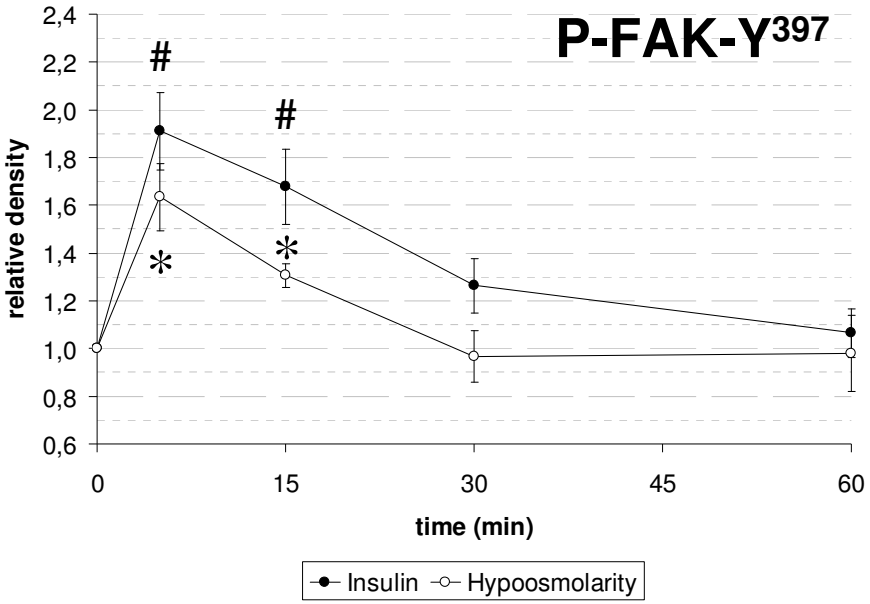
*Supplemental Figure 1*

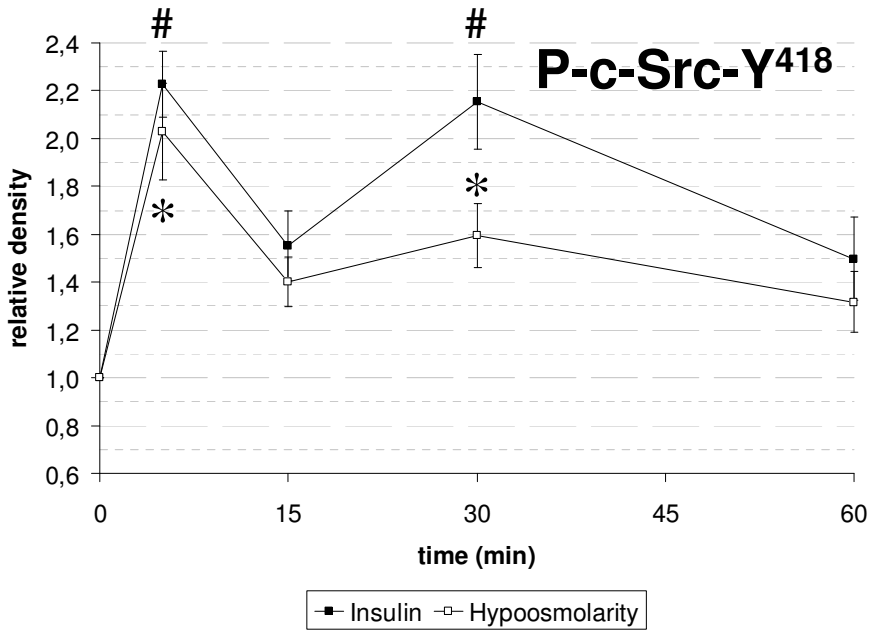


*Supplemental Figure 2*

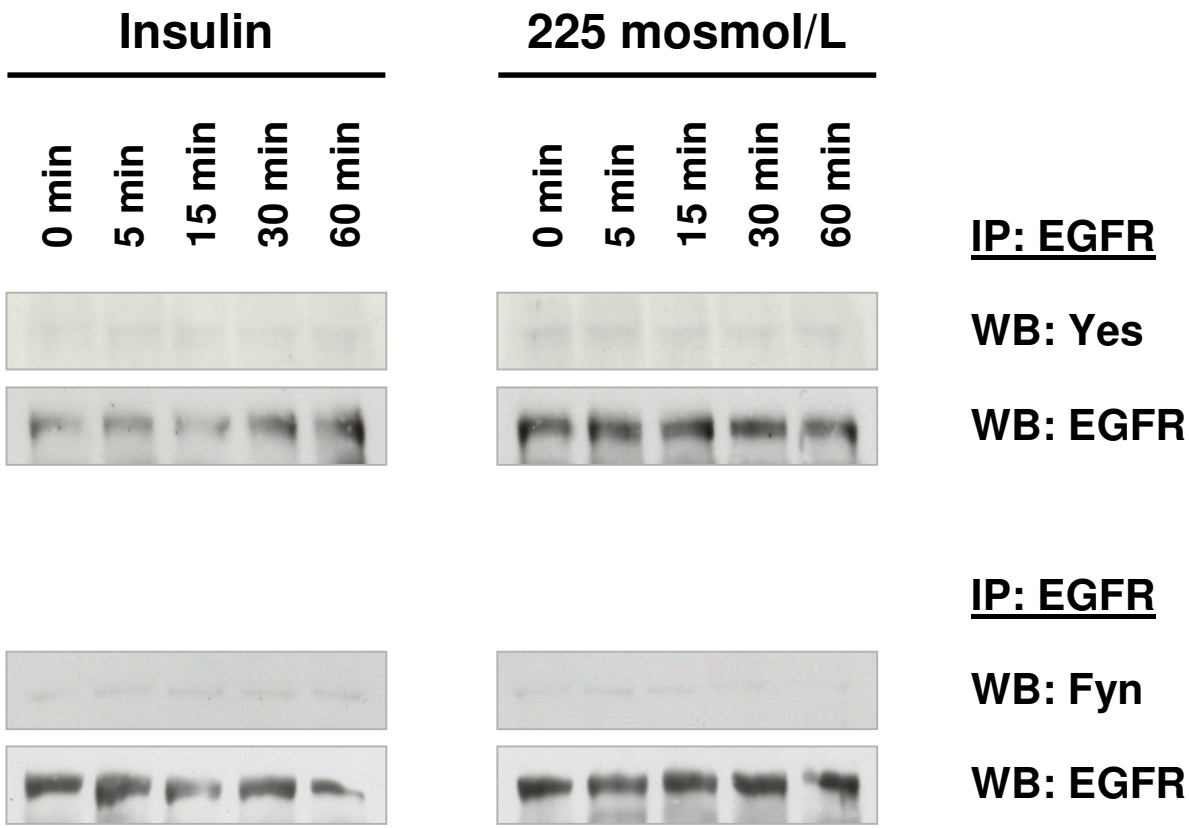


*Supplemental Figure 2*

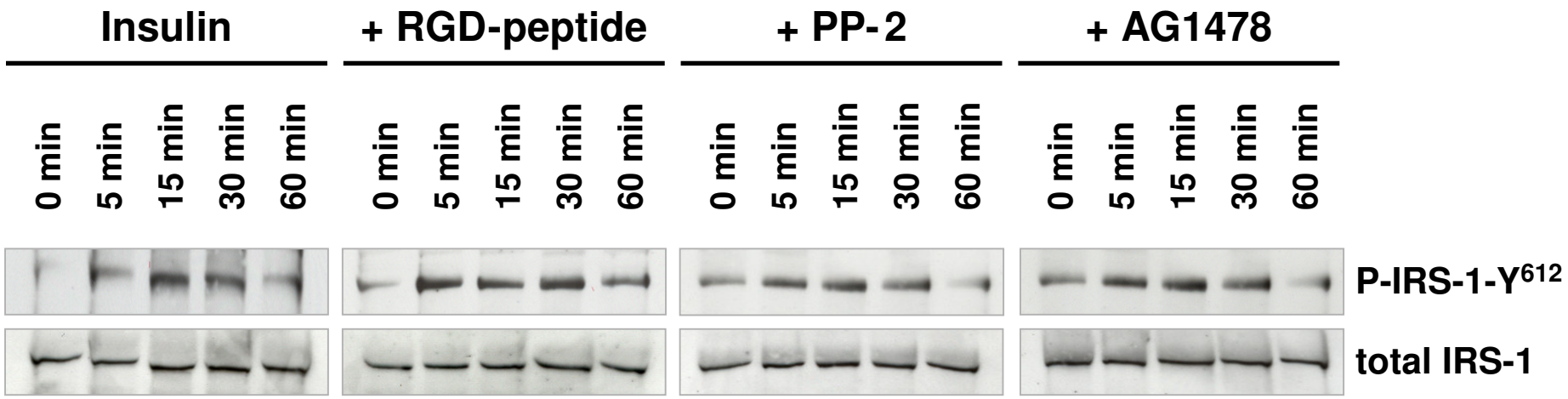


*Supplemental Figure 3*



*Supplemental Figure 4*



*Supplemental Figure 4*