METABOLISM

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

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The short-chain fatty acid propionate is a potent inhibitor of molds that is widely used as a food preservative and endogenously produced by gut microbiota. Although generally recognized as safe by the U.S. Food and Drug Administration, the metabolic effects of propionate consumption in humans are unclear. Here, we report that propionate stimulates glycogenolysis and hyperglycemia in mice by increasing plasma concentrations of glucagon and fatty acid–binding protein 4 (FABP4). *Fabp4*-deficient mice and mice lacking liver glucagon receptor were protected from the effects of propionate. Although propionate did not directly promote glucagon or FABP4 secretion in ex vivo rodent pancreatic islets and adipose tissue models, respectively, it activated the sympathetic nervous system in mice, leading to secretion of these hormones in vivo. This effect could be blocked by the pharmacological inhibition of norepinephrine, which prevented propionate-induced hyperglycemia in mice. In a randomized, double-blind, placebo-controlled study in humans, consumption of a propionate-containing mixed meal resulted in a postprandial increase in plasma glucagon, FABP4, and norepinephrine, leading to insulin resistance and compensatory hyperinsulinemia. Chronic exposure of mice to a propionate dose equivalent to that used for food preservation resulted in gradual weight gain. In humans, plasma propionate decreased with weight loss in the Dietary Intervention Randomized Controlled Trial (DIRECT) and served as an independent predictor of improved insulin sensitivity. Thus, propionate may activate a catecholamine-mediated increase in insulin counter-regulatory signals, leading to insulin resistance and hyperinsulinemia, which, over time, may promote adiposity and metabolic abnormalities. Further evaluation of the metabolic consequences of propionate consumption is warranted.

INTRODUCTION

According to the International Diabetes Federation, about 415 million people worldwide suffer from diabetes (*1*). Despite extensive research efforts, medical and surgical treatments, prevention programs, and public health policies designed to curb this trend, the rate of diabetes incidence is projected to further increase by more than 50% by 2040, becoming one of the greatest threats to global health (*1*). The dramatic increase in the incidence of obesity and diabetes over the past 50 years cannot be attributed solely to genetics and thus must involve contributing environmental and dietary factors. Among these, one factor that warrants attention is the extensive use of chemicals in the processing, preservation, and packaging of foods. It was recently suggested that the lack of evidence linking the wide use of chemicals and food additives to metabolic health is due to the absence of detailed studies evaluating these possibilities (*2*). Propionate (propionic acid), a naturally occurring short-chain fatty acid (SCFA), is a potent mold inhibitor that is widely used as a food preservative in cheeses and baked goods, as well as in animal feeds and artificial flavorings (*3*, *4*). The metabolic actions of propionate were first described in 1912 by Ringer, who demonstrated a significant increase in glucose production after administration of propionate to dogs and concluded that this three-carbon molecule is converted to glucose through gluconeogenesis (*5*), although he also recognized that more glucose was produced than could be theoretically explained by stoichiometric conversion of propionate to glucose (*5*). Subsequently, propionate was shown to strongly stimulate endogenous glucose production in other mammals (*6*–*8*). Given the unique property of propionate to increase glucose production, it is widely used as an energy source for dairy cows and sheep to increase the concentration of glucose in milk (*9*). Recently, elegant studies using labeled propionate demonstrated that the direct conversion to glucose could explain no more than 5% of the increase in endogenous glucose production observed in propionate-infused rats, and the remainder was attributed to increased pyruvate carboxylase activity through an, as of now, unclear mechanism (*7*). In a small study in healthy human volunteers, propionate added to bread as a food preservative resulted in higher postprandial insulin compared to placebo-supplemented bread, suggesting a potential induction of postmeal insulin resistance (*10*).

The hyperglycemia and hyperinsulinemia observed after exposure to exogenous propionate are somewhat in contrast to the beneficial

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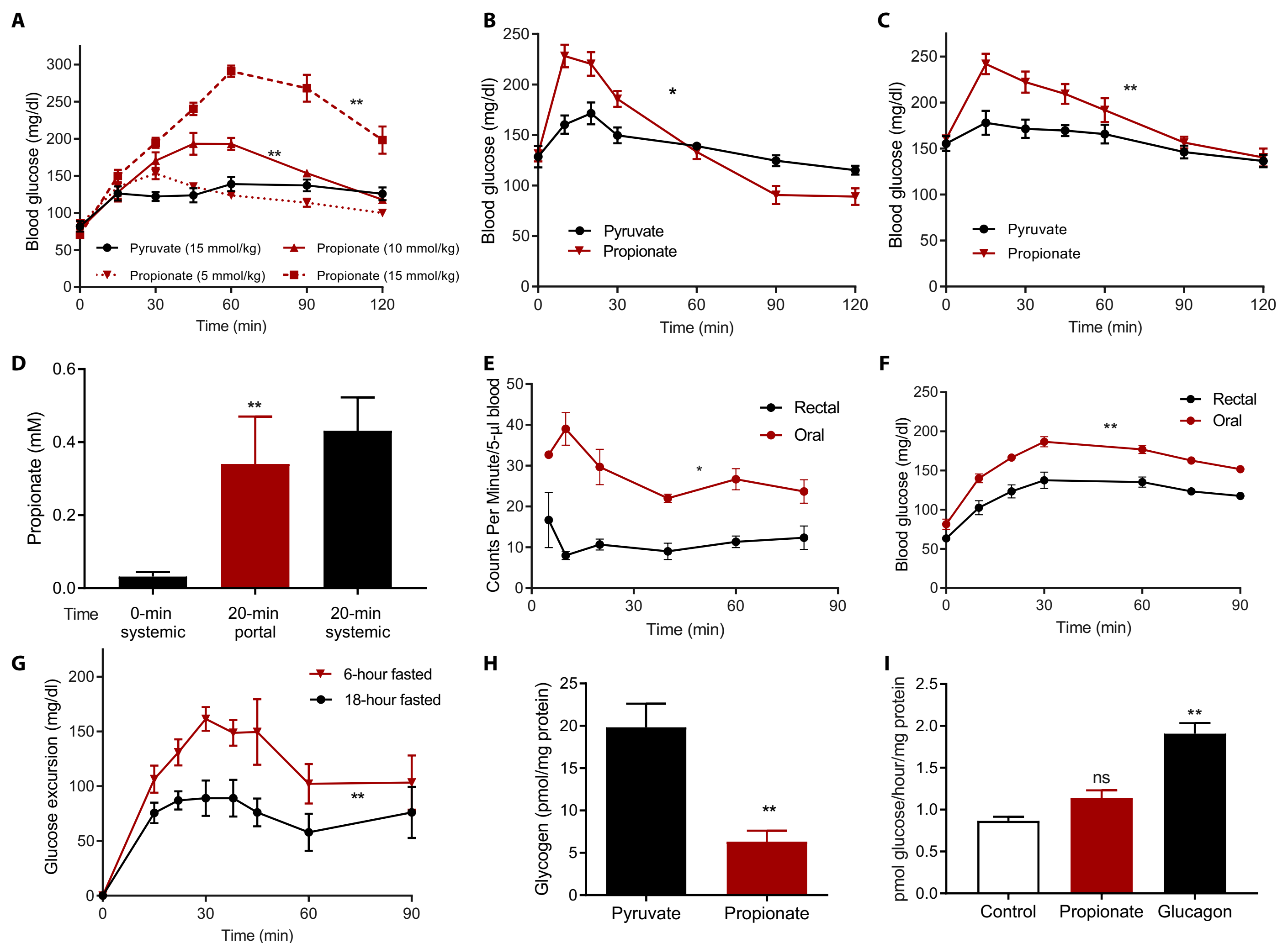

Fig. 1. Propionate induces hyperglycemia in mice. (**A**) Time course of the change in blood glucose was analyzed in wild-type mice after intraperitoneal injection of pyruvate or propionate (at three different doses) ($n = 4$ mice per group, $N = 2$). (**B** and **C**) Propionate and pyruvate (15 mmol/kg each) were injected intraperitoneally (B) or administered orally (C) to female mice, and blood glucose was measured over 2 hours ($n = 5$ mice per group or $n = 4$ mice per group, respectively, $N = 2$; delivered dose was 15 mmol/kg). (**D**) Propionate was measured using gas chromatography–mass spectrometry (GC-MS) in the systemic circulation of mice at 0 and 20 min after oral administration of propionate and in the portal circulation 20 min after oral administration of propionate. Propionate was orally administered at a dose of 15 mmol/kg, 5 hours after food withdrawal ($n = 5$ mice per time point; experiment was performed once). (**E** and **F**) After a propionate bolus (1 g/kg), administered orally or rectaly to male mice, blood glucose and the appearance of radiolabeled propionate were measured in blood ($n = 3$ mice per group; experiment was performed once). (**G**) Glycemic response to intraperitoneal injection of propionate (15 mmol/kg) after 18 hours of fasting ($n = 6$ mice per group; experiment was done once). (**H**) Biochemical analysis of liver glycogen 15 min after intraperitoneal injection of propionate or pyruvate (15 mmol/kg) ($n = 8$ mice per group; administration was after a 5-hour fasting, $N = 2$). (**I**) Glycogen-loaded primary rat hepatocytes were treated with propionate (1 mM) or glucagon (100 nM) in substrate-free Dulbecco's modified Eagle's medium (DMEM) for 3 hours, and glucose appearing in the medium was measured using a glucose oxidase–based assay ($n = 4$ wells per treatment, $N = 3$). All results are reported as means ± SEM. Statistical differences between two groups were determined using unpaired two-tailed Student's t test; differences among three or more groups were compared using one-way analysis of variance (ANOVA) and Tukey post hoc analysis. Responses to glucose tolerance tests between mice groups were compared using two-way ANOVA with Bonferroni post hoc analysis. $^*P < 0.05$; $^{**}P < 0.005$.

metabolic effects attributed to endogenously produced propionate and other SCFAs. In the colon, these molecules are produced primarily by fermentation of undigested carbohydrates (*11*, *12*). Several health-related benefits of dietary fibers such as increased postmeal satiety and decreased body weight and fat mass have been attributed to the production of SCFAs from fermentation (*13*, *14*). However, the direct biological and metabolic effects of SCFAs, in general, and propionate, in particular, are not defined, and their potential link to human obesity and metabolic alterations remains to be established. For example, one study in humans showed that fecal concentrations of propionate, which reflect that in the portal and systemic circulation (*12*), were found to directly correlate with body mass index (BMI) (*15*). In an independent study, higher amounts of propionate and its intestinal transporter were found in overweight and obese participants compared to lean volunteers (*16*). This observation also has been reported recently in obese children (*17*). Moreover, small interventional trials conducted in humans failed to demonstrate any beneficial effects of propionate administration (*18*, *19*). Although

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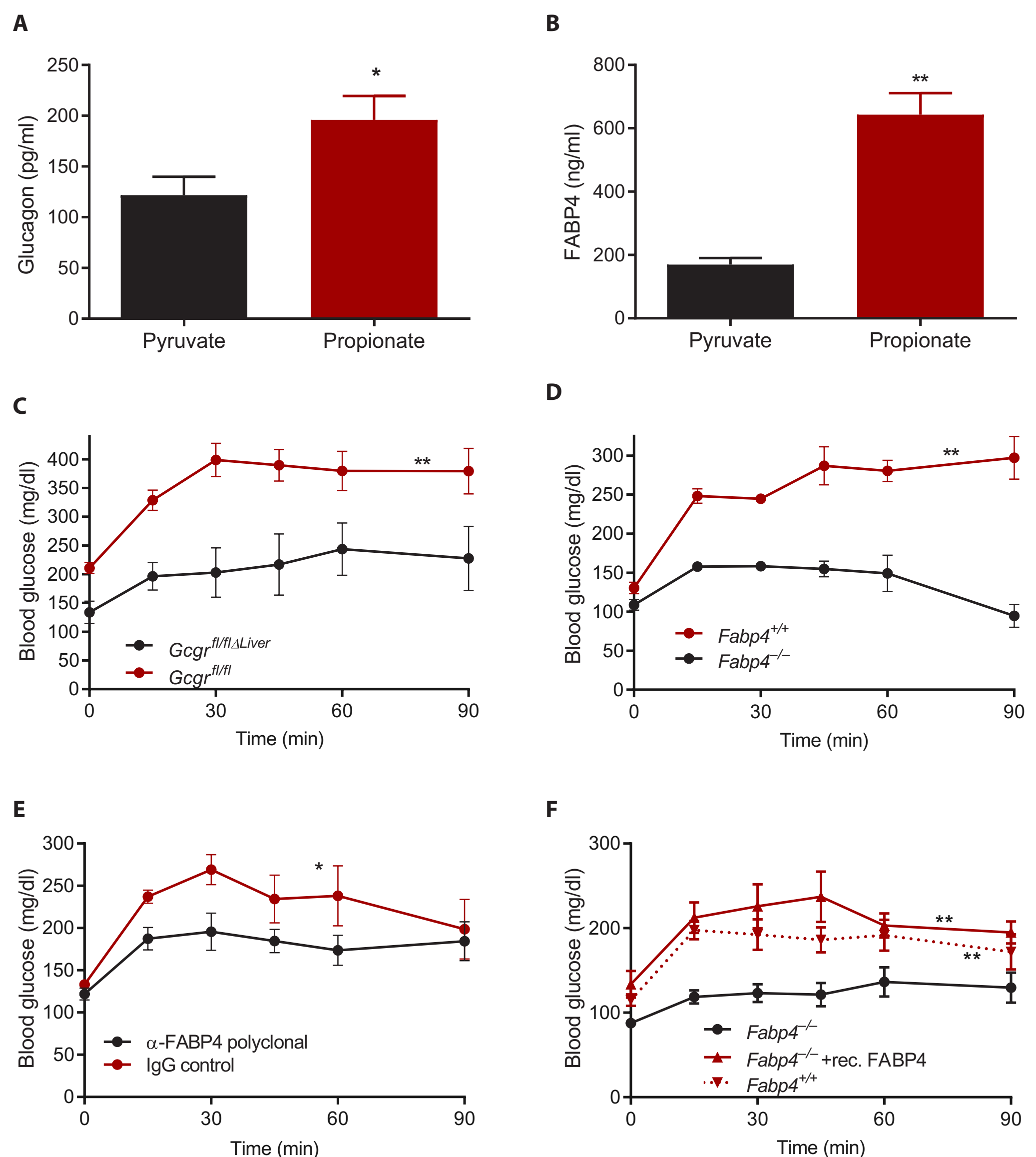

Fig. 2. Glucagon and FABP4 are required for propionate-induced hyperglycemia. (**A** and **B**) Plasma concentrations of glucagon and FABP4 were measured 30 min after propionate or pyruvate administration given 5 hours after food withdrawal (*n* = 8 male mice per group). All experiments were performed using either sodium propionate or sodium pyruvate (15 mmol/kg body weight) (*N* = 2). (**C** and **D**) Genetic deletion of the glucagon receptor in the liver ($Gcgr^{fl/fl\ \Delta Liver}$) or total genetic ablation of FABP4 protected mice from propionate-induced hyperglycemia as shown by blood glucose measurements (*n* = 4 male mice per group, *n* = 5 male mice per group, *N* = 2). (**E**) Tail vein injection of polyclonal antibody against FABP4 (0.2 mg/kg) resulted in an attenuated response to propionate as shown by blood glucose measurements (*n* = 4 male mice per group, *N* = 2). IgG, immunoglobulin G. (**F**) Reconstitution of circulating FABP4 using recombinant FABP4 (50 μg/kg) abolished the protective effect of *Fabp4* genetic deficiency as shown by blood glucose measurements (*n* = 8 male mice per group). All results are reported as means ± SEM. Statistical differences between two groups were determined using unpaired two-tailed Student's *t* test; responses to glucose tolerance tests between mice groups were compared using two-way ANOVA with Bonferroni post hoc analysis. $^*P < 0.05$; $^{**}P < 0.005$.

one study reported reduced postprandial glucose concentrations after propionate treatment, this was found to be secondary to impaired digestion of the meals and not because of improved glucose tolerance (*20*). Despite decreased absorption, propionate administration resulted in increased triglycerides and decreased high-density lipoprotein cholesterol, a common lipid abnormality observed in insulin-resistant states (*21*).

Here, we report that propionate induced an increase in hepatic glucose production in mice, mediated by activation of the sympathetic nervous system, and a subsequent surge in circulating concentrations of fasting counter-regulatory hormones: norepinephrine, glucagon, and fatty acid–binding protein 4 (FABP4 or aP2). Mice lacking either Fabp4 or the liver glucagon receptor were resistant to the effects of propionate and did not show an increase in endogenous glucose production. To assess the potential translational impact of our observations, we conducted a double-blinded, placebo-controlled crossover study in healthy human volunteers. The addition of propionate to a mixed meal at a concentration similar to that used as a food preservative resulted in an increase in postprandial glucagon, FABP4, and norepinephrine concentrations compared to a placebo-supplemented meal, leading to an increase in blood glucose and compensatory hyperinsulinemia. Chronic treatment of mice with a low dose of propionate, equivalent to the amount used in food preservation, resulted in increased adiposity and insulin resistance.

RESULTS

To study the mechanisms by which propionate could induce hyperglycemia, we used C57BL/6J mice. Sample size was chosen on the basis of pilot experiments that ensured a power of 90% and a significance of 5%. We first compared the ability of propionate and another tricarboxylic acid cycle substrate, pyruvate, to increase blood glucose in mice when given at equal molar ratios. Acute intraperitoneal administration of propionate was significantly more potent than pyruvate at increasing blood glucose, resulting in hyperglycemia in both male (Fig. 1A and fig. S1A; $P < 0.005$) and female

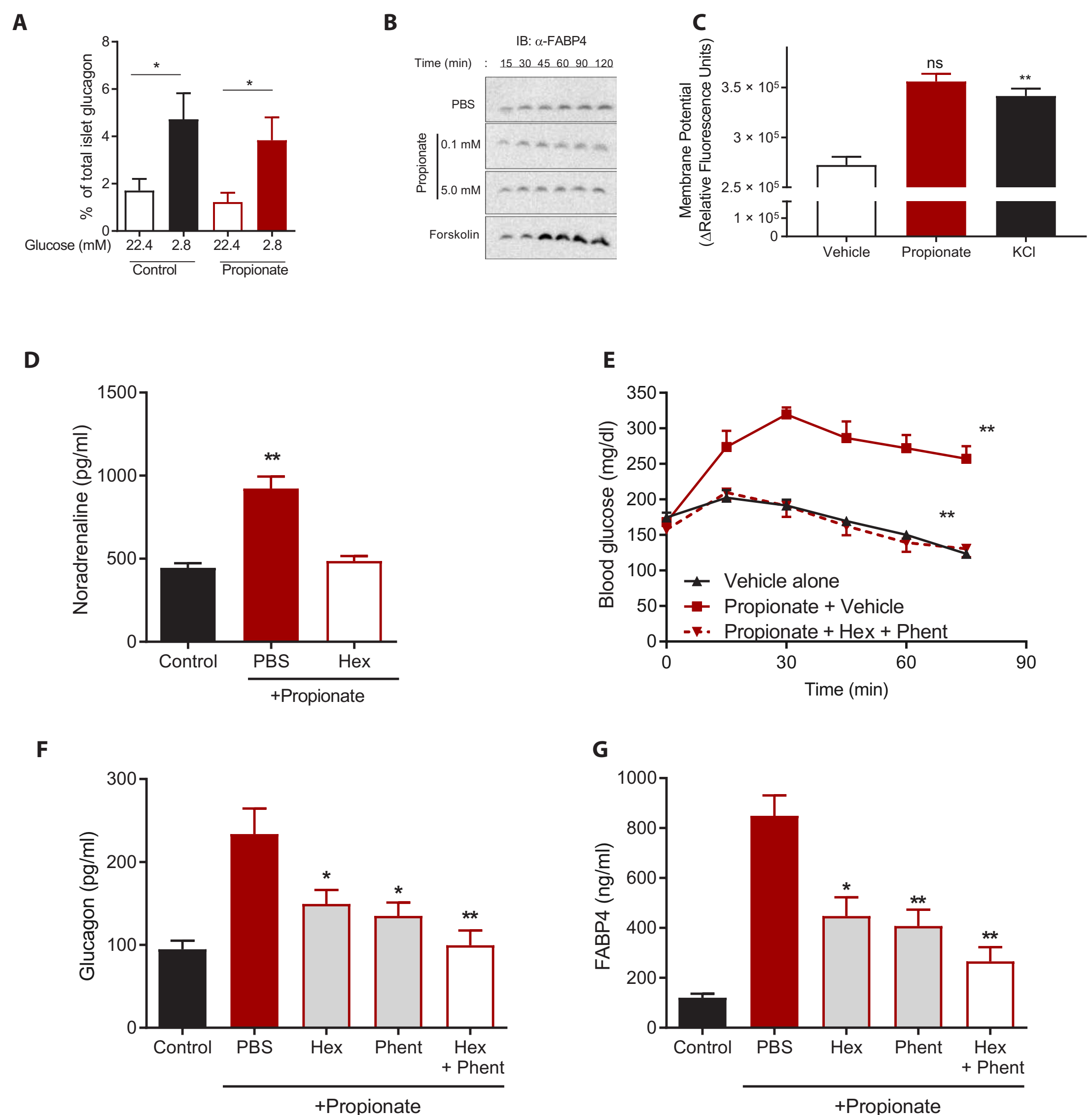

Fig. 3. The hormonal and metabolic effects of propionate are mediated by activation of the sympathetic nervous system. (**A**) Glucagon secretion was measured in isolated pancreatic islets from three wild-type C57/B6 mice. Glucagon was measured in the culture medium at low- and high-glucose concentrations in the presence or absence of 1 mM propionate (n = 15 islets for glucagon production assay). (**B**) Western blot (IB) showing the amount of FABP4 secreted from mouse adipose tissue explants at the indicated concentrations of propionate. Forskolin was used as a positive control (representative blot of n = 3). (**C**) Differentiated Neuro2A cells were treated with propionate or KCl (10 mM final), and the change in plasma membrane potential was measured (n = 8 wells per treatment; repeated with three consecutive passages of cells). ns, not significant. (**D**) Norepinephrine measurements were taken 15 min after intraperitoneal injection of propionate (15 mmol/kg) or PBS. Hexamethonium (Hex; 20 mg/kg) or PBS were intraperitoneally injected 7 min before the injection of either propionate or PBS (n = 5 mice per group, N = 2). (**E**) Blockade of sympathetic nervous system activity with phentolamine (Phent; 1 mg/kg), hexamethonium (20 mg/kg), or both (Hex + Phent) before intraperitoneal injection of propionate (15 mmol/kg), inhibited propionate-induced hyperglycemia as shown by blood glucose measurements. (**F**) Glucagon and (**G**) FABP4 plasma concentrations were collected at 30 min after intraperitoneal injection of propionate or PBS as described in (E) (n = 5 mice per group; experiment was done once). All results are reported as means ± SEM. Islet glucagon secretion was compared using a two-way ANOVA statistical test, and there was no statistical difference between treatment groups. Statistical differences between three or more groups were compared using one-way ANOVA and Tukey post hoc analysis. Responses to a glucose tolerance test between mouse groups were compared using two-way ANOVA with Bonferroni post hoc analysis. *$P < 0.05$; **$P < 0.005$.

(Fig. 1B; $P < 0.05$) C57BL/6 mice, with a concordant increase in plasma insulin concentration (fig. S1B). The ability of propionate to markedly increase blood glucose was dose related, with about two- and fourfold increases in glycemic response after increasing concentrations of propionate (Fig. 1A). This effect was similarly observed when propionate was administered by oral gavage (Fig. 1C). After oral administration, blood propionate concentrations rose rapidly and appeared in comparable amounts in portal and systemic circulations (Fig. 1D). Oral administration of ^{14}C-labeled propionate induced and maintained higher concentrations of propionate in the circulation compared to rectal administration (Fig. 1E). Accordingly, rectal administration of propionate to mice had an attenuating effect on glycemia compared to orally administered propionate (Fig. 1F), suggesting that the oral propionate may have had more pronounced systemic metabolic effects. The rapid hyperglycemic response, with blood glucose reaching as high as 300 mg/dl in nonobese, nondiabetic mice, suggested that it was unlikely to be explained entirely by increased substrate flux via gluconeogenesis or by a defect in insulin response and instead argued in favor of a possible contribution by hepatic glycogen stores. Whereas propionate induced a much greater hyperglycemic response in vivo, pyruvate was found to be superior to propionate as a gluconeogenic substrate when assessed directly in primary rat hepatocytes in vitro (fig. S1C). In support of glycogenolysis being the primary mechanism for propionate-induced

Table 1. Characteristics of participants in the clinical trial. Demographic, anthropometric, and biochemical characteristics of the 14 healthy participants randomized to a double-blind, placebo-controlled, crossover study assessing the effects of the food preservative propionate on postprandial metabolism after an 8-hour fasting. Baseline measurements were taken at the screening visit. WBC, white blood cells; TSH, thyroid-stimulating hormone.

n	14
Mean age (years)	41 ± 14
Male, *n* (%)	9 (64%)
Race, *n* (%)	
Caucasian	9 (64%)
African-American	2 (14%)
Other	3 (22%)
Body weight (kg)	74 ± 10.3
BMI (kg/m^2)	23.7 ± 2.3
Blood pressure (mmHg)	
Systolic	119 ± 14
Diastolic	75 ± 10
Fasting glucose (mg/dl)	89 ± 8
HbA1c (%)	5.5 ± 0.3
Hemoglobin (g/dl)	13.6 ± 1.2
WBC (cells/mm^3)	5.6 ± 1.2
Creatinine (mg/dl)	0.9 ± 0.1
TSH (mIU/liter)	1.9 ± 0.9

hyperglycemia, a marked increase in blood glucose was observed when food was withdrawn for 5 hours, whereas a much milder response was observed after 18 hours of fasting, when liver glycogen was largely depleted (Fig. 1G). In agreement with this, direct measurement demonstrated a marked depletion of liver glycogen stores after propionate administration as compared to pyruvate (Fig. 1H). To assess whether propionate directly stimulated glycogenolysis, we treated primary rat hepatocytes with propionate after glycogen loading. Although glucagon resulted in a significant increase in glucose released into the culture medium as expected ($P < 0.005$), no direct effect of propionate on glycogenolysis in isolated primary rat hepatocytes was observed (Fig. 1I), suggesting the presence of additional hormonal mediators in vivo.

Given that the increase in hepatic glucose production seemed to be hormonally regulated, we next evaluated the effects of propionate on circulating concentrations of glucagon and FABP4, an adipocyte-derived hormone that we recently demonstrated to stimulate hepatic glucose production (*22*). Glucagon, a potent inducer of glycogenolysis, was significantly elevated immediately after an intraperitoneal injection of propionate into mice (Fig. 2A; $P < 0.05$) and was accompanied by a marked increase in circulating FABP4 (Fig. 2B). To dissect the potential role of glucagon and FABP4 in mediating propionate-induced hyperglycemia, we first used liver-specific glucagon receptor knockout mice generated by crossing the floxed glucagon receptor mice (GCGR$^{fl/fl}$) with albumin gene promoter–driven Cre recombinase mice (*23*). This mouse model has been demonstrated to have no expression of the glucagon receptor in the liver, whereas intact expression was observed in other tissues such as the kidneys and the jejunum (*23*). Although propionate administration significantly increased blood glucose in GCGR$^{fl/fl}$ control mice, almost no increase was observed in mice lacking the liver glucagon receptor (GCGR$^{fl/fl\Delta Liver}$; $P < 0.005$; Fig. 2C). These results indicated that glucagon's actions on the liver were required for propionate-induced hyperglycemia. We next used Fabp4-deficient (Fabp4$^{-/-}$) mice to assess the potential contribution of this adipokine to propionate-induced hyperglycemia. Although propionate administration resulted in marked hyperglycemia in wild-type (Fabp4$^{+/+}$) mice, only a minor increase in blood glucose was observed in Fabp4$^{-/-}$ mice (Fig. 2D; $P < 0.005$), despite an intact increase in glucagon (fig. S2). In further support of a role for FABP4 in mediating the hyperglycemic effects of propionate, treatment of Fabp4$^{+/+}$ mice with a polyclonal anti-mouse FABP4 antibody (*22*) significantly attenuated the hyperglycemic response (Fig. 2E; $P < 0.05$). In addition, coadministration of recombinant mouse FABP4 to Fabp4$^{-/-}$ mice restored propionate-induced hyperglycemia (Fig. 2F; $P < 0.005$). Together, these results demonstrate that the hyperglycemia induced by acute propionate administration was mediated by the fasting hormones glucagon and FABP4.

We next asked whether the increase in blood concentrations of these hormones induced by propionate administration resulted from a direct effect on the corresponding end organs (pancreatic islets and adipose tissue). Glucagon secretion from isolated mouse pancreatic islets increased when islets were transferred from a high (22.4 mM) to a low (2.8 mM) concentration of glucose (Fig. 3A). This expected response of the pancreatic α-cells was not affected by the presence or absence of propionate in the culture medium, excluding a direct effect of propionate on pancreatic islets. Similarly, when mouse gonadal adipose tissue explants were examined ex vivo for FABP4 release, no increase in its secretion into the culture medium was observed after propionate treatment, in contrast to the robust response to forskolin stimulation as previously reported (Fig. 3B) (*22*, *24*). Given that ex vivo propionate treatment did not have any direct effects on glucagon or FABP4 secretion by islets or adipose tissue, we postulated that the observed in vivo effect may involve the activation of the sympathetic nervous system. This postulate was driven by recent observations of the ability of propionate to promote sympathetic outflow and norepinephrine release from adrenergic neurons (*25*) and because adrenergic stimulation is known to increase both glucagon (*26*, *27*) and FABP4 secretion (*22*, *24*). As a proof-of-principle experiment, we measured changes in membrane potential in differentiated Neuro2a cells (a neuroblastoma cell line) treated with propionate or potassium chloride (KCl, positive control). Propionate treatment was comparable to KCl in changing membrane potential, consistent with earlier observations (Fig. 3C) (*25*). Propionate was able to stimulate the sympathetic nervous system in vivo in mice, as observed by an acute increase in plasma norepinephrine shortly after propionate administration. This increase in norepinephrine could be completely blocked by pretreating mice with the nicotinic acetylcholine receptor antagonist hexamethonium (Fig. 3D). To assess whether the propionate-mediated increase in norepinephrine could explain the hyperglycemic response to propionate, we injected mice with either phosphate-buffered saline (PBS) (control), hexamethonium, phentolamine (an α-adrenergic antagonist), or both, 7 min before propionate administration. Accordingly, blocking norepinephrine release with hexamethonium, norepinephrine action with phentolamine, or both significantly prevented propionate-induced hyperglycemia (Fig. 3E and fig. S3A; $P < 0.005$). In agreement with our model, blocking sympathetic nervous system signaling also significantly

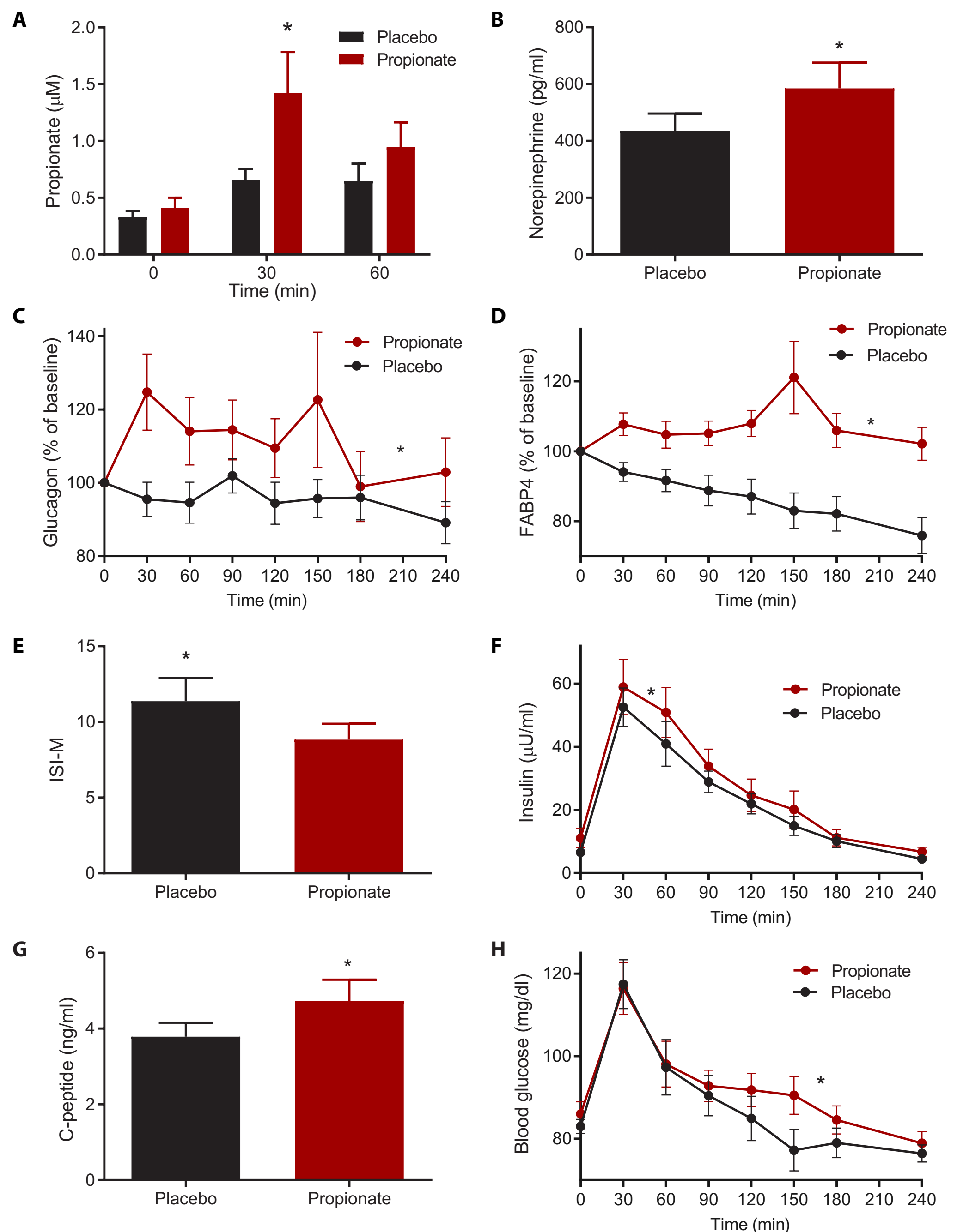

Fig. 4. The metabolic and hormonal effects of propionate in human participants. (**A**) At 30 and 60 min after consumption of a meal supplemented with either placebo or 1 g of calcium propionate, plasma propionate was measured in 14 healthy participants by GC-MS. (**B**) At 30 min after meal consumption, plasma norepinephrine was assayed. Time course of the change in plasma glucagon (**C**) and serum FABP4 concentrations (**D**) from baseline were analyzed at 30-min intervals after the mixed-meal challenge. (**E**) The Matsuda insulin sensitivity index (ISI-M) was calculated using blood glucose and serum insulin during the mixed-meal test. (**F**) Time course of the change in serum insulin at 30-min intervals and serum C-peptide (**G**) at 30 min in response to the mixed meal. (**H**) Plasma glucose concentrations during the 240-min postprandial time course. The meal was consumed after an 8-hour fasting. All results are reported as means ± SEM. Statistical differences between two groups at indicated time points were compared using paired *t* test. Hormonal and glucose response to placebo or propionate throughout the entire sampling was compared using repeated-measures two-way ANOVA with Bonferroni post hoc analysis. *$P < 0.05$.

attenuated the propionate-induced increase in both glucagon (Fig. 3F) and FABP4 (Fig. 3G) plasma concentrations ($P < 0.005$ for both). Acute administration of these drugs alone did not cause a significant drop in blood glucose (fig. S3, B and C).

Humans are exposed to increasing amounts of propionate through consumption of preserved processed foods and artificial flavorings. Thus, to assess the translational relevance of our findings, we designed a randomized, placebo-controlled, double-blinded crossover study (ClinicalTrials.gov identifier no. NCT01889446) to evaluate the metabolic effects of dietary propionate consumption in humans. We enrolled 14 lean and healthy participants (baseline characteristics summarized in Table 1) and randomized them into two groups. After 8 hours of fasting, they were provided a mixed meal with or without 1 g of calcium propionate (also known as E282) provided to us by Niacet Corporation. One week later, participants were provided with an identical mixed meal again, after crossover of the groups. Blood samples were collected at time 0, after which the mixed meal supplemented with placebo or propionate was consumed within 15 min, and blood samples were serially collected every 30 min thereafter for 4 hours. This propionate dose of 1 g is equivalent to the most commonly used amount of 0.3% (w/w) to which humans are exposed when consuming a single processed food–based meal (*10*). This minimal dose of propionate resulted in a significant increase in postprandial plasma propionate (Fig. 4A; $P < 0.05$). Similar to our

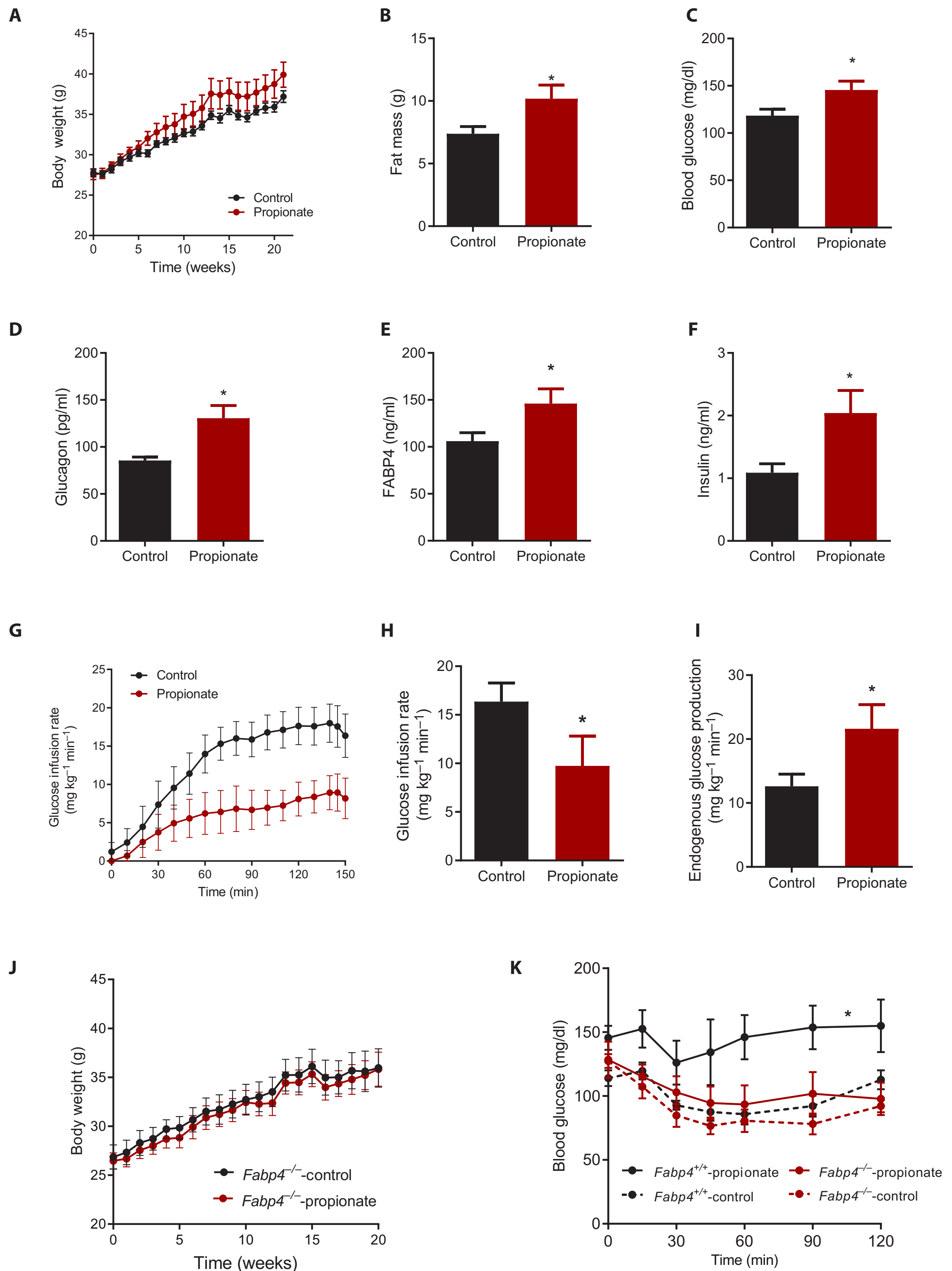

Fig. 5. Chronic propionate treatment induces FABP4-dependent weight gain and impairs glucose homeostasis in mice. (**A**) Weekly body weight measurements were taken during chronic treatment of mice with low-dose sodium propionate (150 μg/ml) or sodium chloride (91.2 μg/ml, to provide a molar equivalent of sodium as the experimental group) that was added to the drinking water ($n = 7$ mice per group, $N = 2$). (**B**) Whole-body fat mass of the two groups was determined by dual-energy x-ray absorptiometry at the end of the experiment. (**C**) Blood glucose measurements were taken in conscious animals 6 hours after food withdrawal at week 18 of treatment with either sodium propionate or sodium chloride. (**D**) Plasma glucagon measurements were taken in conscious animals 6 hours after food withdrawal at week 18 of treatment with either sodium propionate or sodium chloride. (**E**) Plasma FABP4 measurements were taken in conscious animals 6 hours after food withdrawal at week 18 of treatment with either sodium propionate or sodium chloride. (**F**) Plasma insulin measurements were taken in conscious animals 6 hours after food withdrawal at week 18 of treatment with either sodium propionate or sodium chloride. (**G**) Hyperinsulemic-euglycemic clamp studies were performed in conscious mice at the end of the intervention with either sodium propionate or sodium chloride ($n = 8$ animals per group). (**H**) Average glucose infusion rate in the clamp experiment during steady state. (**I**) Calculated endogenous glucose production rate in the clamp experiment during steady state. (**J**) Fabp4$^{-/-}$ mice were placed on chronic sodium propionate or sodium chloride treatment, and body weight was monitored weekly. (**K**) An insulin tolerance test (0.75 U/kg) was performed on Fabp4$^{-/-}$ mice (red lines) and wild-type littermate controls (black lines) after chronic propionate treatment (solid lines) or sodium chloride treatment as a control (dashed lines) ($n = 8$ animals per group). All results are reported as means ± SEM. Statistical differences between two groups were determined using unpaired two-tailed Student's t test. Response to insulin tests between mouse groups and weight gain over time were compared using two-way ANOVA with Bonferroni post hoc analysis. $^*P < 0.05$.

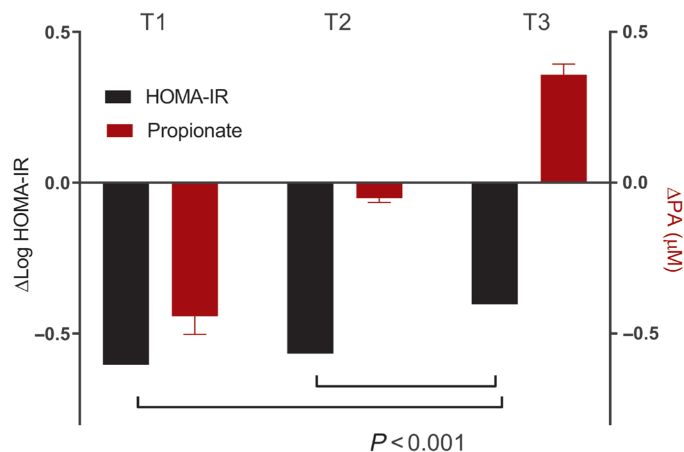

Fig. 6. Correlation of plasma propionate with insulin resistance in overweight and obese human participants. Plasma propionate was measured in 160 overweight or obese participants in the DIRECT study, at baseline and at 6 months after a dietary intervention. The study population was divided into tertiles (T1, T2, and T3) based on the change in circulating propionate from baseline to 6 months (red bars). The change in insulin resistance was assessed for each group using the HOMA-IR calculation (black bars). Correlations between plasma propionate concentrations and metabolic outcomes were performed by general linear models after adjustment for age, sex, dietary group, and baseline value of the respective outcome.

observations in mice, the propionate-containing meal taken by human participants resulted in a significant increase in plasma norepinephrine (Fig. 4B; $P < 0.05$) and a postprandial increase in both glucagon (Fig. 4C; $P < 0.05$) and FABP4 (Fig. 4D; $P < 0.005$) as compared to a placebo-supplemented meal. This rise in postprandial insulin counterregulatory responses by propionate led to a significant decrease in postmeal insulin sensitivity as assessed using the Matsuda index (ISI-M) (Fig. 4E; $P < 0.05$), with a compensatory increase in serum insulin (Fig. 4F; $P < 0.05$) and C-peptide (Fig. 4G; $P < 0.05$). This postprandial hormonal dysregulation–mediated insulin resistance with secondary hyperinsulinemia may underlie the relatively mild postprandial hyperglycemic responses among these healthy, nondiabetic volunteers compared to placebo controls (Fig. 4H). These results support the conclusion that oral consumption of a very low dose of propionate was sufficient to liberate norepinephrine and release the fasting hormones glucagon and FABP4.

We next assessed the metabolic consequences of chronic, low-dose exposure to propionate by adding a daily dose of sodium propionate (15 mg/kg) to the drinking water of wild-type C57BL/6J mice. On the basis of measurements of daily food and water intake (*28*), this concentration resulted in a daily exposure comparable to supplementation of food with 0.15 to 0.3% (w/w) of propionate, which is equivalent to the amount in a processed food–based diet for humans (*3*). This very low concentration of propionate corresponded to a negligible addition of no more than 0.033% of a daily caloric intake for a 25-g C57BL/6J mouse. To control for the minimal increment in sodium content present in sodium propionate, the same molar ratio of sodium chloride was added to the drinking water of the control group. Water intake was comparable between the two groups (fig. S4A). Although kept on a regular chow diet, the propionate-treated group gained significantly more weight over time (10.2 ± 0.59 g versus 7.77 ± 0.19 g; $P = 0.002$; Fig. 5A). This difference could be attributed to an increase in fat mass (Fig. 5B). After 6 weeks of propionate treatment and before significant changes in body weight were observed, there was an increase in blood glucose (Fig. 5C), hyperglucagonemia (Fig. 5D), elevated circulating FABP4 (Fig. 5E), and hyperinsulinemia (Fig. 5F). To directly study the effects of chronic propionate treatment on liver glucose metabolism, we conducted a euglycemic-hyperinsulinemic clamp study to assess insulin sensitivity (fig. S4B). We observed a decrease in glucose infusion rates (Fig. 5, G and H) and an increase in endogenous glucose production (Fig. 5I) in the propionate-treated mice, with no significant change in the rate of glucose disappearance (fig. S4C). Insulin plasma concentrations during the clamp studies are depicted in fig. S4D.

Given that the chronic daily exposure to low amounts of propionate resulted in a hormonal response similar to that observed after acute propionate treatment in mice or a propionate-containing mixed meal in humans, we next studied whether genetic deletion of Fabp4 could prevent these long-term metabolic alterations. In contrast to the effect on wild-type control mice, $Fabp4^{-/-}$ mice did not exhibit excessive weight gain when chronically treated with propionate (Fig. 5J). In addition, as opposed to a decreased insulin response in propionate-treated $Fabp4^{+/+}$ mice, $Fabp4^{-/-}$ mice exhibited unaltered insulin sensitivity after chronic propionate treatment (Fig. 5K). These results suggested that the effects of chronic propionate treatment are mediated, at least in part, by FABP4.

To further assess the translational relevance of these findings in mice to human obesity, we next analyzed plasma propionate concentrations among participants of the dietary intervention randomized controlled trial (DIRECT; ClinicalTrials.gov identifier no. NCT00160108) (*29*) to examine the effects of weight loss interventions on circulating propionate concentrations and metabolism. The DIRECT study was a 2-year nutritional randomized trial conducted to study the effects of the following weight-loss diets: a low-fat, restricted-calorie diet; a Mediterranean restricted-calorie diet; and a low-carbohydrate, nonrestricted-calorie diet. Overweight and obese participants were included in the study (mean age, 52 ± 7 years; 86% men; mean BMI, 31 ± 4 kg/m^2). Serum samples from 160 participants at baseline, 6 and 24 months after the start of the intervention were subjected to metabolomics analysis to determine changes in serum propionate concentrations.

At baseline, plasma propionate concentrations directly correlated with insulin resistance as evaluated using the homeostatic model assessment of insulin resistance (HOMA-IR) index (Pearson's $r = 0.156$, $P = 0.049$). In a general linear model adjusted for age, sex, initial BMI, and the extent of weight loss after 6 months of dietary interventions, a greater decline in serum propionate (from baseline to 6 months) was associated with a significant improvement in insulin resistance ($\beta = 0.632$ and $P = 0.008$; Fig. 6). Thus, in human obese participants, circulating propionate directly correlated with insulin resistance, and a greater reduction in propionate during dietary intervention was associated with a greater improvement in HOMA-IR index, regardless of the dietary intervention or initial body weight.

DISCUSSION

In this study, we report that exposure to the SCFA propionate, a food preservative, led to a rapid activation of the sympathetic nervous system and concomitantly an increase in the fasting hormones, glucagon and FABP4, in the postprandial state in mice. This increase resulted in enhanced endogenous glucose production, primarily

most likely due to hepatic glycogenolysis, leading to hyperglycemia and compensatory hyperinsulinemia. Human consumption of propionate at a dose used to extend shelf life and preserve food was sufficient to reproduce the hormonal response to acute propionate exposure observed in mice. Furthermore, chronic exposure of mice to an equivalent daily propionate dose resulted in an increase in plasma concentrations of the insulin counter-regulatory hormones, glucagon and FABP4, and the development of insulin resistance, hyperinsulinemia, and gradual weight gain. The relevance of propionate to insulin resistance and obesity in humans was also suggested in a large, long-term, dietary interventional study (DIRECT), in which the reduction in plasma propionate in response to a weight-loss diet was independently associated with improved insulin sensitivity.

The unfavorable metabolic effects that we observed upon both acute and chronic administration of propionate contrast with some reports of metabolic benefits attributed to propionate. Some studies have demonstrated that propionate suppresses food intake (*30*–*32*), inhibits lipolysis, and reduces plasma fatty acid content (*18*, *33*, *34*). However, these effects were reported when high concentrations of propionate (>4% of food) were used, more than 10-fold higher than the concentrations used for preserving foods. In studies where lower concentrations of propionate [0.5 to 2% (w/w) of food] were used, no beneficial effects could be demonstrated (*32*). In addition, despite the suggested favorable metabolic effects associated with gut microbiota–derived SCFAs, mice lacking one of the potential propionate receptors (the G protein–coupled receptor 43) were protected against the effects of a high-fat diet with a lower body fat mass and improved glucose control and insulin sensitivity (*35*). Reciprocally, a recently published study in Toll-like receptor 5–deficient mice demonstrated that the metabolic pathologies previously reported in this model are mediated by increased portal delivery of gut microbiota–derived propionate and other SCFAs to the liver (*36*). Furthermore, in line with our findings, Perry *et al*. (*7*), directly measured pyruvate cycling relative to mitochondrial pyruvate metabolism in rats and demonstrated that propionate administration increased this ratio by ~30-fold, with a concomitant increase in the rate of endogenous glucose production up to 100%. Thus, a combination of multiple mechanisms may account for the increase in endogenous glucose production observed after propionate administration to rodents. These include activation of the sympathetic nervous system leading to glucagon and FABP4 release and glycogenolysis, as well as direct activation of gluconeogenesis by increased pyruvate cycling. Thus, the sensitivity of the endogenous glucose production machinery to propionate not only makes propionate an unsuitable tracer to assess hepatic glucose metabolism (as suggested by Perry *et al.*) (*7*) but also indicates that propionate may act as a "metabolic disruptor" in the postprandial state when added to human foods.

Data in humans also indirectly support a detrimental rather than a beneficial metabolic effect of propionate. Association studies in humans have indicated a relationship between propionate and an increase in BMI (*15*–*17*). Consistent with our findings (Fig. 4), the interventional study showed a higher postprandial insulin concentration with similar blood glucose concentration in healthy participants consuming propionate-containing bread (*10*). In addition, impaired postprandial suppression of SCFAs was also observed after eating propionate-containing bread, suggesting inefficient suppression of adipose tissue lipolysis, a condition that is known to result in augmented adipocyte release of FABP4 (*24*). Neither catecholamines nor glucagon, which stimulate both lipolysis and FABP4 release (*22*, *24*), were measured in these studies (*10*). Together, the current literature suggests that orally delivered propionate does not mimic the beneficial metabolic effects attributed to SCFAs derived from bacteria in the colon and may result in adverse metabolic effects, including insulin resistance and glucose intolerance. These differential effects of oral propionate versus colonic propionate were also apparent in our tracer experiments (Fig. 1, E and F). This apparent discrepancy may be explained by different doses and routes of administration and local effects of propionate on proximal enterocytes versus distal colonocytes, as has been recently suggested for acetate (*37*). This apparent discrepancy may also be explained by the interactions of propionate and the colonic mucosa with other SCFAs and metabolites produced by the gut microbiota. The observation of a potential increase in endogenous glucose production leading to postprandial hyperinsulinemia is of concern, especially given the addition of propionate to processed foods and the compelling evidence that chronic hyperinsulinemia can drive obesity and metabolic abnormalities (*38*–*40*). Additional studies in larger human populations, with longer exposure to various doses of propionate, are needed to better elucidate the various metabolic effects of propionate in humans.

An additional conclusion arising from our work is that there is an apparent interaction between the biological activities of both FABP4 and glucagon. The inability of propionate to induce hyperglycemia when Fabp4 was either genetically deleted or pharmacologically neutralized, despite an intact increase in plasma glucagon, is intriguing. This finding suggests that FABP4 may be required for glucagon-induced glycogen breakdown and may serve as a bona fide counter-regulatory endocrine signal. Glucagon bioactivity has not been evaluated in the Fabp4$^{-/-}$ mouse, but this may provide more insight into the type 2 diabetes-resistant phenotype of these mice under high-fat diet conditions. Unraveling the potential biological and mechanistic interactions of FABP4 and glucagon represents an important area of future investigation.

Our study has several limitations. First, the acute metabolic effects of propionate observed in the human study and the association between insulin resistance and propionate concentration in the DIRECT study do not demonstrate a direct causal relationship between oral propionate consumption and the global epidemics of obesity and diabetes. In addition, we also did not examine the metabolic impact of chronic dietary propionate exposure in humans. Larger interventional studies targeting a reduction in environmental propionate and assessing metabolic and anthropometric outcomes are necessary to fully translate our findings in mice to humans. In addition, blood propionate concentrations reflect not only the systemic absorption of exogenous propionate (environmental exposure and production by gut bacteria) but also endogenously produced propionate. Propionate is the degradation product of some amino acids and of odd-chain fatty acids. Thus, blood propionate is a composite of various metabolic pathways and we cannot estimate the differential contribution of propionate-based preserved foods on systemic propionate concentrations. We were able to demonstrate that even trace amounts of propionate added to a single mixed meal were sufficient to rapidly increase blood propionate concentrations. Last, participants in the acute study were healthy, nonobese, and normoglycemic. Thus, they were able to mount a hyperinsulinemic response to overcome (at least in part) the postprandial insulin resistance induced by propionate. Therefore, the potential hyperglycemic effects

of propionate-containing foods on prediabetic and overtly diabetic participants cannot be concluded from this study.

Our findings may have implications for the current practice of food preservation. Given that the U.S. Food and Drug Administration has declared propionate to be generally recognized as safe with no known adverse effects, there is currently no limitation on its utilization other than as required by good manufacturing practice (*3*). Here, we report that exogenous propionate leads to a rapid activation of the sympathetic nervous system, resulting in an increase in both glucagon and FABP4. The increase of both of these fasting hormones in the postprandial state drives enhanced endogenous glucose production, likely due to glycogenolysis, leading to hyperglycemia and compensatory hyperinsulinemia. The hormonal responses to high-dose propionate observed in mice were also observed in humans given a much lower dose that was equivalent to that in processed foods, although the hyperglycemic response was milder. Nonetheless, repeated daily exposure to propionate for prolonged periods, as evident in our chronic propionate treatment of mice, may have important implications for public health and should stimulate a renewed interest in examining the potential actions and underlying mechanisms associated with food components such as propionate in humans. There are alternatives that could be used for food preservation [for review, see (*41*)], and if those molecules prove to be neutral in their metabolic activities, then simple alterations in manufacturing practices may yield public health benefits.

MATERIALS AND METHODS

Study design

The objectives of the study were to assess whether the commonly used food preservative propionate affects postprandial glucose metabolism, both in mice and in humans, and to identify its mechanism of action. Experiments conducted in mice included glucose tolerance tests in response to propionate or pyruvate and blood biochemistry measurements after test compound administration. The Harvard Medical Area Standing Committee on Animals approved all in vivo studies. Sample size for mouse groups was chosen on the basis of pilot experiments that ensured a power of 90% and a significance of 5%. All samples were included in the analysis unless they fell more than 2 SDs from the mean. No randomization was used for animal studies. Investigators analyzing data were blinded to mouse genotype and treatment groups. Individual level data for the mouse experiments are included in data file S1.

We conducted a double-blind, randomized, placebo-controlled, crossover clinical study (#NCT 01889446) to test whether propionate, given as a food supplement to humans, resulted in altered postprandial metabolism. The rationale was to use an amount of propionate that was similar to that used for food preservation. Thus, we supplemented a 500-kcal propionate-free meal with 1000 mg of calcium propionate or placebo as detailed below. Inclusion criteria for the study were age of 18 to 65 years, good health as evidenced by history and physical exam, and BMI of 20 to 29.9 kg/m^2. Exclusion criteria were fasting plasma glucose > 110 mg/dl, HbA1c > 6.0%, current illness other than treated hypothyroidism, blood pressure > 135/85 or systolic blood pressure < 90 mmHg, hepatic disease (transaminase more than three times normal), renal impairment (creatinine clearance < 60 ml/min), history of drug or alcohol abuse, participation in any other concurrent clinical trial, pregnant women, and participants with a history of food allergies. Baseline characteristics were collected at the screening visit. Fourteen healthy volunteers were randomized into two groups, provided with a mixed meal after 8 hours of fasting that did or did not contain 1000 mg of calcium propionate (also known as E282) provided to us by Niacet Corporation. After a 1-week washout, participants were crossed over to the other arm and provided with the other mixed meal. Blood samples were collected at time 0, after which the mixed meal supplemented with placebo or propionate was consumed within 15 min, and blood samples were serially collected every 30 min thereafter for 4 hours. All assays of blood, including those for C-peptide, glucagon, and catecholamine, were analyzed at the LabCorp laboratories using validated assays that are used for clinical care of patients. Determination of FABP4 was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems). The ISI-M, which was found to correlate with the rate of whole-body glucose disappearance during a euglycemic insulin clamp study (*42*), was calculated using glucose and insulin values obtained during the mixed-meal test. ISI-M = $10{,}000/(G_0 \times I_0 \times G_{\text{mean}} \times I_{\text{mean}})^{1/2}$, where G and I represent plasma glucose (mg/dl) and insulin (μU/ml) concentrations, respectively, and "0" and "mean" indicate the fasting value and mean value during administration of the mixed-meal test, respectively. The Brigham and Women's Hospital (BWH) Institutional Review Board (IRB) approved this study, which was conducted at the outpatient facility of the BWH Clinical and Translational Science Center. Informed consent was obtained from all participants.

An additional patient cohort from DIRECT was used to study the effects of weight loss interventions on plasma propionate concentrations and metabolic characteristics. The DIRECT study was a workplace dietary intervention trial that took place in Dimona, Israel (*29*). Briefly, 322 overweight or obese participants (age, 52 ± 7 years; women, 14%; BMI, 31 ± 4) were randomized to one of the following weight-loss diets: a low-fat, restricted-calorie diet; a Mediterranean restricted-calorie diet; and a low-carbohydrate nonrestricted-calorie diet. Here, we subjected plasma samples from the DIRECT study for metabolomics analysis. These plasma samples were obtained from 160 participants at the baseline visit and after 6 months of the dietary intervention (the weight loss nadir). The samples were analyzed by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany using electrospray tandem mass spectrometry (*43*). Correlation between propionate and metabolic outcomes was performed by general linear models after adjustment for age, sex, dietary group, and baseline value of the respective outcome. This study was approved by the IRB of Sheba Medical Center, Tel-Hashomer, Israel.

Animals

Male littermates in the C57BL/6J background at 6 weeks of age were obtained from the Jackson laboratory (Bar Harbor, ME, stock no. 000664). All mice were maintained on a 12-hour light/dark cycle in a pathogen-free barrier facility with free access to water and food. The Harvard Medical Area Standing Committee on Animals approved all in vivo studies. Sample size was chosen on the basis of pilot experiments that ensured a power of 90% and a significance of 5%. All samples were included in analysis unless they fell more than 2 SDs from the mean. No randomization was used for animal studies. Investigators analyzing data were blinded to mouse genotype and treatment groups.

Wild-type or Fabp4-deficient (Fabp4$^{-/-}$) mice (*44*) on C57BL/6J background were used to evaluate the effects of propionate in the

presence or absence of the adipokine FABP4. The liver-specific glucagon receptor–deficient mice were generated by crossing the floxed G-coupled glucagon receptor mice ($GCGR^{fl/fl}$, provided to us by D. Drucker, University of Toronto) (*23*) with albumin gene promoter–driven Cre recombinase mice (Alb_Cre, B6.Cg-*Speer6-ps1*$^{Tg(Alb-cre)21Mgn}$/J; the Jackson laboratory, Bar Harbor, ME, stock no. 003574). The characterization of this mouse model was previously described in detail (*23*). For studies assessing the metabolic consequences of chronic, low-dose exposure to propionate, we conducted three independent experiments in which either C57BL6 mice or wild-type and $Fabp4^{-/-}$ mice (6 weeks old) were exposed to propionate (~15 mg/kg) (the food preservative sodium propionate; Sigma-Aldrich, St. Louis, MO) added to the drinking water. On the basis of measurements of daily food and water intake (*28*), we estimate this concentration to correspond to supplementation of food by 0.15 to 0.3% (w/w) of propionate, equivalent to the propionate content in a processed food–based diet (*3*). The same molar ratio of sodium chloride was added to the drinking water of the control group. Weekly body weight and blood glucose were measured. Blood was collected at 6 weeks of intervention for assessment of glucagon, insulin, and FABP4 concentrations. Insulin tolerance test (0.75 units/kg body weight) was performed after 6-hour food withdrawal. Hyperinsulinemic-euglycemic clamp studies were performed as previously described (*22*). Briefly, 4 days before experiments, mice were anesthetized, and the right jugular vein was catheterized with a PE10 polyethylene tube (inside and outside diameters, 0.28 and 0.61 mm, respectively; Becton Dickinson, Franklin Lakes, NJ) filled with heparin solution (100 USP U/ml). The distal end of the catheter was tunneled under the skin, exteriorized in the intrascapular area, heat-sealed, and then placed in a pocket under the skin until the day of the experiment, when it was re-exteriorized. During hyperinsulinemic-euglycemic clamp, steady-state tracer analysis was used for calculations, and glucose-specific activity was verified for steady state.

Propionate and pyruvate tolerance tests

C57BL/6J mice aged 10 to 14 weeks were injected intraperitoneally with propionate at concentrations of 0.5 to 2.0 g/kg body weight (5 to 20 mmol/kg) or equal molar ratios of sodium pyruvate (both from Sigma-Aldrich, St. Louis, MO). All studies were performed after 5 hours of food withdrawal, unless stated otherwise in the figure legend. Similar experiments were performed using an oral gavage instead of intraperitoneal injections. PBS was used as a vehicle. All treatments were administered after adjustment of the pH to 7.4. Blood glucose was measured at baseline and at 15- to 30-min intervals (as indicated in the figures) using a Breeze2 glucometer (Bayer, Leverkusen, Germany). Hexamethonium (20 mg/kg) and/or phentolamine (1 mg/kg) or PBS were injected 7 min before the intraperitoneal injection of propionate, and both blood glucose and hormones were measured as indicated in the figure legends. A polyclonal anti-mouse FABP4 antibody (Santa Cruz Biotechnology, Dallas, TX) or an immunoglobulin G control were intravenously injected to the tail vein of wild-type mice 1 week before the propionate tolerance test. A concentration of 50 μg/kg body weight of a recombinant FABP4 protein produced in *Escherichia coli* (or PBS as a vehicle control) was injected with propionate into $Fabp4^{-/-}$ mice.

Rectal propionate administration

Anesthetized animals were either orally gavaged or rectally administered 1-^{14}C–labeled propionate (0.1 mCi/kg) (MP Biomedicals, Santa Ana, CA) mixed with sodium propionate (1 g/kg) and trace amounts of dextran blue (for visual confirmation of administration). Rectal administration was performed via 3-cm PE50 tubing attached to a blunt 23G needle. After administration, animals were sampled via tail vein bleeding for blood collection. At the end of the experiment, animals were euthanized via cervical dislocation, and digestive tract was removed for visual inspection of dextran blue. At all instances, orally administered bolus was confined to stomach and rectally administered bolus was confined between duodenum and distal colon. Radioactivity was measured by decolorizing 5 μl of blood with hydrogen peroxide and mixing it with Ecoscint H (National Diagnostics, Atlanta, GA) at 1:10 (v/v) ratio and reading on a MicroBeta2 instrument (PerkinElmer, Waltham, MA).

Hormonal response to propionate or pyruvate treatment

Mouse serum concentration of FABP4 was determined using a commercially available ELISA system (BioVendor, Czech Republic). Noradrenaline was measured using an ELISA obtained from Eagle Biosciences Inc. (Nashua, NH). Plasma for glucagon determination was collected in EDTA-containing tubes supplemented with aprotinin and was assayed using a Glucagon Quantikine ELISA system (R&D Systems, Minneapolis, MN). Plasma concentration of insulin was measured using the Mercodia Mouse Insulin ELISA (Uppsala, Sweden).

Liver glycogen determination

Liver glycogen content was determined using a phenol-sulfuric acid–based colorimetric method after perchloric acid extraction (*45*). Briefly, about 100 mg of liver tissue was snap-frozen in 2-ml round bottom Eppendorf tubes in liquid nitrogen until sample processing. On the day of sample processing, liver was homogenized in 10% perchloric acid using 1:3 (v/v) ratio of tissue to 1.0-mm zirconium oxide beads (#ZrOB10; Next Advance, Troy, NY). Homogenized samples were aliquoted into three equal parts after centrifugation. One aliquot was saved for analysis of free sugars (S_0). The other homogenate was mixed with concentrated sulfuric acid (1:10, v/v) for hydrolysis of sugars (S_h). Although vortexing, 5% phenol was added (1:2, v/v) to the sample for color development. After 30-min incubation, 200 μl of the sample was transferred to a microplate for absorbance measurement at 490 nm. Glycogen from bovine liver (#G0885, Sigma-Aldrich) was used to generate the standard curve for glycogen, and dextrose was used to generate standard curve for free sugars. Glycogen content was calculated by subtracting free sugars from final glycogen hydrolyzed homogenate ($S_h - S_0$). The last aliquot was used to measure protein concentration. Curve fitting and linear regression analysis was done using SoftMax Pro version 5.1.

Membrane polarization in Neuro2a cells

Neuro2a cells were obtained from the American Type Culture Collection (#CCL-131; Manassas, VA) at passage 27 and maintained in DMEM with 10% fetal bovine serum (FBS) supplementation. Before the experiments, the cells were seeded onto a 96-well black, clear-bottom plates, and differentiation was induced with serum deprivation. After 2 days, when neurite growth was observed, cells were washed with Dulbecco's phosphate-buffered saline (DPBS; #59331C, Sigma-Aldrich) and incubated with Screen Quest Membrane Potential Assay Kit reagents (#36005; AAT Bioquest, Sunnyvale, CA) as per the manufacturer's instructions. Measurements were performed on a Spectramax Paradigm instrument (Molecular Devices, San Jose, CA).

FABP4 secretion from adipose tissue

Perigonadal adipose depots were removed for preparation of explants. Adipose tissue samples were washed in PBS and serum-free DMEM consecutively and minced into roughly 2-mm-sized pieces with scissors. Explants were washed with DMEM and incubated for 1 hour in the same medium for recovery. After recovery, fresh DMEM was added and secreted FABP4 in culture medium was measured every 15 min in the presence or absence of increasing concentrations of propionate or forskolin (20 μM). Samples were subjected to Western blot analysis using an anti-FABP4 antibody (Santa Cruz Biotechnology).

Glucagon secretion from isolated pancreatic islets

The methods for isolating islets from mice were described previously (*46*). Briefly, the pancreatic duct was perfused with 2.5 ml of Liberase RI (3 mg/ml) (Roche, Penzberg, Germany), after which it was excised and disaggregated by shaking for 24 min at 37°C. The islets were partially isolated by sedimentation and then hand-picked from the acinar tissue debris under a dissecting microscope. To stimulate glucagon secretion, islets were transferred from high glucose (22.4 mM) to low glucose (2.8 mM) medium. Conditioned medium was assayed for glucagon production using a commercial glucagon ELISA system (R&D Systems).

Glucose production in primary hepatocytes

Primary rat hepatocytes plated onto 24-well collagen-coated plates were acquired from the Massachusetts General Hospital Cell Resource Core (Boston, MA). All cells were changed to low serum medium (199 medium supplemented with 0.1% FBS, penicillin/streptomycin, and 1 μM dexamethasone) overnight upon arrival. For glycogenolysis experiments, cells were washed with DPBS (Thermo Fisher Scientific, Waltham, MA) and changed to glycogen loading medium [phenol red–free DMEM supplemented with 10 mM glucose, 15 mM fructose, and Humulin (100 mU/ml) (Eli-Lilly, Indianapolis, IN)] for 2 hours. At the end of glycogen loading, cells were washed three times with DPBS and changed to substrate-free DMEM and incubated in the presence or absence of stimulants for 3 hours. For gluconeogenesis experiments, cells were washed three times with DPBS and incubated with substrate-free DMEM and 1 μM dexamethasone for 3 hours. At the end of the glycogen depletion period, cells were washed again and fresh substrate-free DMEM with indicated substrates was added to the cells. Medium was harvested at the end of 2 hours. At the completion of each experiment, cells were lysed with 0.5 N NaOH and neutralized with 0.5 N HCl before protein concentration determination using commercial Bradford assay. Glucose secreted into the medium was assayed using Amplex Red–based glucose oxidase assay (Thermo Fisher Scientific, #A22189).

Statistics

All values are presented as means ± SEM unless stated otherwise in the figure legends. Paired *t* tests were used to compare changes in hormone concentrations at 30 min versus the baseline within groups of mice or human participants. Paired *t* tests were also used to compare the 30-min concentrations of norepinephrine and C-peptide between treatment groups in the crossover human study. Unpaired *t* tests were used to compare FABP4, glucagon, and norepinephrine amounts between propionate and pyruvate treatment groups in mice. For the propionate and pyruvate tolerance tests in mice and for comparing glucagon and FABP4 responses in the human study, we used a two-way ANOVA with Bonferroni post hoc analysis. One-way ANOVA and Tukey post hoc analysis were used to compare area under the curve between multiple groups. A two-tailed P value <0.05 was considered statistically significant. All statistical analyses were done using GraphPad Prism 7.0.

SUPPLEMENTARY MATERIALS

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Fig. S1. Propionate induces hyperglycemia and hyperinsulinemia in mice.
Fig. S2. Fabp4 deficiency does not affect glucagon secretion in response to propionate administration.
Fig. S3. The effect of sympathetic blockade on blood glucose.
Fig. S4. Results of hyperinsulinemic-euglycemic clamp studies.
Fig. S5. Proposed model for data presented in this study.
Data file S1. Source data for Figs. 1 to 6 and figs. S1 to S4.

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