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Manuscript

MICROBIOTA-PRODUCED SUCCINATE IMPROVES GLUCOSE HOMEOSTASIS VIA INTESTINAL GLUCONEOGENESIS

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SUMMARY

Beneficial effects of dietary fiber on glucose and energy homeostasis have long been described, focusing mostly on the production of short-chain fatty acids by the gut commensal bacteria. However, bacterial fermentation of dietary fiber also produces large amounts of succinate and, to date, no study has focused on the role of succinate on host metabolism. Here, we fed mice a fiber-rich diet and found that succinate was the most abundant carboxylic acid in the cecum. Dietary succinate was identified as a substrate for intestinal gluconeogenesis (IGN), a process that improves glucose homeostasis. Accordingly, dietary succinate improved glucose and insulin tolerance in wild-type mice, but those effects were absent in mice deficient in IGN. Conventional mice colonized with the succinate producer *Prevotella copri* exhibited metabolic benefits, which could be related to succinate-activated IGN. Thus microbiota-produced succinate is a previously unsuspected bacterial metabolite improving glycemic control through activation of IGN.

INTRODUCTION

Type 2 diabetes is the consequence of an imbalance in glucose homeostasis, which results in fasting hyperglycemia with deleterious effects on health. It is well established that improving the quality of diet is a main factor to tackle this disease. Management of metabolic diseases can be facilitated by an increased consumption of dietary fiber (Anderson et al., 2009). Several studies have pointed out the benefits of fiber-rich diets in both lean (Robertson et al., 2003) and obese diabetic subjects (Mendeloff, 1977). While most of these studies attribute the benefits of dietary fiber to the production of short-chain fatty acids (SCFAs) by bacterial fermentation in the gut (Flint et al., 2012), no study has analyzed the role of other organic acids (such as succinate or lactate) produced by the gut microbiota during this fermentation process.

Little is known about the function of succinate in the body. Classically described as a key intermediate in microbial propionate synthesis (Miller and Wolin, 1979; Reichardt et al., 2014), succinate should not accumulate in the bowel to a substantial extent (Macfarlane and Macfarlane, 2003). Interestingly, its concentration in the cecum is increased by feeding dietary fibers (Everard et al., 2014; Jakobsdottir et al., 2013), and this increase is even more significant when dietary fiber is fed in conjunction with high-fat diet (Jakobsdottir et al., 2013; Zhong et al., 2015).

In a recent study (De Vadder et al., 2014), we specified that the SCFAs butyrate and propionate activate a gut-brain neural circuit involving the induction of intestinal gluconeogenesis (IGN), a process initiating multiple metabolic benefits (Mithieux, 2014; Mithieux and Gautier-Stein, 2014). In particular, propionate acts as a glucose precursor in the gut, which leads to the activation of a portal glucose sensor (Delaere et al., 2012), resulting in improved glucose tolerance and insulin sensitivity (De Vadder et al., 2014). Propionate is first

metabolized to propionyl-CoA, further carboxylated to methylmalonyl-CoA, which is finally transformed to succinyl-CoA and incorporated into the Krebs cycle in the gluconeogenesis pathway. Glutamine, another important substrate for gluconeogenesis is metabolized *via* glutamate and alpha-ketoglutarate before incorporation into the Krebs cycle (Croset et al., 2001; Mithieux et al., 2004). As an integral intermediary metabolite of the Krebs cycle (just downstream of succinyl-CoA), we hypothesized that succinate could be efficiently converted into glucose in the intestinal mucosa and postulated that succinate production by commensal bacteria could improve glucose metabolism through increased IGN. Interestingly, in a recent study we showed that the gut commensal *Prevotella copri*, a known succinate producer, improves glucose homeostasis by a yet-to-be-defined mechanism of communication with the host (Kovatcheva-Datchary et al., 2015).

Here we evaluated the possibility of succinate serving as a precursor of IGN. Using dietary intervention studies we assessed the impact of a diet enriched in fiber or in succinate on the host glucose homeostasis. Using mice with an intestinal-specific knockout of *G6pc*, the catalytic subunit of glucose-6-phosphatase (I-G6pc -/- mice) (Penhoat et al., 2011), we tested whether IGN had a causal role in the metabolic impact of fiber or succinate feeding.

RESULTS

Dietary fructo-oligosaccharides (FOS) increase cecal succinate concentration with concomitant changes in the gut microbiota composition, independently of the genotype

Diet strongly alters the composition of the gut microbiota (David et al., 2014; Turnbaugh et al., 2008; Wu et al., 2011), thus possibly altering the production of microbial metabolites. Accordingly, we fed wild type (WT) mice a high-fat/high-sucrose (HF-HS) diet supplemented with FOS and we observed a marked increase in succinate concentration in the cecum (Figure 1A). Our previous results have shown that metabolic benefits induced by FOS

feeding are absent in mice lacking *G6pc*, a key enzyme in gluconeogenesis, specifically in the intestinal mucosa (I-G6pc -/- mice) (De Vadder et al., 2014). When these mice were fed the same diet as WT mice, we observed the same increase in cecal succinate, independently of the genotype (two-way ANOVA, P = 0.37). Furthermore, among all carboxylic acids that we measured in the cecum, succinate exhibited the largest relative increase in response to FOS feeding (Figure 1B, Figure S1A-D). No such changes in succinate concentration occurred in portal vein or vena cava plasma after FOS feeding (Figure S1E-F), suggesting that most succinate produced in the cecum is metabolized in the intestine.

In our previous study we observed that the colonic microbiota was modified after FOS feeding in both wild-type and I-G6pc-/- mice, this was independent of the genotype (De Vadder et al., 2014). Here we analyzed the microbiota composition in the cecum, where the bulk fermentation occurs. In line with our previous findings, FOS feeding strongly modified the cecal microbiota, independently of the genotype (Figure 1D-E). As previously described (Murphy et al., 2010), Firmicutes and Bacteroidetes represented more than 80% of the reads in the cecal microbiota (Figure 1C). FOS feeding had a major impact on the ratio between these two main phyla in the cecal microbiota, associated with a significant decrease in the Firmicutes/Bacteroidetes ratio in both WT and I-G6pc -/- mice (Figure 1D). In line with the increased succinate concentration after FOS feeding is the relative enrichment in Bacteroidetes (Figure 1E), a phylum that comprises species known to be major propionate and/or succinate producers in the intestine (Miller and Wolin, 1979; Reichardt et al., 2014). Furthermore, among all Bacteroidetes we found that *Bacteroides* exhibited the largest relative abundance increase in response to FOS feeding (Figure 1E). Furthermore, caecal succinate concentration correlated significantly with the abundance of *Bacteroides* (Figure 1F), whose species can produce both succinate and propionate in levels dependent on the nutrient availability in the gut (Fischbach and Sonnenburg, 2011; Miller and Wolin, 1979). It is

noteworthy that we did not find any sequence corresponding to *Prevotella* species, which are important succinate producers from the Bacteroidetes phylum that have previously been linked to improved glycemic control ((Tilg and Kaser, 2011; Kovatcheva-Datchary et al., 2015). This may be due to the fact that *Prevotella* growth is promoted by diet enriched in dietary fibers and depleted in presence of fat (De Filippo et al., 2010; Wu et al., 2011), whereas *Bacteroides* abundance in humans has been associated with diet enriched in protein and animal fat (Wu et al., 2011).

Succinate feeding improves glucose homeostasis in an IGN-dependent manner

To determine whether succinate feeding promoted beneficial effects similar to those of FOS, and whether these effects were the consequence of induced IGN, we fed WT and I-G6pc -/- mice with a HF-HS diet supplemented with succinate. Supplementation with succinate significantly improved glucose and insulin tolerance in WT, but not I-G6pc -/- mice, compared with mice fed control diet without succinate supplementation (Figure 2A-F). Thus, the succinate-mediated improvement in glucose control requires IGN. Using two-way ANOVA, we evaluated the relative effects of diet and genotype in the observed phenotypes. Regarding glucose tolerance, we observed significant interaction between diet and genotype ($P = 0.046$, 8.9% of total variance), with diet being the major driver of the beneficial effects ($P < 10^{-4}$, 46.9% of total variance). Diet also had a major effect ($P = 0.031$, 20.3% of total variance) for the improved insulin tolerance.

The levels of propionate or other SCFAs were not altered in the cecum, suggesting that succinate had direct effects on IGN (Figure S2A-B). Cecal succinate levels did not increase (Figure S2C), suggesting that most dietary succinate was absorbed prior to the cecum. Taken together, these data suggest that IGN plays a causal role in the improvement in glucose homeostasis associated to succinate feeding.

Interestingly, these modifications in glucose metabolism were associated with a resistance to weight gain only in succinate-fed WT mice (Figure 2G-I). However, this decrease in body weight was not linked to a decrease in food intake (Figure 2J), suggesting that succinate feeding increases energy expenditure, as previously observed with FOS and propionate feeding (De Vadder et al., 2014).

Succinate is converted into glucose by the gut and decreases hepatic glucose production

We next quantified intestinal glucose production (IGP) in rats fed a succinate-enriched chow diet (composition available in Table S1) during three weeks. We used an approach combining glucose tracer infusion at steady state and arterio-portal glucose difference determination, allowing us to quantify IGP and total endogenous glucose production (EGP) concomitantly, as previously described (Croset et al., 2001; De Vadder et al., 2014). Upon infusion of [3-^{3}H] glucose, the [3-^{3}H] glucose specific activity was significantly decreased by 6% in the portal vein compared to the carotid artery (Figure 3A), highlighting that unlabeled (newly synthetized) glucose had been released by the intestine. In line with tracer dilution, the calculated IGP accounted for about 16-17% of total EGP (Figure 3A). We therefore investigated whether carbons from succinate could be detected in *de novo* synthetized glucose released by the intestine. We fasted rats for 24 hours and then infused a [U-^{14}C]-succinate solution into the jugular vein, as previously described (De Vadder et al., 2014). As shown in Figure 3B, after the infusion there was a 4.9% increase in [^{14}C]-glucose specific activity in the portal vein plasma. This suggests that the intestine is able to efficiently convert succinate into glucose. Since we previously reported that IGP is hardly detectable under normal feeding conditions, one can here estimate that the bulk of intestinal glucose produced stems from succinate.

Furthermore, we quantified G6Pase activity in the intestine of C57Bl/6 mice fed HF-HS diet with or without succinate. Unlike other microbiota-produced metabolites such as

propionate and butyrate (De Vadder et al., 2014), succinate did not induce G6Pase activity in the small intestine of these mice (Figure 3C). Similarly, there was no increase in the phosphoenolpyruvate carboxykinase protein (the other key regulatory enzyme of gluconeogenesis) studied by Western-blot, in the intestine of succinate-fed mice (data not shown). It should be noted that G6Pase activity is assessed at maximal velocity, so any variation in the activity of the enzyme depends only on the amount of active enzyme. This suggests that succinate acts as a glucose precursor in the gut, without affecting the amount of gluconeogenic enzymes. However, since dietary succinate did not seem to reach the caecum (see above), gene expression analyses were performed in the jejunum. Thus, one cannot rule out a possible effect of succinate on gene expression in the distal gut, when succinate is produced there through fermentation of fiber. Interestingly, we observed a significant decrease in the capacity of hepatic glucose production in WT mice. Indeed, we noted a 25% decrease in the liver G6Pase activity in succinate-fed WT mice (Figure 3D). In agreement with a decrease in the glucose-6 phosphate hydrolysis in the liver, we observed significant increases in both liver glucose-6-phosphate and glycogen contents in these mice (Figure 3E-F). This suppression in hepatic glucose production was absent in succinate-fed I-G6pc -/- mice, further emphasizing the link between succinate-induced IGN and the suppression of hepatic glucose production (Figure 3E-F). Statistical analysis showed a major role of genotype in the observed variations in hepatic metabolism (e.g. genotype accounting for 68% of total variance in glycogen content, $P < 10^{-4}$), with strong interaction between diet and genotype (glycogen content: 17% of total variance, $P < 10^{-4}$). These data highlight the key role of IGN in driving the improvements associated to succinate feeding.

Colonization of conventional mice with the succinate producer *Prevotella copri* increases cecal succinate and inhibits hepatic glucose production

In populations with a diet essentially based on dietary fiber, metagenomics studies have shown an increase in the abundance of bacteria from the genus *Prevotella* (Schnorr et al., 2014). Furthermore, we previously showed that mono-colonization of germ-free (GF) chow-fed Swiss-Webster mice with *P. copri* significantly increases the levels of succinate in the cecum, with no increase in any other carboxylic acid, and improves glucose tolerance with concomitant increase in glycogen storage in the liver (Kovatcheva-Datchary et al., 2015). To assess whether this succinate-producing bacterium could exert its metabolic benefits via succinate and IGN, we orally administered conventionally raised (CONV-R) C57Bl/6 WT and I-G6pc -/- mice daily with live culture of *P. copri*. For both genotypes, the presence of *P. copri* increased glucose tolerance (Figure S3A-B), with similar changes in insulin secretion (Figure S3C-D). This was associated with a reduction in hepatic G6Pase activity (Figure S3E), with a very significant contribution of both the genotype and treatment (respectively 28% and 55% of total variance, $P < 10^{-4}$, two-way ANOVA). It is noteworthy that CONV-R WT mice that received oral gavage with *P. copri* had increased cecal succinate but no increased portal succinate when compared to controls (Figure S3G-H). This suggests that succinate could be utilized by the gut mucosa, presumably to glucose synthesis. No increase in cecal succinate was observed in I-G6pc -/- mice colonized with *P. copri*. Taken together, this suggests that *Prevotella* produced succinate can be a substrate for IGN, but *Prevotella* has also additional succinate-independent effects that improve glucose metabolism, in agreement with our previous observations (Kovatcheva Datchary et al., 2015).

DISCUSSION

While succinate has been shown to decrease the proliferation rate in the colonic mucosa of rats (Inagaki et al., 2007), so far no study has reported the effect of succinate on intestinal metabolism or on glucose control. Here we examined the effect of microbiota-produced succinate on glucose homeostasis, with a special focus on IGN, an intestinal

function that has been previously described as a key regulator of energy homeostasis (De Vadder et al., 2014; Troy et al., 2008). We found that succinate could be produced by the gut microbiota in response to FOS-enriched diets and incorporated as a substrate in IGN, thus improving glycemic control and energy metabolism *via* beneficial effects on hepatic glucose production and body weight. Following our data relating to propionate (De Vadder et al., 2014), we here identify another bacteria-produced metabolite that can directly modulate glucose metabolism in the host's intestine and influence systemic energy homeostasis.

Dietary interventions, especially *via* supplementation with soluble fibers, are efficient for modulation of the gut microbiota and improvement of host physiology (Neyrinck et al., 2012). However, the ability of the host to modify its intestinal glucose metabolism in response to such dietary changes appears to have the causal role in the observed metabolic improvements (De Vadder et al., 2014). Here we show that succinate-induced benefits are, as for propionate, dependent on the capacity of the host to induce IGN. We confirmed the effect of IGN using I-G6pc -/- mice (i.e. mice that cannot convert succinate into glucose in the intestine), and show that the anti-diabetic and anti-obesity effects of succinate supplementation are absent in these mice. However, we cannot exclude that succinate-mediated improvement of glucose metabolism may be caused at least in part by the recently described receptor *Sucnr1*, which is expressed in both the liver and intestine (He et al., 2004). Despite this, even if IGN cannot solely account for all the beneficial effects observed, we clearly show that the improved glucose metabolism deriving from succinate can only occur in the presence of IGN.

Unfortunately, our experiments of colonization with *P. copri* did not allow us to conclude about the role of succinate production and IGN in the metabolic benefits associated with this bacterium (Kovatcheva-Datchary et al., 2015). Indeed, our data were consistent with metabolic benefits dependent on IGN activated by succinate only in CONV-R WT mice.

However, in CONV-R I-G6pc -/- mice, metabolic benefits took place in absence of IGN, suggesting that the bacterium *per se* can have beneficial effects independently of succinate. It is remarkable that in I-G6pc -/- mice colonized with *Prevotella* there was no increase in the concentration of succinate in the cecum. This suggests that, specifically in these mice, the host intestinal succinate metabolism and/or the microbial ecology could be altered in the presence of *P. copri*. How the deficiency in IGN and/or the presence of the probiotic modify the microbial ecology in these mice needs to be studied further.

We have previously shown that altered microbiota composition after prebiotic treatment with FOS or barley kernels ameliorates glucose control and favors glycogen storage in the liver (De Vadder et al., 2014; Kovatcheva-Datchary et al., 2015). In this study, we extend these data by deciphering that IGN is activated by succinate, an organic acid deriving from FOS. We also show that probiotic supplementation with succinate-producing bacteria such as *P. copri* in a conventional context (CONV-R WT) mice increases the production of succinate, this being associated with the inhibition of hepatic glucose production, independently of any nutritional intervention. This emphasizes the relevance of the colonization with *Prevotella* to improve glucose control in a physiological context.

In conclusion, despite the generally accepted dogma that an intermediary metabolite is unlikely to exert a regulatory role, we here decipher the previously unsuspected beneficial effect of a microbiota-derived metabolite: succinate. The latter acts as a substrate of IGN and leads to an inhibition of hepatic glucose output and to dramatic improvements in glucose and energy metabolism. An increase in glucose release by the liver is considered a causal factor of insulin resistance and type 2 diabetes, while its suppression prevents obesity and diabetes (Abdul-Wahed et al., 2014). We confirm that IGN is essential to translate the changes in the microbiota metabolites in response to prebiotic diets into metabolic benefits, providing mechanistic insights on how the microbiota function may influence host metabolism. This

further emphasizes the interest of a mechanism that could be targeted for treating and/or preventing impaired glucose metabolism, paving the way for future innovative approaches of dietary and/or probiotic interventions to treat metabolic diseases such as obesity and diabetes.

EXPERIMENTAL PROCEDURES

Animals

All protocols in this work were performed according to the recommendations of our local animal ethics committees for animal experimentation, which gave their authorization (number DR-2013-23 for University Lyon 1; number 339-2012 for University of Gothenburg).

Adult C57BL/6J mice, aged 12-14 weeks at the beginning of the experiments, were housed in a climate-controlled room (22 ± 2°C) subjected to a 12 h light/dark cycle (7:00 AM - 7:00 PM), with free access to water and food. Male Sprague-Dawley rats (Charles River), aged 6 to 8 weeks and weighing 275-300 g at the time of their arrival, were housed under similar conditions. I-G6pc -/- mice were generated as described previously (Penhoat et al., 2011), and experiments were performed 5 weeks after gene deletion. For colonization experiments, C57Bl6/J male mice were given daily gavage for 7 days with live *P. copri* strain DSM18205 (DSMZ - German Collection of Microorganisms and Cell Cultures). Sodium succinate (Sigma) or FOS (Orafti P95, kindly donated by Beneo) was incorporated into the diet at 5% wt/wt (succinate) or 10% wt/wt (FOS). Standard diet was SAFE A04 (Augis, France) and HF-HS diet was prepared at Unité de Préparation des Aliments Expérimentaux (INRA Jouy-en-Josas, France; composition in Table S1). Prior to diet change, animals were fed standard diet and groups were designed to match food intake and body weight. Animals were then fed the special diet for 21 days.

Sample collection for microbial and SCFA analysis

Mice were fasted for 6 h and euthanized by cervical dislocation. Blood samples were

collected from the portal vein and vena cava. The intestine (including the colon) and liver were sampled and immediately put in liquid nitrogen. For plasma analysis, blood samples were centrifuged and plasma collected and stored at -80°C before the assay.

SCFA assay

SCFAs were measured in 50 µL of plasma samples after acidification and extraction into diethyl ether by gas chromatograph coupled with mass spectrometer detector (7890A and 5975C, Agilent Technologies). Details of the assay are given in Supplemental Experimental Procedures.

Genomic DNA purification, 16S rRNA gene amplification, and sequence analyses

Genomic DNA was isolated from colon segments as described by Salonen et al., 2010. The V1-V2 region of bacterial 16S rRNA gene was amplified using the 27F and 338R primers fused with 454 Titanium sequencing adapters. PCR was performed and samples were pooled and sequenced using Roche 454 GD-FLC system. Details are given in Supplemental Experimental Procedures.

Glucose (GTT) and insulin tolerance tests (ITT)

Animals were fasted for 16 (GTT) or 6 hrs (ITT) and then received an injection of glucose (1 g/kg b.w., i.p.) or insulin (0.5 U/kg b.w., Insulatard, Novo Nordisk). Blood glucose was monitored for 120 minutes using a glucometer (Accu-Check, Roche) on samples collected from the tip of the tail vein.

Biochemical assays

G6Pase activity and glycogen and G6P assays were performed based on the protocols described by Baginski et al., 1974 and Pfleiderer, 1974.

Determination of intestinal glucose fluxes

After a 6-hr fast, rats were anesthetized with 2% isoflurane and fitted with polyethylene catheters inserted into the right jugular vein for [3-^{3}H] glucose (Perkin-Elmer) infusion and

the left carotid artery for blood sampling. For succinate incorporation studies, rats were fasted for 24 hrs and fitted with catheters as described above. [U-^{14}C] Succinc acid sodium salt (Hartmann Analytic) was infused for 90 min. Sampling and calculations are described in detail by Croset et al., 2001.

Statistical analyzes

Data are presented as mean ± SEM or as box plots show maximum, minimum, median and interquartile range. The appropriate test that was used is described in the figure legends. $P < 0.05$ was considered as statistically significant.

AUTHOR CONTRIBUTIONS

F.D.V., P.K.-D., F.B. and G.M. conceived the experiments; F.D.V., P.K.-D., C.Z. and A.D. performed the experiments; F.D.V., P.K.-D, F.B. and G.M. wrote the manuscript. All authors read and agreed on the final version of the manuscript.

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FIGURE LEGENDS

Figure 1: FOS-feeding increases succinate abundance in the cecum, independent of the genotype

A and B. Succinate **(A)** and total SCFA **(B)** content in the cecum of mice fed regular high fat/high sucrose (HF-HS) or FOS-supplemented (HF-HS + FOS) diet. **C.** Abundance plot of the most important phyla in each group. **D and E.** Relative ratio of Firmicutes/Bacteroidetes **(D)** and abundance of genus in the Bacteroidetes phylum **(E)** in the cecum. **F.** *Bacteroides* abundance in the cecum positively correlates with the amount of succinate.

*, $P < 0.05$ (when not indicated HF-HS vs. HF-HS + succinate); **, $P < 0.01$; ****, $P < 10^{-4}$, adjusted *P*-value, two-way ANOVA followed by Tukey's test corrected for multiple comparisons; § indicates significant effect of genotype ($P = 0.0117$, two-way ANOVA). Data

are mean ± SEM; box plots represent minimum, maximum, interquartile range and median. $N = 5 - 6$ mice per group.

Figure 2: Succinate-mediated improvements in glucose tolerance and insulin sensitivity are absent in I-G6pc -/- mice

A to C. Glucose tolerance test was performed in 16 hour-fasted wild type **(A)** or I-G6pc -/- **(B)** mice after 21 days on succinate-enriched high fat/high sucrose diet. Total glucose area under the curve (AUC) was calculated **(C)**. **D to F.** Insulin tolerance test was performed in 6 hour-fasted wild type **(D)** or I-G6pc -/- **(E)** mice after 25 days on succinate-enriched high fat/high sucrose diet. Total glucose area under the curve (AUC) was calculated **(F)**.

G to I. Evolution of body weight gain after one **(B)**, two **(C)** and three **(D)** weeks of diet. **J.** Mean food intake over 2 weeks in mice fed high fat/high sucrose (HF-HS) or succinate-supplemented (HF-HS + succinate) diet.

*, $P < 0.05$ (when not indicated vs. HF-HS); **, $P < 0.01$; ***, $P < 0.001$, adjusted *P*-value, two-way ANOVA followed by Tukey's test corrected for multiple comparisons. Data are mean ± SEM of $N = 6$ mice per group.

Figure 3: Effect of succinate enrichment on glucose production in jejunum and liver

A. Endogenous glucose production (EGP) and intestinal glucose fluxes were determined in rats fed a succinate-enriched diet for 3 weeks. **B.** Determination of intestinal incorporation of [$U^{14}C$]-succinate into glucose in 24 hour-fasted rats. SA: specific activity.

**, $P < 0.01$ vs. value in artery, Student's two-tailed *t*-test for paired values.

C. Effect of succinate enrichment on intestinal G6Pase activity in wild type mice fed a high fat/high sucrose diet. **D to F.** Effect of succinate supplementation on liver G6Pase activity **(D)** and G6P **(E)** and glycogen **(F)** contents in wild type and I-G6pc -/- mice fed a high fat/high sucrose diet.

*, $P < 0.05$; ****, $P < 10^{-4}$, adjusted P-value, two-way ANOVA followed by Tukey's test corrected for multiple comparisons.

Data are mean ± SEM of $N = 6$ animals per group.

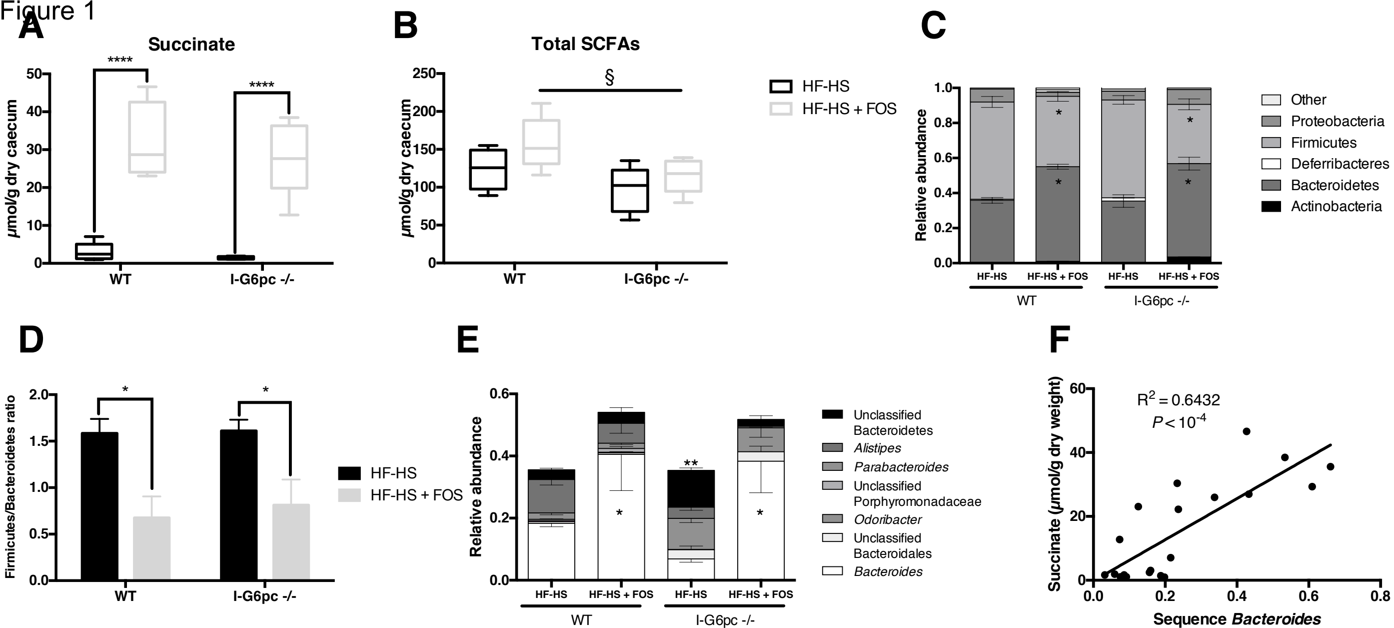
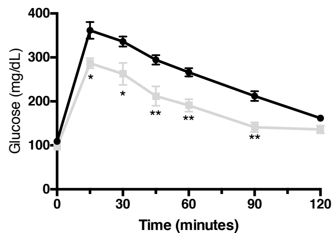
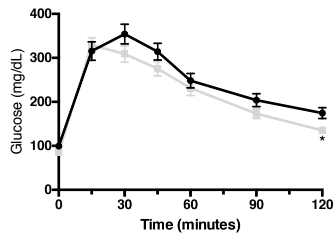
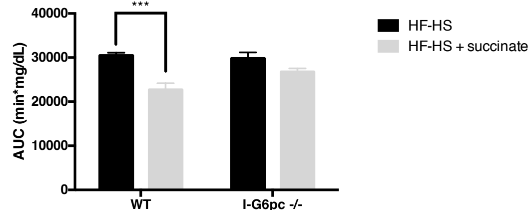


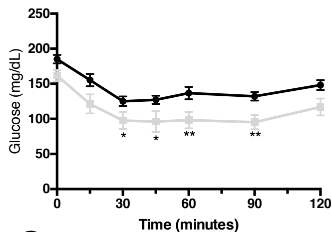
Figure 1
A
Succinate
****
****
µmol/g dry caecum
WT
I-G6pc -/-
B
Total SCFAs
§
µmol/g dry caecum
WT
I-G6pc -/-
HF-HS
HF-HS + FOS
C
Relative abundance
HF-HS
HF-HS + FOS
HF-HS
HF-HS + FOS
WT
I-G6pc -/-
Other
Proteobacteria
Firmicutes
Deferribacteres
Bacteroidetes
Actinobacteria
D
Firmicutes/Bacteroidetes ratio
WT
I-G6pc -/-
HF-HS
HF-HS + FOS
E
Relative abundance
HF-HS
HF-HS + FOS
HF-HS
HF-HS + FOS
WT
I-G6pc -/-
Unclassified Bacteroidetes
Alistipes
Parabacteroides
Unclassified Porphyromonadaceae
Odoribacter
Unclassified Bacteroidales
Bacteroides
F
$R^2 = 0.6432$
$P < 10^{-4}$
Succinate (µmol/g dry weight)
Sequence Bacteroides

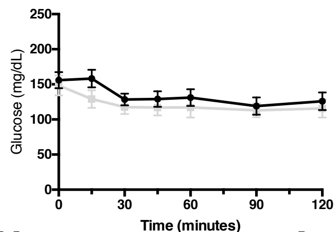
Figure 2 **WILD TYPE** **I-G6pc -/-**

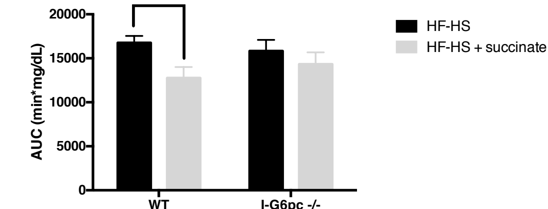


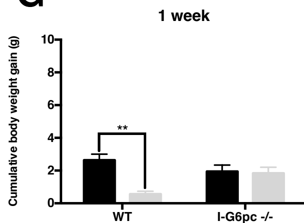


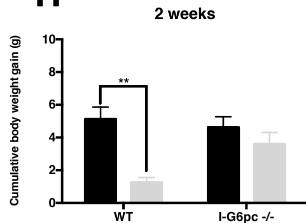


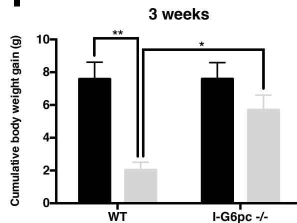


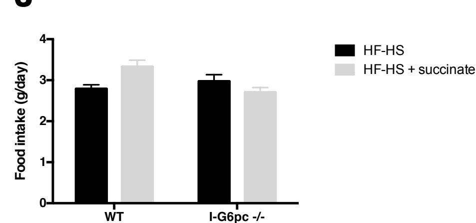












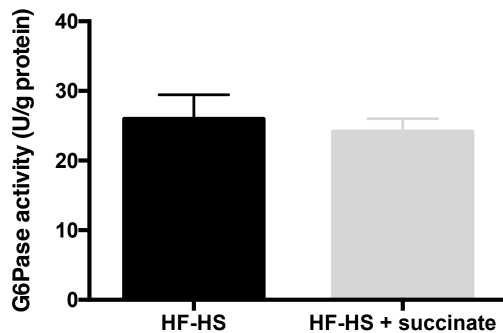
Figure

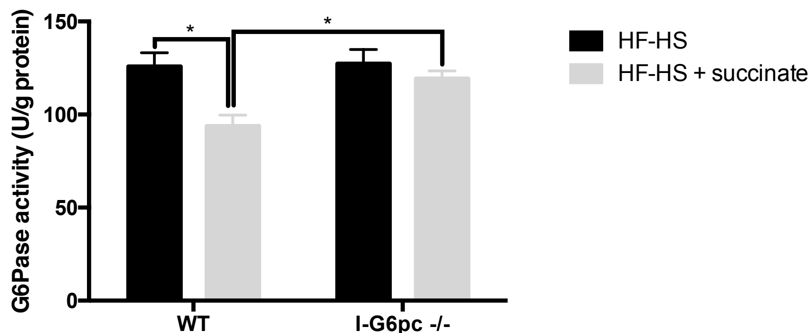
A

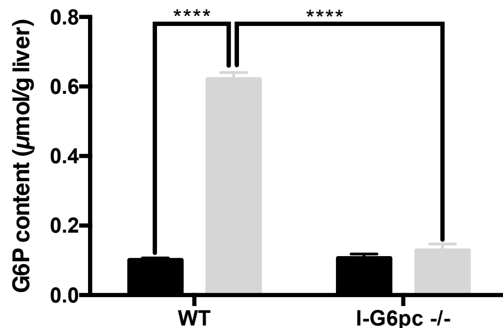
Diet	[3-^{3}H] glucose SA (dpm/nmol) Artery	[3-^{3}H] glucose SA (dpm/nmol) Vein	Glucose concentration (mmol/L) Artery	Glucose concentration (mmol/L) Vein	IGP (µmol/kg/min)	IGP (%EGP)	EGP (µmol/kg/min)
Succinate	11.3 ± 0.3	10.6 ± 0.3**	11.20 ± 0.26	10.98 ± 0.20	10.5 ± 2.0	16.4 ± 3.1	64.0 ± 1.7

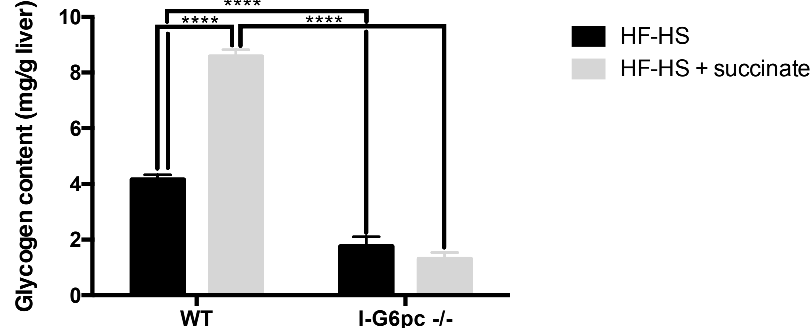
B

Precursor infused	[^{14}C] glucose SA (dpm/µmol) Artery	[^{14}C] glucose SA (dpm/µmol) Vein
[U-^{14}C] Succinate	1,558 ± 133	1,635 ± 118
	($\Delta_{v\text{-}a}$ = 4.9%, P = 0.044)	









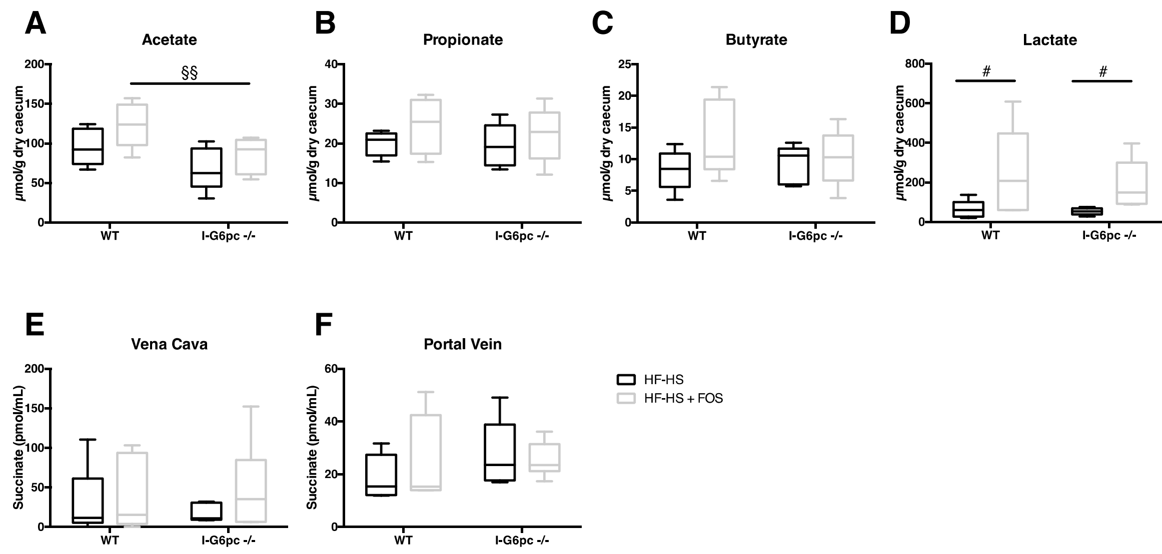


Figure S1

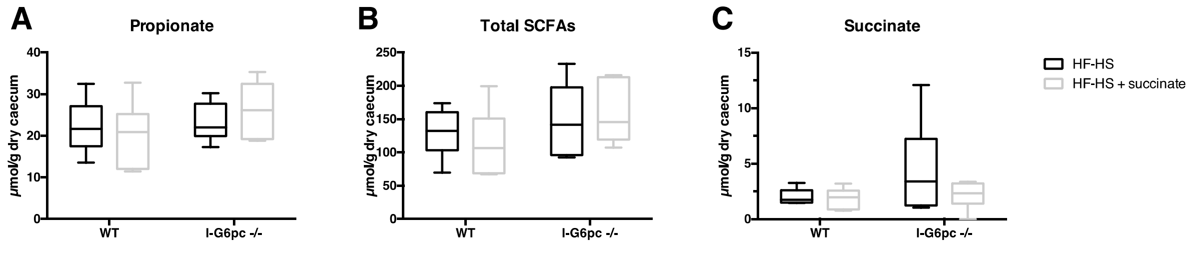


Figure S2

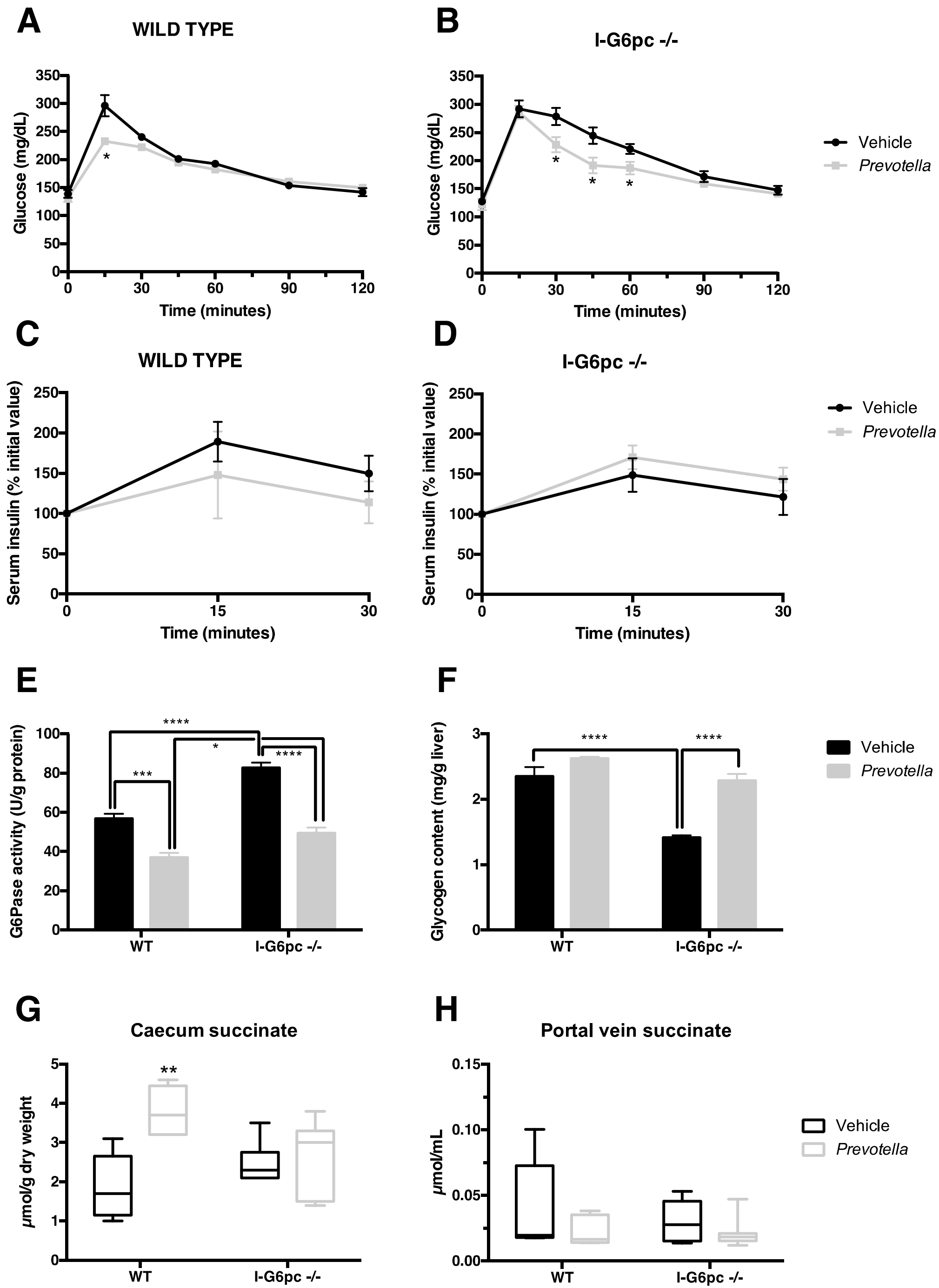


Figure S3

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Concentration of organic acids in caecum and plasma after FOS feeding, related to Figure 1

A to D. Caecum concentration of acetate **(A)**, propionate **(B)**, butyrate **(C)** and lactate **(D)** of wild-type (WT) and I-G6pc -/- mice.

E and F. Serum concentration of succinate in vena cava **(E)** and portal vein **(F)** serum of wild-type (WT) and I-G6pc -/- mice.

§§ indicates significant effect of genotype ($P = 0.0085$, two-way ANOVA); # indicates significant effect of diet ($P = 0.012$, two-way ANOVA).

Box plots represent minimum, maximum, interquartile range and median. $N = 5 – 6$ mice per group.

Figure S2: Concentration of organic acids in caecum after succinate feeding, related to Figure 2

Caecum concentration of propionate **(A)**, succinate **(B)** and total SCFAs **(C)** of wild-type (WT) and I-G6pc -/- mice.

Box plots represent minimum, maximum, interquartile range and median. $N = 6$ mice per group.

Figure S3: Probiotic treatment with *Prevotella copri* improves glucose tolerance and inhibits hepatic glucose production, related to Figure 3

A to D. Glucose tolerance test was performed in 16 hour-fasted wild type **(A)** or I-G6pc -/- **(B)** mice after 7 days of gavage with *Prevotella copri* or vehicle solution. Serum insulin was measured 0, 15 and 30 minutes after glucose injection in wild type **(C)** and I-G6pc -/- **(D)** mice.

E and F. Liver G6Pase activity **(D)**, and glycogen content **(E)** of the aforementioned mice.

G and H. Succinate content in the cecum **(G)** and in the portal vein **(H)** of the aforementioned mice.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 10^{-4}$, adjusted *P*-value, two-way ANOVA followed by Tukey's test corrected for multiple comparisons. Data are mean ± SEM; box plots represent minimum, maximum, interquartile range and median. $N = 5$-7 mice per group.

Table S1. Composition (% of dry weight) of diets used, Related to Experimental Procedures

	SAFE A04	HF-HS
Starch	68	17
Protein	18	22
Dietary Fiber	4.5	0
Lipids	3.4	36
Sucrose	0	17

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

SCFA assay. A mix of 1 M [1-^{13}C] acetate, 0.2 M [6-^{2}H] propionate and 0.2 M [4-^{13}C] butyrate, 0.5 M [^{13}C]-lactate and 40 mM [$^{13}C_4$]-succinic acid was added as internal standard. Prior to injection, the samples were derivatized with *N*-tert-butyldimethylsilyl-*N*-methyltrifluoracetamide (MTBSTFA; Sigma) at room temperature. Quantitation of the measured metabolites was completed in selected ion monitoring acquisition mode by comparison to labeled internal standards. The *m*/*z* ratios of monitored ions were as follows: 117 (acetic acid), 131 (propionic acid), 145 (butyric acid), 261 (lactic acid), 289 (succinic acid), 121 ([1-^{13}C]-acetate), 136 ([6-^{2}H] propionate), 149 ([4-^{13}C] butyrate), 264 (^{13}C-lactate) and 293 ([4-^{13}C]-succinic acid).

Genomic DNA purification, 16S rRNA gene amplification, and sequence analyses

Three independent 25 µL PCR reactions were performed for each sample using 1.5 U of FastStart Taq DNA Polymerase (Roche) and PCR was performed at conditions: one cycle of 3 min at 95°C, 25 cycles: 20 s at 95°C, 30 s at 52°C and 60 s at 72°C, and 10 min at 72°C. The resulting product was checked for size and purity on 0.8% Agarose-GelRed gel, further purified (NucleoSpin 740609, Macherey-Nagel, Germany) and quantified with the Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, CA). All samples were pooled in equal amounts (20 ng/µL) and purified again with magnetic beads (AMPure XP, Beckman, Danvers, MA) to remove short amplification products. The purified pooled products were sequenced (Roche *454 GS-FLX* system*, Titanium chemistry,* by *GATC*, Konstanz, Germany*)*. 454 reads were denoised using the denoiser_preprocess.py and denoiser.py, tools available in QIIME and sequences were further analyzed as described in detail by (Larsson et al., 2012). We retained 98,502 sequences for 21 mouse colon samples with an average of 4,924 sequences per sample (3,509 to 6,038 sequences). One of the samples contained 9 sequences and therefore was

excluded from analysis.

Plasma insulin. Blood was collected from the tip of the tail vein during the glucose tolerance test at 0, 15 and 30 minutes. Samples were centrifuged and plasma was collected. Insulin concentration was assayed using ultra-sensitive mouse insulin ELISA kit (Crystal Chem 90080), according to the manufacturer's instructions.

SUPPLEMENTAL REFERENCES

Larsson, E., Tremaroli, V., Lee, Y.S., Koren, O., Nookaew, I., Fricker, A., Nielsen, J., Ley, R.E., and Bäckhed, F. (2012). Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. Gut *61*, 1124–1131.