SUPPLEMENTARY DATA

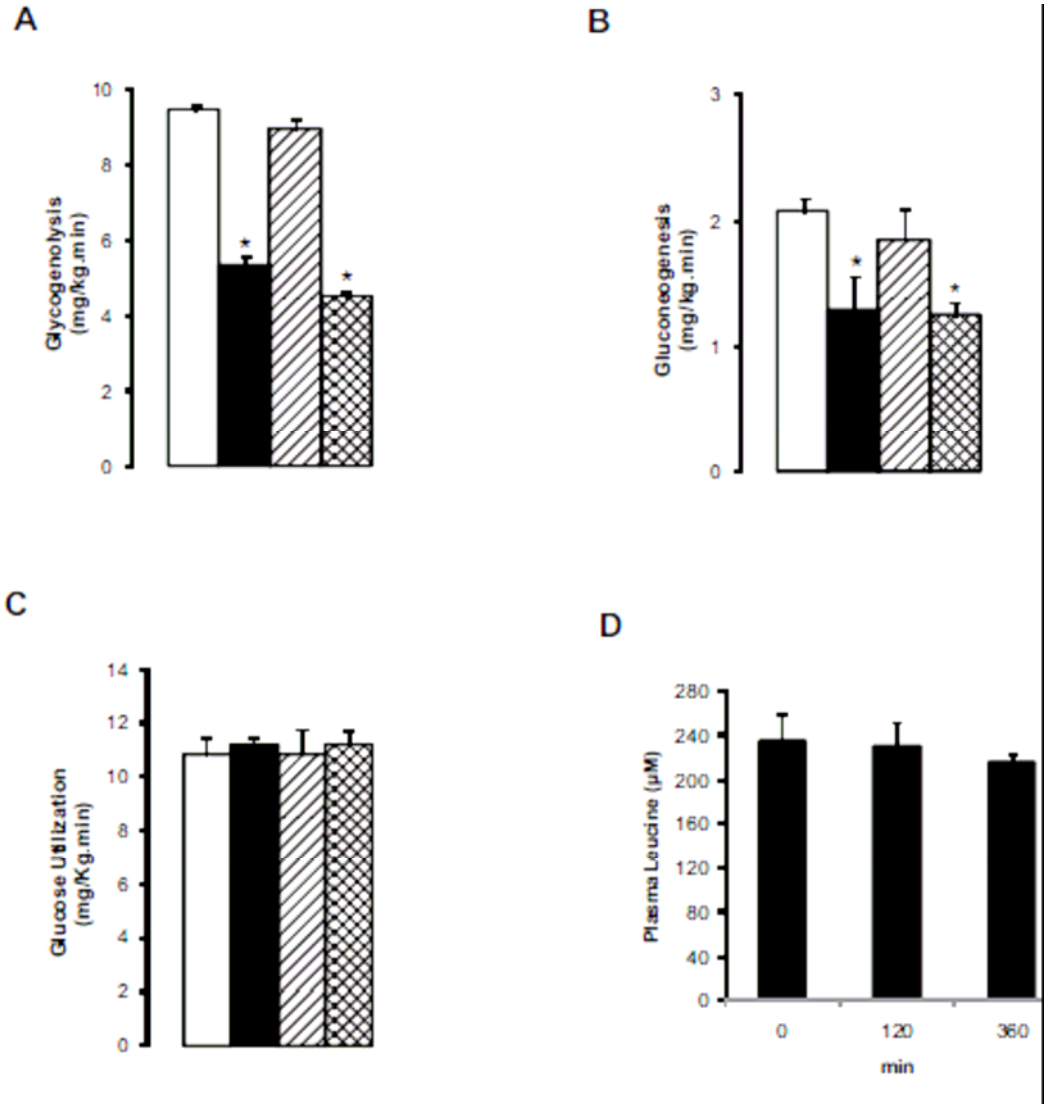
Supplementary Table 1. Characteristics of the experimental groups during the basal (pre-clamp) and clamp period of pancreatic clamp studies

	IH VEH	IH LEU	IH KIC	IH α-CIC
Basal				
Body weight (g)	308±2	305±5	319±4	295±4
Insulin (ng/ml)	1.9±0.3	0.7±0.1*	1.0±0.1*	1.3±0.1
Glucagon (pg/ml)	83±10	85±9	90±7	88±10
Clamp				
Glucose (mM)	8.3±0.4	7.8±0.8	8.0±0.4	7.7±0.4
Insulin (ng/ml)	1.2±0.1	1.3±0.2	1.3±0.2	1.4±0.1
Glucagon (pg/ml)	33±7	30±6	30±4	31.1±4
Adiponectin (ng/ml)	3.7±0.3	3.3±0.4	3.5±0.1	2.6±0.1

IH, intrahypothalamic (mediobasal hypothalamus); VEH, vehicle; LEU, Leucine; KIC, α-Ketoisocaproic acid; α-CIC, α-Chloroisocaproic acid. Data are means±s.e.m.; n=4-6. *, $p<0.05$ versus vehicle.

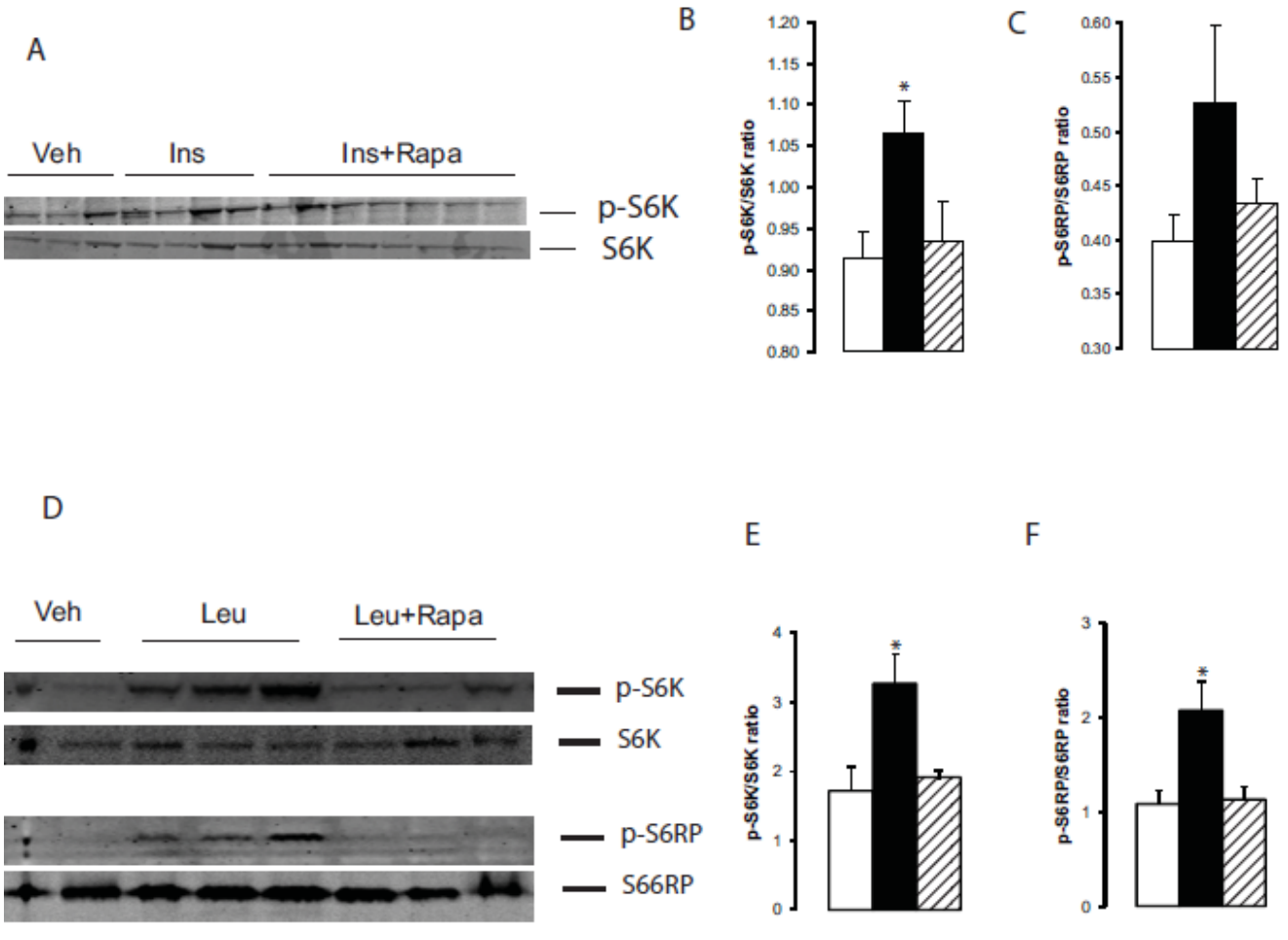
SUPPLEMENTARY DATA

Supplementary Figure 1. Central administration leucine decreases hepatic glucose fluxes during pancreatic clamps. Effect of central (MBH) administration of vehicle (white bar; n=6), leucine (black bar; n=4), leucine plus BCAT inhibitor (hatched bar; n=4), and leucine plus rapamycin (cross- hatched bar; n=4) on (A) hepatic glycogenolysis, (B) hepatic gluconeogenesis, and (C) glucose utilization . D. Effect of intrahypothalamic infusions of leucine on circulating levels of leucine during pancreatic clamp studies (n=6). Values are mean±s.e.m. Asterisk, $P<0.05$ vs. vehicle.



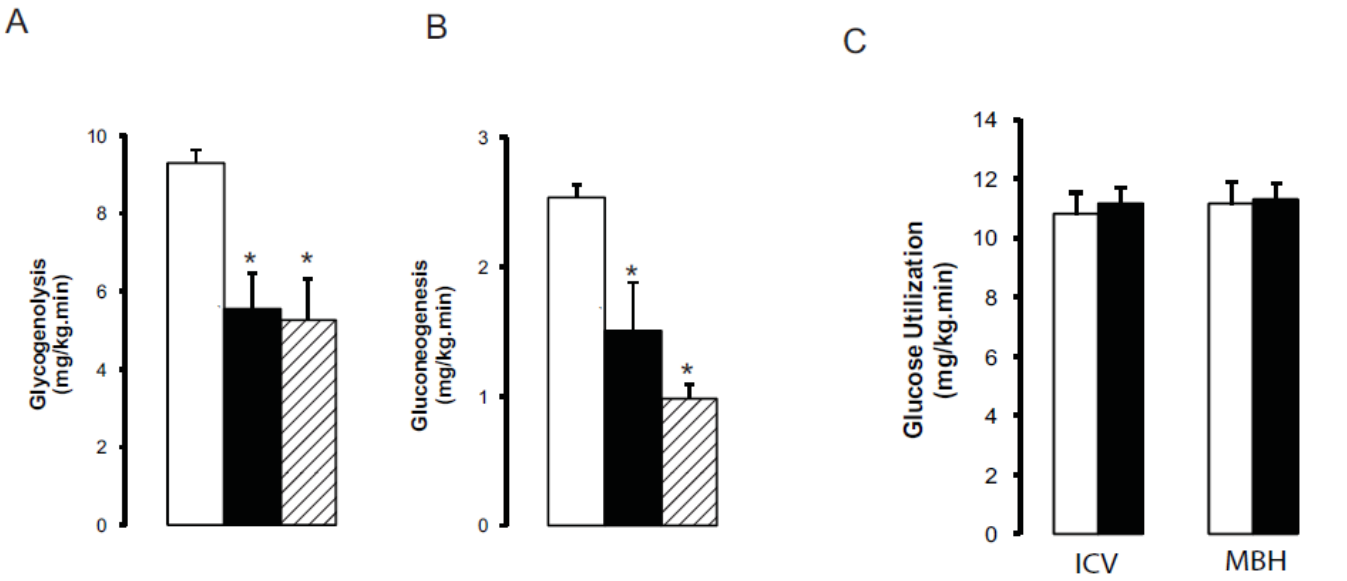
SUPPLEMENTARY DATA

Supplementary Figure 2. Rapamycin prevents the MBH insulin- and leucine- mediated activation of S6K and phosphorylation of S6 ribosomal protein. A, Representative western blot of rapamycin effect on insulin-mediated activation of p70 S6 kinase. B, Quantification of S6K western blots in panel A. C, Quantification of S6 ribosomal protein phosphorylation. Panels B and C: Vehicle (Veh, n=3), white bars; insulin (Ins, n=4), black bars; insulin plus rapamycin (Ins+Rapa, n=7), hatched bars. D, Representative western blot of rapamycin effect on leucine-mediated activation of p70 S6 kinase (top panel) and S6RP phosphorylation (bottom panel). E-F, Quantification of effect of rapamycin on S6K activation (E) and S6RP phosphorylation (F). Panels B and C: Vehicle (Veh, n=4), white bars; Leucine (leu, n=5), black bars; leucine plus rapamycin (Leu+Rapa, n=4), hatched bars. Abbreviations: p-S6K, phospho-p70 S6 Kinase; S6K, dephospho-p70 S6K; p-S6RP, phospho-S6 ribosomal protein; S6RP, dephospho-S6 ribosomal protein. Values are mean±s.e.m. Asterisk, $P<0.05$ vs. vehicle.

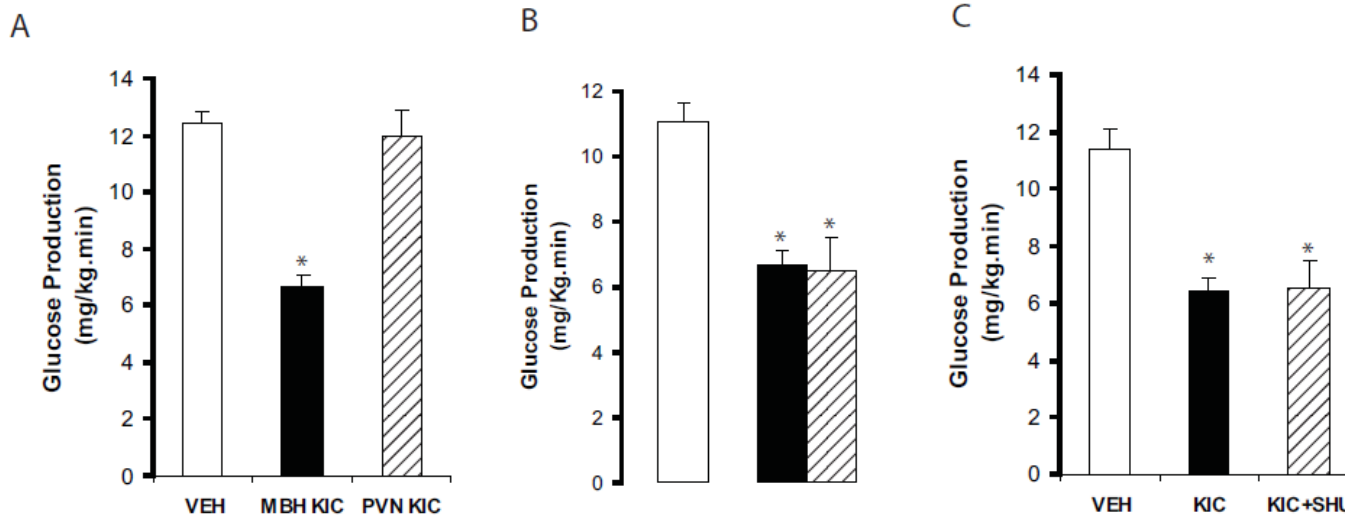


SUPPLEMENTARY DATA

Supplementary Figure 3. Central administration of KIC decreases hepatic glycogenolysis and gluconeogenesis during pancreatic clamps. A,B, Effect of central (into the MBH) administration vehicle (white bar) and KIC (black bar) on hepatic glycogenolysis and gluconeogenesis. C, Effect of central administration of either vehicle (white bars) or KIC (black bars) into the third ventricle (i.c.v.) or into the MBH on glucose utilization during pancreatic clamps. Values are mean±s.e.m of 5-6 experiments. *, $P<0.05$ vs. vehicle.

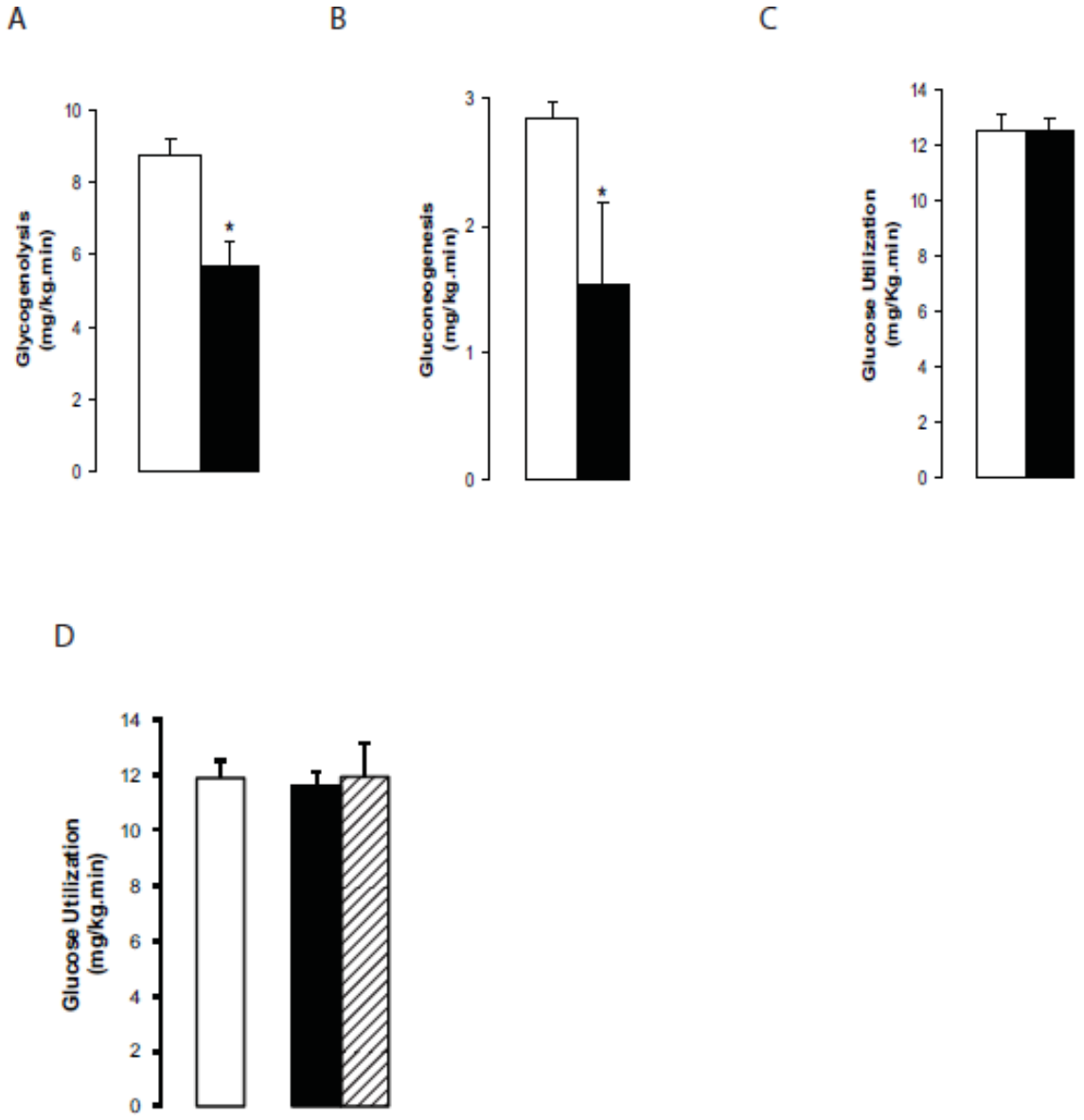


Supplementary Figure 4. A, KIC in the MBH but not in the PVN inhibits hepatic glucose production. MBH, mediobasal hypothalamus (includes the arcuate nucleus); PVN, paraventricular nucleus of the hypothalamus. B. BCAT inhibition does not modify the effect of central KIC on hepatic glucose production. Vehicle, white bar; KIC, black bar; KIC+BCAT inhibitor, hatched bar. C, The melanocortin antagonist SHU9119 does not interfere with the effect of central KIC on hepatic glucose metabolism. Values are mean±s.e.m. of 4-6 experiments. Asterisk, $P<0.05$ vs. vehicle.



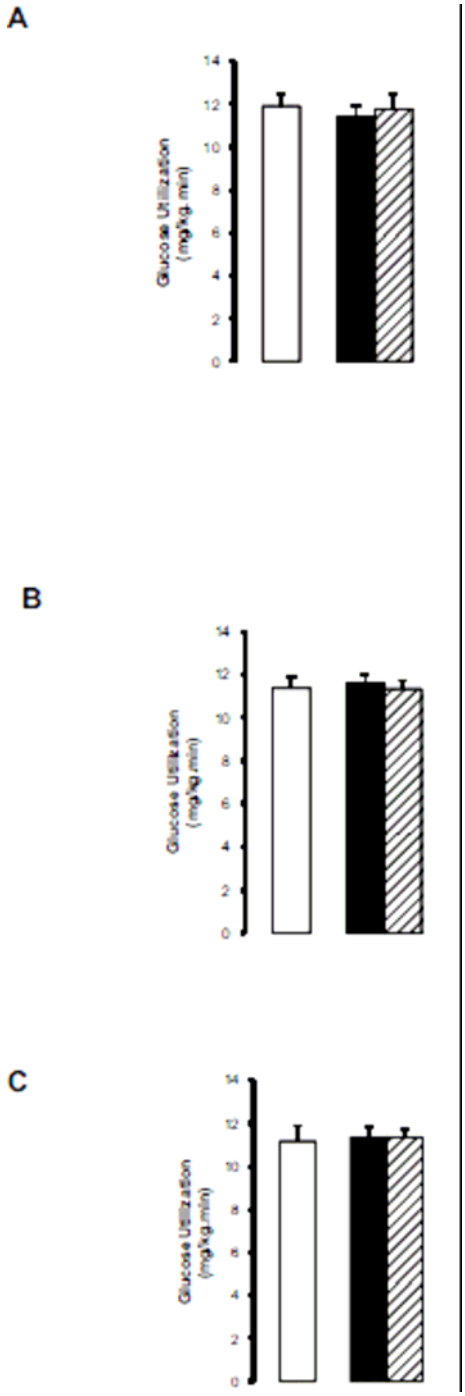
SUPPLEMENTARY DATA

Supplementary Figure 5. A-C, Central inhibition of BCKDH kinase (BCKDK) decreases hepatic glucose fluxes during pancreatic clamps. Effect of central (MBH) administration of vehicle (white bar; n=6), and α-chloroisocaproate (black bar; n=5) on hepatic (A) glycogenolysis, (B) gluconeogenesis, and (C) whole body glucose utilization. D, Effect of central (MBH) leucine on glucose utilization during pancreatic clamps in animals over-expressing BCKDK in the MBH. Effect of leucine in animals injected into the MBH with either AAV-GFP (black bar; n=5) or AAV-BCKDK (hatched bar; n=4), and effect of vehicle in AAV-GFP treated animals (white bar; n=5). All values represent mean±s.e.m. Asterisk denotes $p<0.05$ vs vehicle.



SUPPLEMENTARY DATA

Supplementary Figure 6. Glucose utilization during pancreatic insulin clamps in animals receiving central (MBH) infusions of (A) vehicle (white bar; n=6), KIC (black bar; n=6), KIC plus AICAR (gray bar; n=5); (B) leucine in animals over-expressing malonyl-CoA decatboxylase in the MBH:Vehicle (white bars; n=5) in animals over-expressing GFP in the MBH; leucine in animals over-expressing either GFP (black bar; n=5) or MCD (hatched bars; n=5) in the MBH; and (C) vehicle (white bar; n=6), KIC (black bar; n=5), KIC plus glibenclamide (hatched bar; n=5). All values represent mean±s.e.m.



SUPPLEMENTARY DATA

Supplementary Figure 7. A-B, Effect of systemic administration of leucine on plasma isoleucine (A) and valine (B). C, Effect of systemic leucine infusions on glucose utilization during pancreatic clamps. Panels A-C: white bars, vehicle (n=5); black bars, leucine, infusion rate 120 µmol/kg.h (n=4); hatched bars, leucine, infusion rate 240 µmol/kg.h (n=4). D, Effect of central (MBH) BCAT inhibition on glucose utilization during pancreatic clamps with systemic leucine infusions. White bars, MBH and i.v vehicle (n=6); black bars=MBH vehicle and i.v. leucine (n=6); hatched bar=MBH BCAT inhibitor and i.v. leucine (n=6). Asterisk denotes $p<0.05$ vs vehicle. Values represent mean±s.e.m. E-F, Western blot analysis of the effect of systemic leucine infusions on mTOR pathway activation in peripheral tissues. E, Skeletal muscle; F, liver.