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Supplementary Materials for

The short-chain fatty acid propionate increases glucagon and FABP4 production, impairing insulin action in mice and humans

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/489/eaav0120/DC1)

Data file S1 (Microsoft Excel format). Source data for Figs. 1 to 6 and figs. S1 to S4.

Supplementary Material

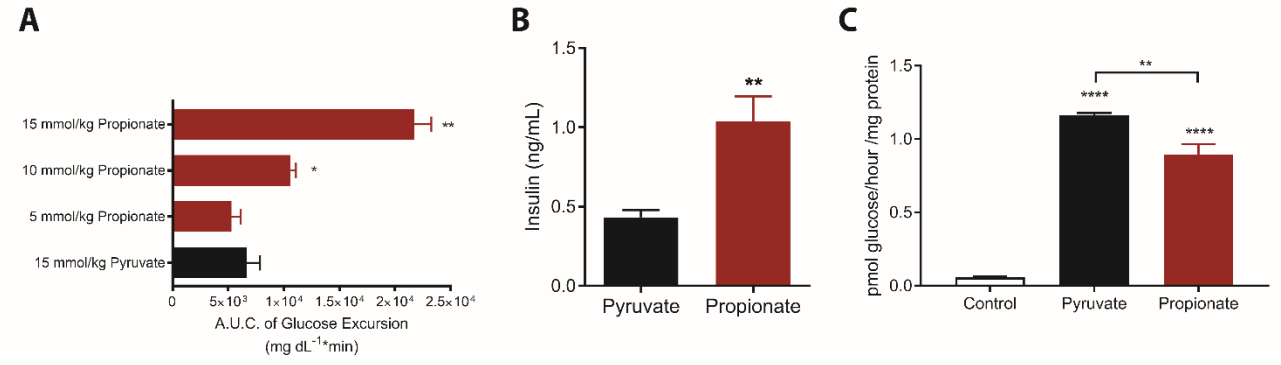

Fig. S1. Propionate induces hyperglycemia and hyperinsulinemia in mice. (A) Area under the curve for Figure 1A. **(B)** Plasma insulin was measured 30 minutes following i.p injection of 15 mmol/kg pyruvate or propionate (n=8 mice/group). **(C)** Glycogen-depleted primary rat heptocytes were treated with propionate (10mM) or pyruvate (10mM) in substrate-free DMEM for 2 hours and glucose appearance in the media was measured using a glucose oxidase based assay (n=4 repeated with 3 different isolations of hepatocytes). All results are reported as arithmetic mean ± S.E.M. Statistical differences between two groups were determined using unpaired two-tailed Student's t test, differences between three or more groups were compared using one-way-ANOVA and Tukey post-hoc analysis. *p<0.05, ** p<0.005, ****p<0.0001.

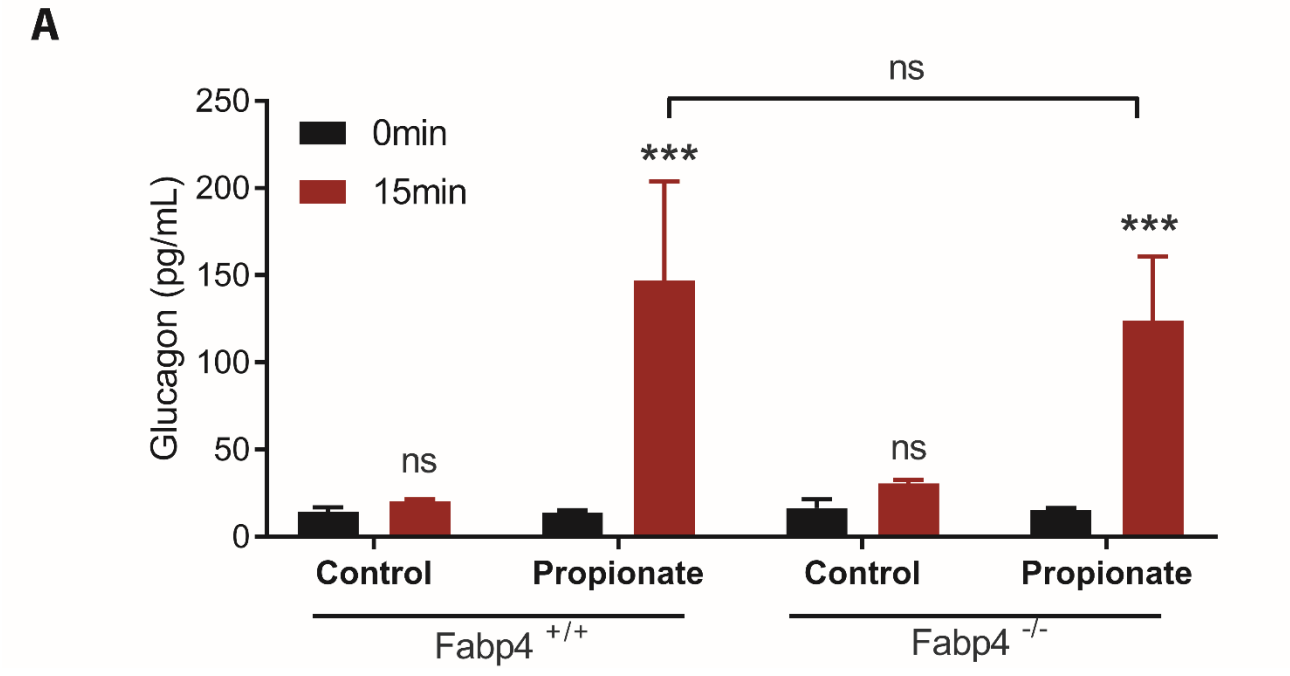

Fig. S2. Fabp4 deficiency does not affect glucagon secretion in response to propionate administration. (A) Plasma glucagon was measured before and after 15 minutes following i.p. injection of 15 mmol/kg propionate or vehicle control (PBS) following 5 hour food withdrawal (n=4-6 mice/group). All results are reported as arithmetic mean ± S.E.M. Statistical differences between two groups were determined using unpaired two-tailed Student's t test. ns, not significant *** $p<0.001$.

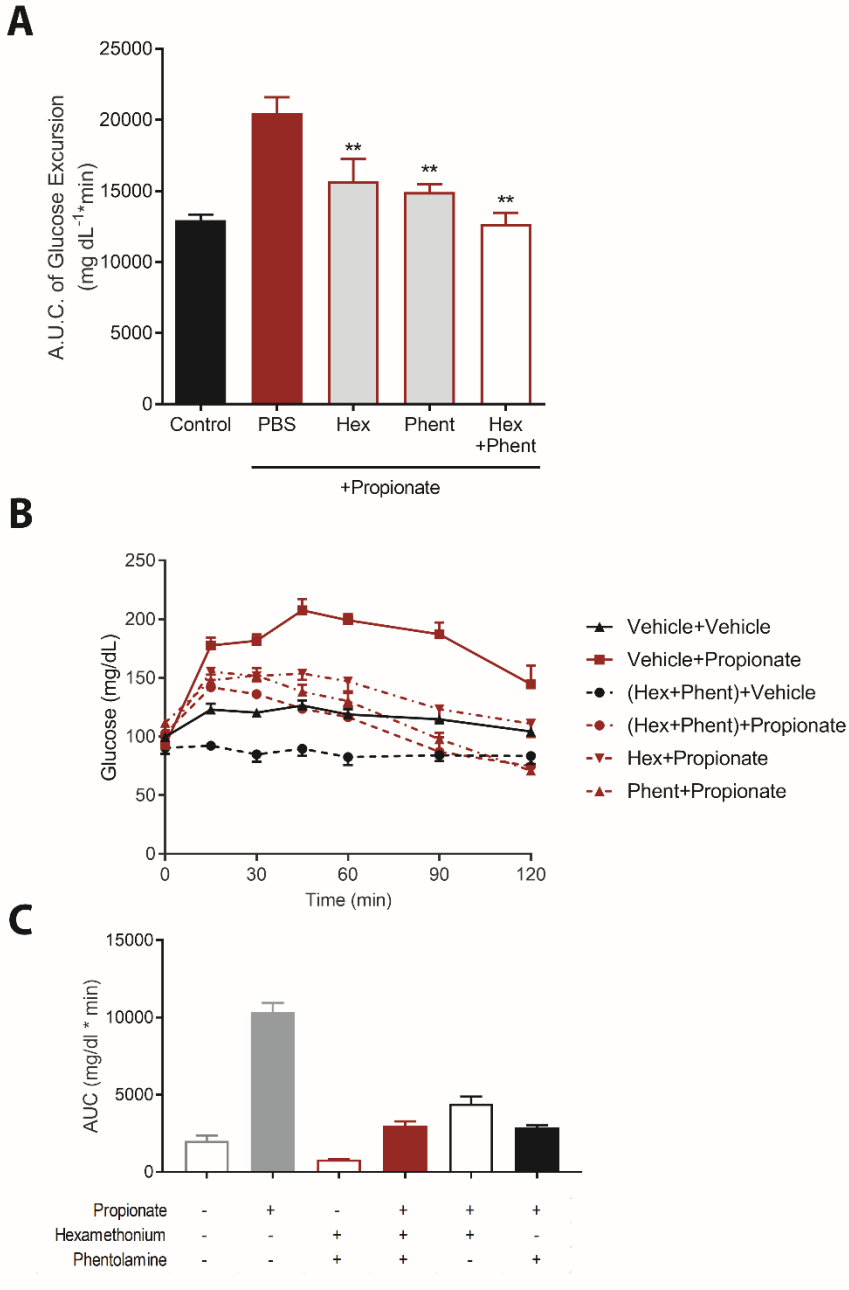

Fig. S3. The effect of sympathetic blockade on blood glucose. (A) Area under the curve calculations for Figure 3E. **(B)** Sympathetic blockade prior to i.p injection of 15 mmol/kg of propionate or vehicle (PBS) with phentolamine (Phent, 1 mg/kg), hexamethonium (Hex; 20 mg/kg) or both (Hex+Phent) significantly inhibited propionate induced hyperglycemia while not changing baseline blood glucose in control animals (Vehicle+Vehicle and Hex+Phent+Vehicle, black lines) (n=6/group) **(C)**Area under the curve calculations for Figure S3B. All results are reported as arithmetic mean ± S.E.M. Statistical differences between three or more groups were compared using one-way-ANOVA and Tukey post-hoc analysis. Response to tolerance tests between mice groups were compared using two-way-ANOVA with Bonferonni post-hoc analysis *p<0.05, ** p<0.005.

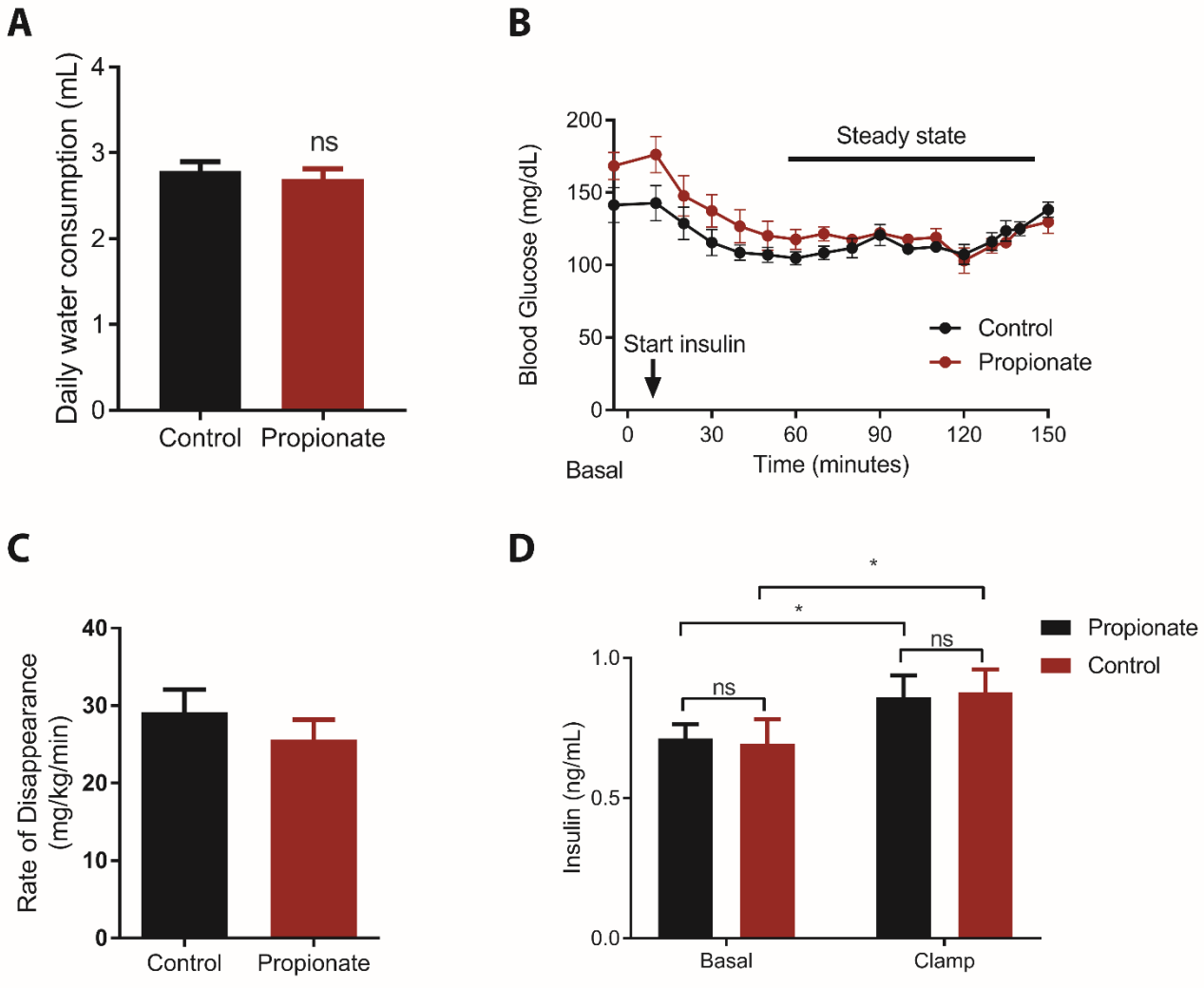

Fig. S4. Results of hyperinsulinemic-euglycemic clamp studies. (A) Daily consumption of propionate or sodium chloride supplemented water. Animals were monitored for three days in individual cages and daily water consumption was recorded at the end of each day. Presented as the average of animals per day. (n=8 animals/group). **(B)** Glycemia profile of groups for the duration of the hyperinsulinemic-euglycemic clamp experiment (n=8 animals/group). **(C)** Rate of glucose disappearance during the steady state period of the hyperinsulinemic-euglycemic clamp experiment. **(D)** Plasma insulin concentrations before and after the clamp period. All results are reported as arithmetic mean ± S.E.M. Statistical differences between two groups were determined using unpaired two-tailed Student's t test. ns, not significant *** $p<0.001$.

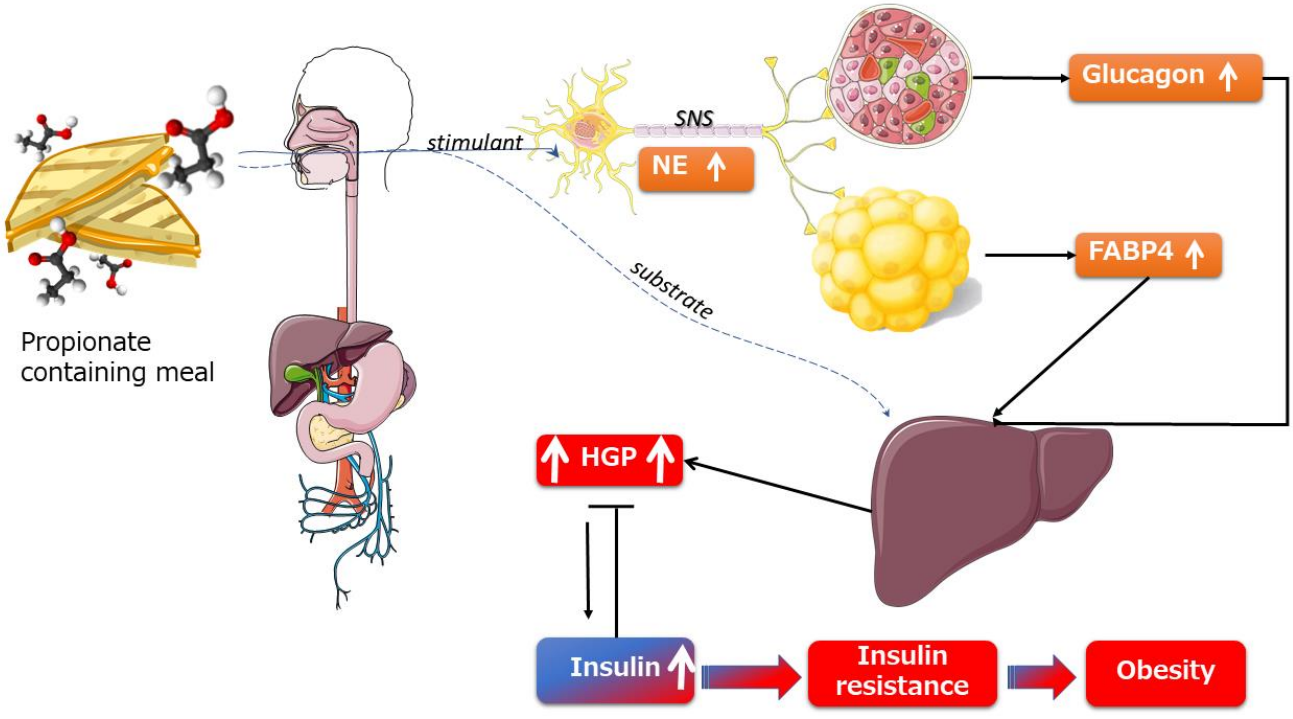

Fig. S5. Proposed model for data presented in this study. Model describing the working hypothesis of the mechanism by which the food preservative propionate acts as a metabolic disruptor, promoting post-prandial insulin resistance by activating the sympathetic nervous system leading to an increase in both glucagon and FABP4. This, in turn, results in increased hepatic glucose production and insulin resistance and compensatory hyperinsulinemia, which over time promotes weight gain.