Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production

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Supplementary Table 1 Diet content of the regular chow and the lard-oil enriched high fat diet.

Calories provided	Chow diet	High fat diet
Carbohydrates (%)	49	44
Protein (%)	33	22
Fat (%)	18	34
Saturated	6.6	14.3
Monounsaturated	7.7	14.8
Polyunsaturated	3.7	4.8
Total metabolizable energy provided (kcal/g)	3.1	3.9

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Supplementary Table 2 Plasma insulin and glucose concentrations of the groups receiving duodenal infusion during the basal and clamp conditions.

	Saline (N = 7)	Metformin (N = 6)	Portal Metformin (N = 5)
Basal			
Insulin (ng/mL)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2
Glucose (mM)	7.9 ± 0.6	8.1 ± 0.3	7.7 ± 0.2
Clamp			
Insulin (ng/mL)	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1
Glucose (mM)	7.9 ± 0.5	7.8 ± 0.2	7.6 ± 0.3

Data are mean ± SEM (Basal: 60-90 min; Clamp: 180-200 min)

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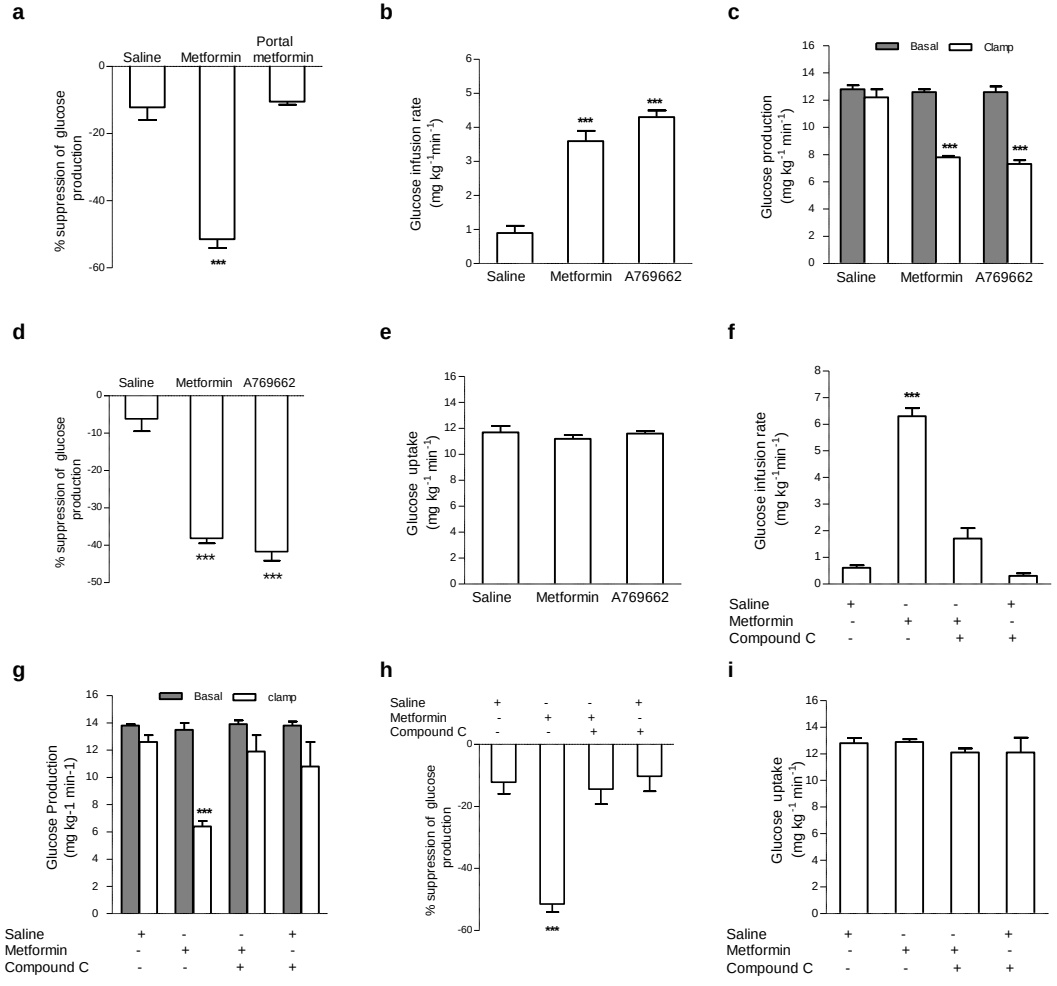
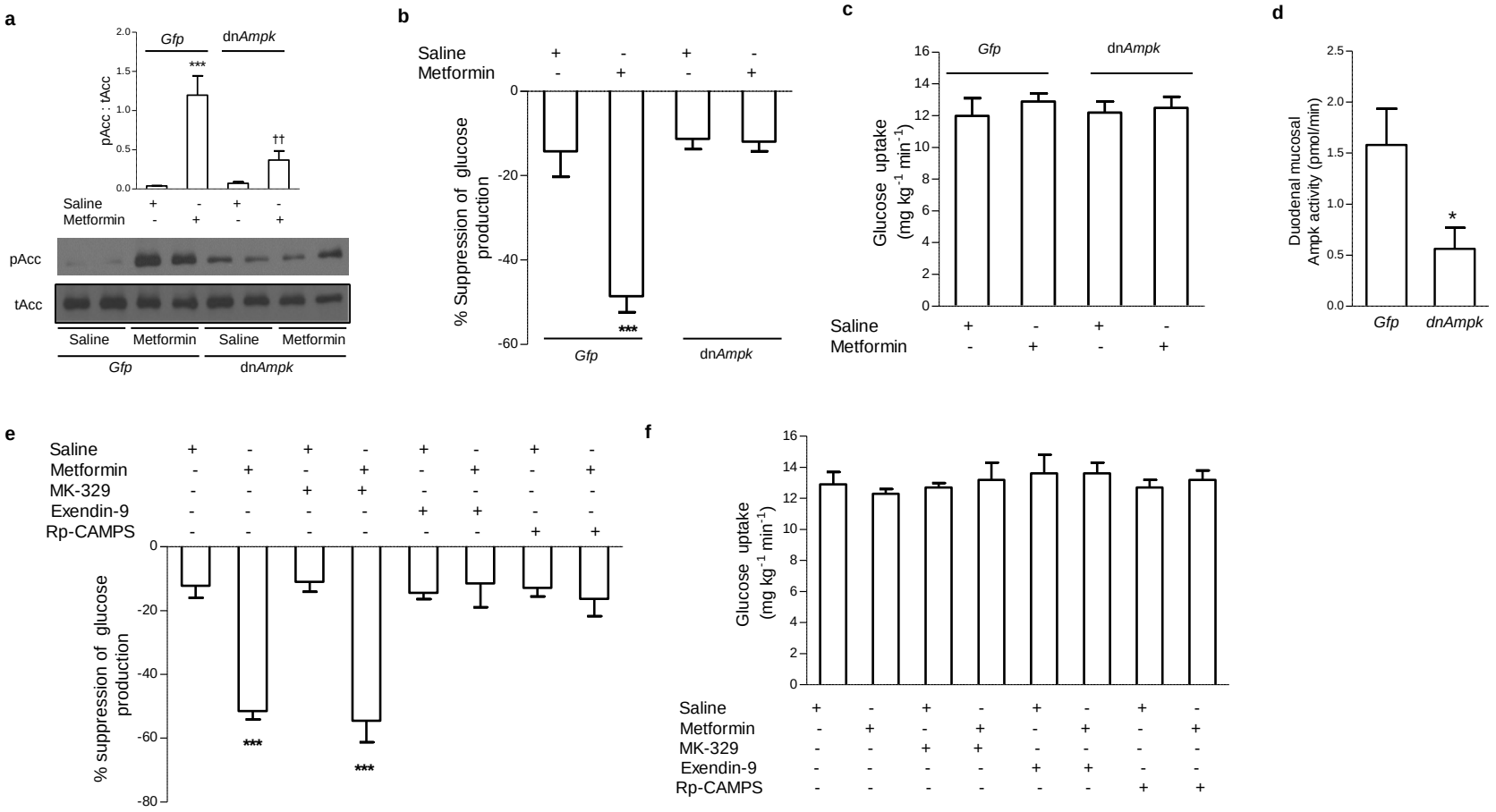
Supplementary Figure 1



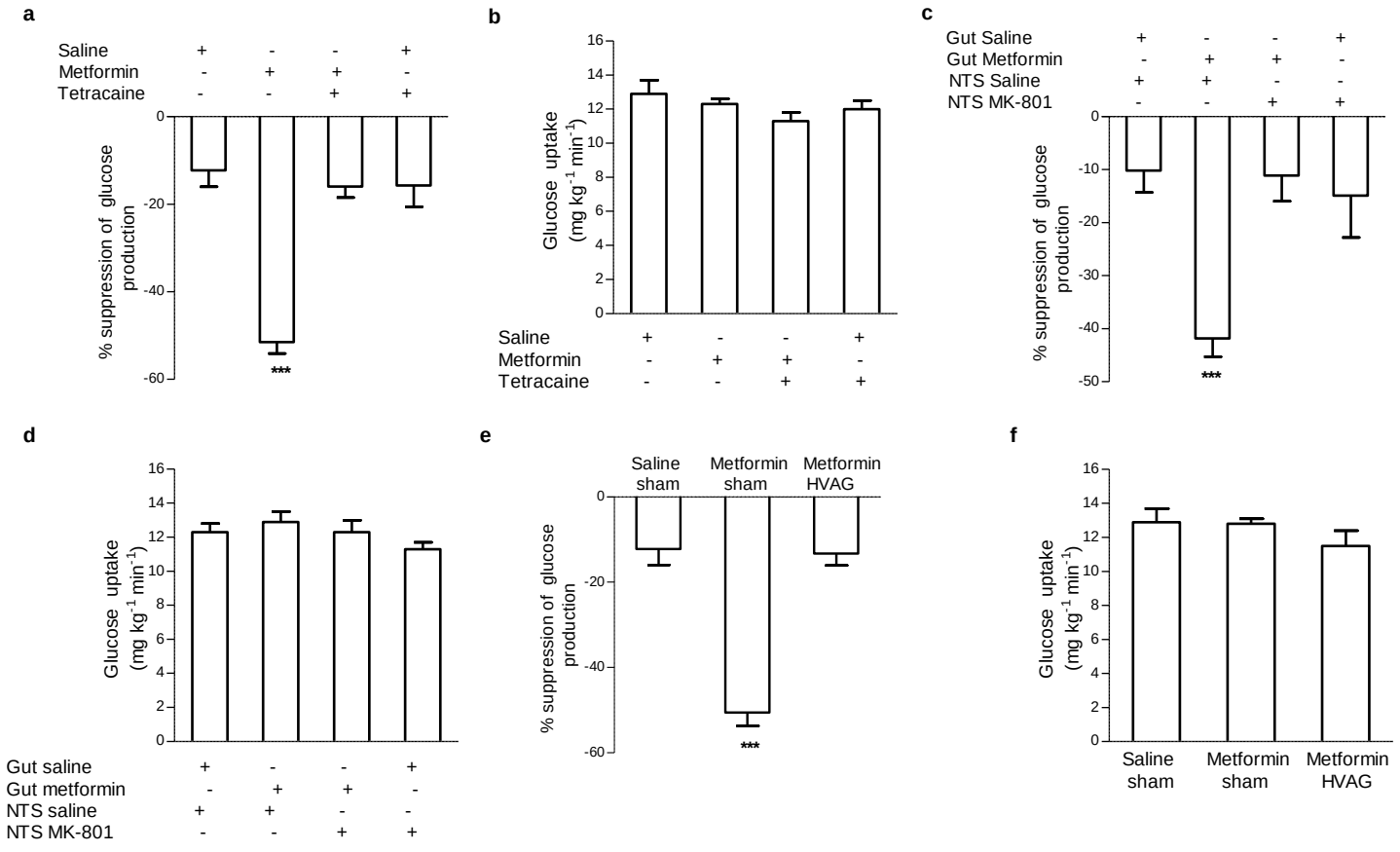
Supplementary Figure 1 Preabsorptive metformin activates duodenal Ampk to lower HGP, independent of changes in glucose uptake. (**a**) HGP percent suppression from basal following duodenal or portal metformin infusion during the pancreatic clamp studies. (**b,c,d,e)** The glucose infusion rate (**b**), rate of HGP (**c**), HGP percent suppression from basal (**d**) and glucose uptake (**e**) during the pancreatic (basal insulin) euglycemic clamp in HFD-fed rats following 50 min intraduodenal infusion of either saline, low dose of metformin (50 mg kg^{-1}), or Ampk activator A769662 (3 mg kg^{-1}; *n* = 6,5,5). (**f,g,h,i**) The glucose infusion rate (**f**), rate of HGP (**g**), HGP percent suppression from basal (**h**) and glucose uptake (**i**) during the pancreatic (basal insulin) euglycemic clamp in HFD-fed rats with intraduodenal compound C administration alone (*n* = 5) or in combination with metformin (*n* = 6). Values are shown as mean ± s.e.m. ***$P < 0.001$, versus all other groups as calculated by ANOVA with Tukey's *post hoc* test.

Supplementary Figure 2



Supplementary Figure 2 A duodenal Ampk – Glp1r – Pka axis is necessary for metformin to lower HGP independent of changes in glucose uptake. (a) Quantitative anaylsis and representative western blot of pAcc protein expression normalized to tAcc in HEK 293 cells infected with either Ad-*Gfp* or dn*Ampk* and treated with saline or metformin for 6 hours (***$P < 0.001$, between saline within viral group; ††$P < 0.01$, between viral group within treatment; as calculated by one-way ANOVA with Tukey's *post hoc* test; $n = 4$ per treatment). (**b,c**) HGP percent suppression from basal (**b**) and glucose uptake (**c**) for duodenal viral injected (Ad-*Gfp* or Ad-dn-*Ampk*) rats receiving duodenal infusion of saline or metformin during the clamp. (**d**) Ampk activity in duodenal mucosal tissue of HFD-fed duodenal viral injected (Ad-*Gfp* or Ad-dn-*Ampk*) rats following 50 minute intraduodenal infusion of metformin (*$P < 0.05$, calculated by unpaired t-test; $n = 5,5$). (**e,f**) HGP percent suppression (e) and glucose uptake (**f**) during the pancreatic (basal insulin) euglycemic clamp in HFD-fed rats with intraduodenal MK-329, exendin-9, and Rp-CAMPS administration alone or in combination with metformin. Values are shown as mean ± s.e.m. $n = 5$ rats per group. ***$P < 0.001$, versus saline as calculated by ANOVA with Tukey's *post hoc* test.

Supplementary Figure 3



Supplementary Figure 3 A gut-brain-liver neuronal axis is required for the HGP lowering effect of metformin, independent of changes in glucose uptake. (**a,b**) HGP percent suppression (**a**) and glucose uptake (**b**) in HFD-fed rats infused with intraduodenal tetracaine alone or in combination with metformin. (**c,d**) HGP percent suppression (**c**) and glucose uptake (**d**) in HFD-fed rats with intraduodenal saline or metformin and NTS saline or MK-801. (**e,f**) The glucose infusion rate (**e**) and rate of HGP (**f**) in HFD-fed rats with intraduodenal metformin with either a sham surgery or hepatic vagotomy. Values are shown as mean ± s.e.m. *n* = 5-6 rats per group. ****P* < 0.001, versus saline, as calculated by one-way ANOVA with Tukey's *post hoc* test.

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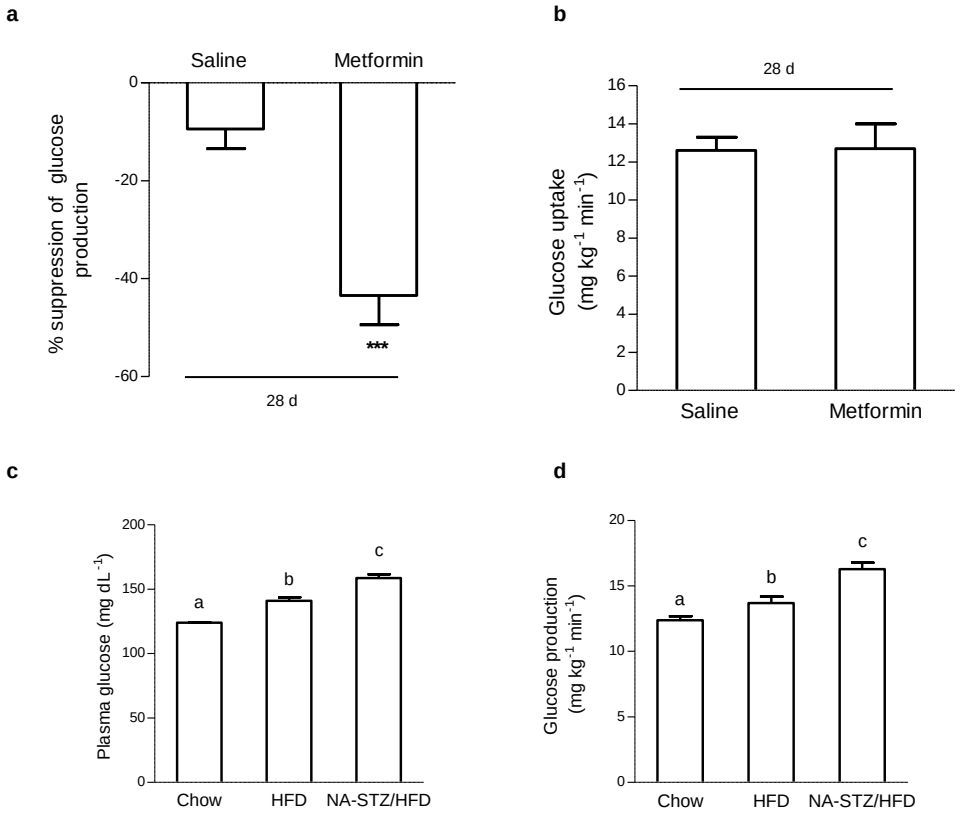
Supplementary Figure 4



Supplementary Figure 4 The effect of duodenal metformin in 28 d HFD-induced obese and insulin resistant model and the establishment of the NA-STZ/HFD type 2 diabetic model. (**a,b**) HGP percent suppression (**a**) and glucose uptake of HGP (**b**) during the pancreatic (basal insulin) euglycemic clamp in 28 day HFD-fed rats with intraduodenal saline or metformin infusion (***$P < 0.001$, versus saline as compared by unpaired t-test). (**c,d**) Basal plasma glucose levels (**c**) and basal HGP (**d**) in NA-STZ/HFD induced hyperglycemic rats (differing letters denotes differences of $P < 0.05$ between groups as calculated by ANOVA with Tukey's *post hoc* test) Values are shown as mean ± s.e.m. n = 6-8 rats per group.

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