SUPPLEMENTARY DATA

Online Supplemental Materials

Insulin regulates hepatic triglyceride secretion and lipid content via signaling in the brain

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Supplementary Figure 1. Acute infusions of low dose insulin through accurately placed and verified intracerebroventricular (ICV) injections activates the insulin signaling cascade in the brain, but not the liver; ICV infusion of Igf–1 infusion does not affect triglyceride (TG) secretion. (**A**) An insulin bolus of 30 μU injected directly into the 3rd ventricle (ICV) of 8-week old male rats increases insulin receptor (IR) expression and Akt phosphorylation in hypothalamic punch-biopsies of the arcuate nucleus as assessed by western blot (n = 6 per group). (**B**) Western blots and quantification assessing Akt phosphorylation at Ser473 in liver tissue samples of ICV insulin or vehicle infused rats used for the experiment in **Fig. 1A** (n = 5 per group). (**C**) Western blots assessing hormone sensitive lipase (Hsl) activation with a phospho-Hsl Ser563 antibody in epididymal white adipose tissue harvested from rats that received infusions of either insulin or vehicle (artificial cerebrospinal fluid) into the MBH and were subjected to a tyloxapol experiment similar to that in **Fig. 1A**. The observed suppression of Hsl activity in WAT is in line with our previous data (1) and suggests that the MBH was correctly targeted. (**D**) Western blot analyses of hepatic *de novo* lipogenic protein expression and activation state in tyloxapol infused rats after ICV insulin or vehicle administration (n = 5 per group; Fas: fatty acid synthase, Atpcl: ATP citrate lyase and Acc: acetyl-CoA carboxylase). (**D**) Since insulin can also signal through the Igf–receptor and the ICV infusion of Igf–1 can affect hepatic glucose production (2), we also tested the effects of ICV Igf– 1 infusion on TG secretion. Along the protocol outlined for insulin in **Fig. 1A**, rats were ICV infused with a total dose of 0.77 μg Igf–1 over 4 hrs. The insert depicts the calculated TG secretion rate (n = 8 per group). All error bars are SEM. *P<0.05 vs. ICV vehicle group

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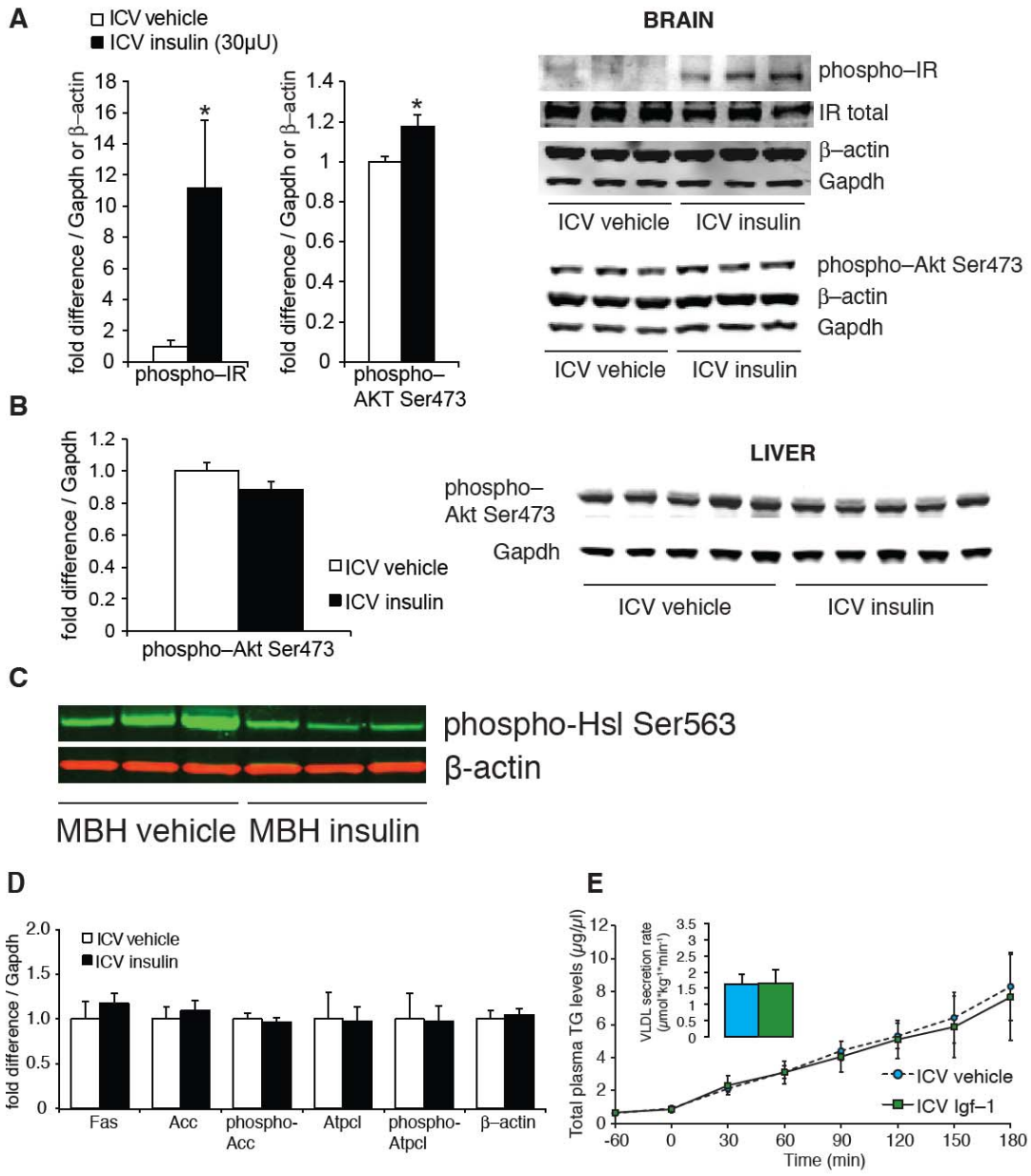

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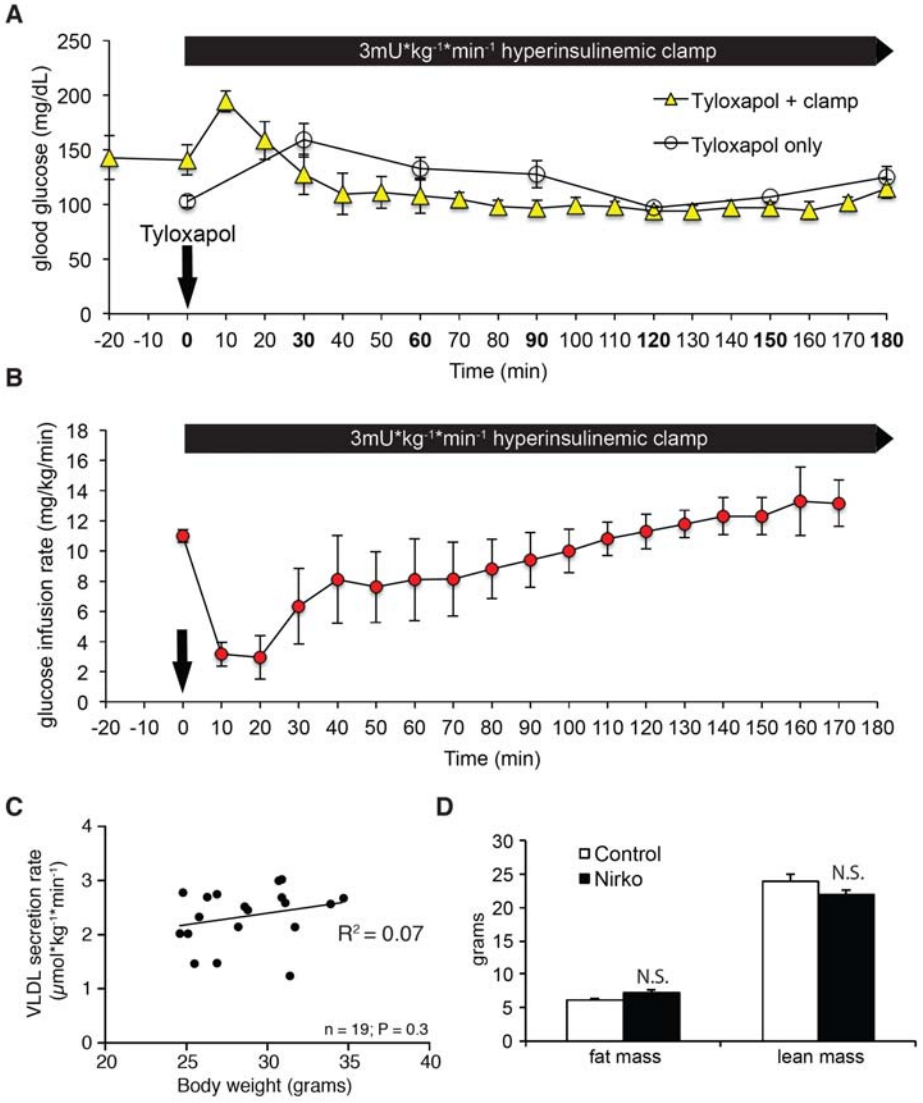
Supplementary Figure 2. Blood glucose levels are controlled during the hyperinsulinemic clamp experiments by adjusting the glucose infusion rate; body weights do not correlate with TG secretion rates; body composition of Nirko and littermate control mice. (**A**) Circulating blood glucose levels of clamped (IV insulin) and non-clamped (ICV insulin and vehicle) tyloxapol infused rats ($n \geq 4$ per group; the arrow indicates the tyloxapol bolus injection). (**B**) Glucose infusion rates required during the 3mU hyperinsulinemic clamp to maintain euglycemia ($n = 4$; the IV insulin group from **Fig. 1** is depicted**;** the arrow indicates the tyloxapol bolus injection). (**C**) Body weights of Nirko and littermate control mice do not correlate with triglyceride secretion rates as assessed by tyloxapol infusion experiments ($n = 19$). (**D**) Body composition of Nirko and littermate controls ($n \geq 7$ per group). All error bars are SEM; N.S. not significant.



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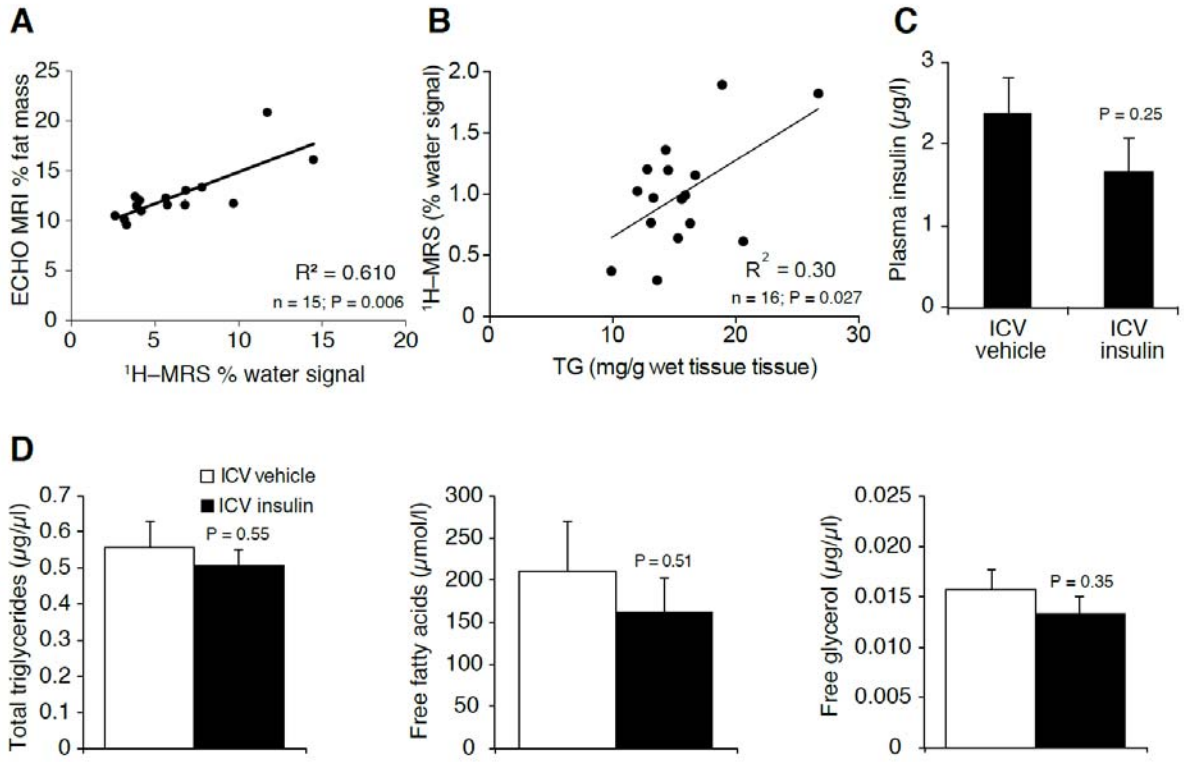
Supplementary Figure 3. Correlation of liver fat content as quantified by ^{1}H–magnetic resonance spectroscopy (^{1}H–MRS) *in vivo* versus EchoMRI™ or chloroform-methanol extraction *ex vivo*; Circulating, insulin, triglyceride, free fatty acid and glycerol levels are unchanged in chronic ICV insulin infused rats. (**A**) To compare the ^{1}H–MRS data to EchoMRI™, liver samples (2 – 3g) from high fat–fed rats (8 weeks on diet #D12492 from Research Diets; 60% of calories from fat) were used for measurement of lipid content by an EchoMRI™–100 (EchoMRI LLC, Houston, TX, USA). The results were compared to data obtained from the same rats with ^{1}H–MRS measurements of liver fat content *in vivo*. Good intra-individual correlation of results obtained with the two quantification methods eliminates the possibility that focal steatosis impairs the data obtained with ^{1}H–MRS (n = 15). (**B**) Liver fat data assessed by ^{1}H–MRS from our chronic ICV experiments (depicted in **Fig. 4A**) correlate with conventional liver triglyceride (TG) measurements using chloroform-ethanol extraction (n = 16). (**C**) Circulating insulin levels are unchanged in chronic ICV insulin-infused rats compared to controls suggesting that there is no spill over of ICV insulin into the circulation. (**D**) Plasma triglyceride, free fatty acid and free glycerol levels of chronic ICV insulin or vehicle infused rats at the end of the infusion protocol depicted in **Fig. 4A** (n = 8 per group).



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References:

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2. Muzumdar RH, Ma X, Fishman S, Yang X, Atzmon G, Vuguin P, Einstein FH, Hwang D, Cohen P, Barzilai N: Central and Opposing Effects of IGF-I and IGF-Binding Protein-3 on Systemic Insulin Action. Diabetes 2006;55:2788-2796