Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome

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Obesity, insulin resistance and the metabolic syndrome are associated with changes to the gut microbiota; however, the mechanism by which modifications to the gut microbiota might lead to these conditions is unknown. Here we show that increased production of acetate by an altered gut microbiota in rodents leads to activation of the parasympathetic nervous system, which, in turn, promotes increased glucose-stimulated insulin secretion, increased ghrelin secretion, hyperphagia, obesity and related sequela. Together, these findings identify increased acetate production resulting from a nutrient–gut microbiota interaction and subsequent parasympathetic activation as possible therapeutic targets for obesity.

Previous studies have shown that both increases1–5 and decreases6,7 in plasma and faecal short-chain fatty acid (SCFA) concentrations can be associated with overfeeding, obesity and the metabolic syndrome. However, whether and how alterations in SCFAs play a causal role in the development of obesity is unknown. Because plasma SCFA concentrations do not fully represent the SCFA load presented to the body, we developed a method to measure whole-body turnover rates of acetate, propionate, and butyrate by gas chromatography–mass spectrometry (GC–MS; as described in the Supplementary Methods) and found that, in contrast to propionate and butyrate, whole-body acetate turnover as well as plasma and faecal acetate concentrations were markedly increased in insulin-resistant rats after 3 days or 4 weeks on a high-fat diet (HFD) (Fig. 1a, b and Extended Data Fig. 2a–j).

Next we sought to determine the source of the increased acetate turnover in HFD-fed rats. We measured tissue acetate concentrations and dilution of the [13C]-acetate label during an infusion of [13C]acetate, and found each to be increased in the luminal contents of the caecum and ascending colon, with HFD-fed rats exhibiting more than a twofold increase in total acetate in the caecum and colon as well as in the brain and in [13C] bicarbonate incorporation into [13C]acetate compared to chow-fed rats (Figs 1c, d, 2a and Extended Data Fig. 2k). In order to determine conclusively the source of the increased acetate production in HFD-fed rats, we conducted four independent in vivo experiments to distinguish colon lumen acetate production from production by the rest of the body: we 1) washed out the contents of the caecum and colon with a saline flush; 2) ligated the portal vein below the splenic juncture; 3) performed an acute colectomy. Each of these interventions reduced whole-body acetate turnover by 75–90% (Fig. 2b–f). Together, these data strongly suggest that the gut microbiota is the source of most of the increase in endogenous acetate production in HFD-fed rats. We next showed that faecal material can generate acetate in vitro from [13C] glucose or [13C]fatty acids, and that boiling or irradiating the faeces prevents the production of acetate, suggesting a role for faecal microbes in generating acetate (Extended Data Fig. 2l–n). Consistent with this hypothesis, treatment of the faecal material with either or both of the broad-spectrum antibiotics vancomycin and gentamycin markedly reduced acetate production (Extended Data Fig. 2o).

Acetate drives insulin secretion

Next we examined glucose-stimulated insulin secretion (GSIS) during a hyperglycaemic clamp and measured marked increases in GSIS in 3-day and 4-week HFD-fed rats (Fig. 3a and Extended Data Fig. 3a–c). To determine whether the associated increases in acetate turnover drove this increased GSIS, we performed hyperglycaemic clamps in chow-fed rats given intra-arterial infusions of acetate to match whole-body acetate turnover to that measured in HFD-fed rats. Acetate infusion in chow-fed rats replicated the increases in GSIS measured in HFD-fed rats (Fig. 3b, c and Extended Data Fig. 3d–g), strongly implicating increased acetate turnover in driving acute increases in GSIS in HFD-fed rodents. In contrast, supplementing butyrate in chow-fed rats to match the turnover rates observed in HFD-fed rats had no effect on GSIS (Extended Data Fig. 3h–m).

To evaluate further the effects of alterations in food intake on gut acetate production, we starved 4-week HFD-fed rats for 48 h and found that this intervention resulted in ~50% reductions in whole-body acetate turnover and in GSIS; however, replacing acetate by arterial infusion of 20 μmol kg⁻¹ min⁻¹ acetate resulted in restoration of GSIS in rats after 48 h food deprivation (Extended Data Fig. 4a–f). Next we performed a series of dietary interventions to assess whether simple caloric excess or variations in nutrient composition8–10 were responsible for the increased acetate turnover measured in HFD-fed rats. Pair-feeding with isocaloric portions of chow or HFD produced no change in acetate turnover or GSIS, whereas dietary interventions resulting in increased caloric intake increased acetate turnover and GSIS proportionally to the total calories consumed (Extended Data Fig. 4g–n; R² = 0.90). To examine the role of the gut microbiota in acetate-induced hyperinsulinaemia, we treated HFD-fed rats with broad-spectrum, poorly absorbable oral antibiotics and measured a 70% reduction in GSIS during a hyperglycaemic clamp. This reduction in GSIS was acutely reversed by infusion of acetate to match plasma acetate turnover in HFD-fed rats (Fig. 4d, e and Extended Data Fig. 4o–s).

To establish a causal relationship between the microbiota and GSIS, we next transferred faecal material from chow- or HFD-fed donor rats to chow- or HFD-fed recipients. Consistent with previous reports4,11–14, culture-independent 16S rRNA sequencing of donor...
and recipient faecal microbiomes revealed an increase in the relative abundance of bacteria belonging to the phylum Firmicutes and a decrease in the relative abundance of representatives of the phylum Bacteroidetes in fresh faecal pellets from HFD-fed donors relative to chow-fed donors, and faecal transplantation altered the recipient animal microbiome to resemble that of the donor (Extended Data Fig. 5a–f). Notably, these faecal transplantations also transferred the corresponding acetate turnover, faecal acetate, and GSIS from the donor group to the recipient group (Fig. 3f, g and Extended Data Fig. 6a–e). However, transplantation of microbiota from chow-fed donors into chow-fed recipients by an identical procedure did not alter microbiota or metabolic phenotypes (Fig. 3f, g and Extended Data Figs 5a–f, 6a–e).

Having found a strong causal relationship between acetate turnover and GSIS, we next examined the mechanism by which increased acetate turnover caused increased GSIS. We first investigated whether acetate could stimulate GSIS through a direct effect on β-cells, perhaps by increasing acetylarnitine concentrations. However, we found that neither acetate nor acetylarnitine stimulated GSIS in isolated islet perifusions, ruling out a direct effect on β-cells (Fig. 3h and Extended Data Fig. 6f–h). In addition, concentrations of β-cell stimulatory amino acids and plasma glucagon were unchanged or reduced in the acetate-infused rats (Extended Data Fig. 6i–l). A small (about 2 pM) but significant (P < 0.05) increase in plasma glucagon-like peptide-1 (GLP-1) concentration was measured in rats after 120 min of acetate infusion (Extended Data Fig. 6m). Because GLP-1 can stimulate GSIS, we treated acetate-infused rats with a GLP-1 inhibitor; this treatment produced no change in GSIS (Extended Data Fig. 6n–s), demonstrating that these small changes in GLP-1 were not responsible for the increased GSIS in acetate-infused rats.

### Acetate drives GSIS via parasympathetic input

As parasympathetic input is a well-known stimulator of β-cell insulin secretion, we next measured plasma gastrin concentrations as a marker of parasympathetic activity in rats acutely infused with acetate. Plasma gastrin increased threefold after 60 min of infusion with 20 μmol kg⁻¹ min⁻¹ acetate (Fig. 4a). Increases in brain acetate concentrations in the acetate-infused animals confirmed the ability of acetate infused systemically to enter the brain circulation (Fig. 4b). Because vagal stimulation has been shown to drive, and vagotomy has been shown to suppress, basal and glucose-stimulated insulin secretion, we hypothesized that vagotomy would reduce GSIS in acetate-infused rats. Consistent with this hypothesis, vagotomized rats infused with acetate exhibited an approximately fourfold reduction in plasma insulin concentrations throughout a hyperglycaemic clamp without any change in plasma glucagon concentrations, when compared with intact rats infused with acetate (Fig. 4c and Extended Data Fig. 7a–h). In addition, treatment with the parasympathetic blocker atropine before the acetate infusion abolished the ability of acetate to stimulate GSIS, without any effect on plasma glucagon concentrations (Fig. 4d and Extended Data Fig. 7i–n), replicating prior studies demonstrating that atropine can suppress basal and glucose-stimulated insulin secretion indirectly in vitro and in vivo. To test whether the effect of parasympathetic stimulation of GSIS is centrally mediated, we administered acetate by intracerebroventricular (ICV) injection at a dose chosen to increase cerebrospinal fluid acetate concentrations by 200 μM, mimicking the increases in plasma acetate concentrations caused by intra-arterial infusion of 20 μmol kg⁻¹ min⁻¹ acetate. ICV acetate tripled GSIS during a hyperglycaemic clamp without inducing any difference in systemic acetate concentrations; however, this effect was blocked by treatment with atropine, and was independent of changes in plasma glucagon concentrations (Fig. 4e, f and Extended Data Fig. 8a–d), suggesting that acetate...
acts centrally to increase GSIS. Because atropine has also been shown to act directly on β-cells to suppress insulin secretion, we infused rats with intra-arterial acetate and examined the effect of ICV methylatropine, an atropine analogue that does not cross the blood–brain barrier. Consistent with acetate driving GSIS via the parasympathetic nervous system, methylatropine fully abrogated the ability of acetate to drive

Figure 3 | Acetate turnover drives GSIS. a, Plasma insulin in a hyperglycaemic clamp. *P < 0.05, **P < 0.01, ***P < 0.001 versus chow-fed rats; §P < 0.05 versus 3-day HFD-fed rats. b, c, Acetate turnover and GSIS in rats given acute acetate. *P < 0.05, **P < 0.01, ***P < 0.001 versus 2 μmol kg⁻¹ min⁻¹; §P < 0.05, §§P < 0.01, §§§P < 0.001 versus 8 μmol kg⁻¹ min⁻¹. d, e, Acetate turnover and GSIS in rats treated with broad-spectrum, non-absorbable oral antibiotics. *P < 0.05, **P < 0.01, ***P < 0.001 versus controls; §P < 0.05, §§P < 0.01, §§§P < 0.001, §§§§P < 0.0001 versus antibiotic-treated rats. f, g, Whole-body acetate turnover and GSIS. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus chow-fed donor and chow-fed recipient; §§P < 0.01, §§§P < 0.001, §§§§P < 0.0001 versus chow-fed donor and HFD-fed recipient. h, GSIS in isolated islets (KRB buffer; n = 4 replicates per group). Data show mean ± s.e.m. Groups were compared by one-way ANOVA with Bonferroni's multiple comparisons test (a–g) or by two-tailed unpaired Student's t-test (h). Unless otherwise specified, n = 6 rats per group.

Figure 4 | Acetate drives increased GSIS via parasympathetic activation. a, Plasma gastrin. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus 2 μmol kg⁻¹ min⁻¹ acetate; §P < 0.05 versus 8 μmol kg⁻¹ min⁻¹ acetate. b, Tissue acetate. c, d, GSIS. e, f, Plasma acetate and GSIS. *P < 0.05, **P < 0.01, ***P < 0.001 versus controls; §P < 0.05, §§P < 0.01, §§§P < 0.001 versus ICV acetate. g, h, Plasma gastrin (120 min) and GSIS. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus acetate. i, j, Plasma gastrin (120 min) and GSIS following acetate injection into the nucleus tractus solitarius. Data are the mean ± s.e.m. of n = 6 animals per group, with groups compared by ANOVA with Bonferroni's multiple comparisons test (a–h) or by two-tailed unpaired Student's t-test (b–d, i, j). In b–d, i, and j, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Chronic increases in acetate drive obesity

We next investigated whether a chronic increase in acetate turnover would promote chronic hyperinsulinaemia, hyperphagia, and weight gain and the associated sequelae of obesity. To answer this question, we performed continuous intragastric acetate infusions for 10 days, treating chow-fed rats with 20 μmol kg⁻¹ min⁻¹ acetate to mimic the increase in gut microbial acetate production measured in HFD-fed rats (Extended Data Fig. 9a, b). Rats that received chronic intragastric acetate infusions exhibited increased insulin secretion during both a hyperglycaemic clamp and an intraperitoneal glucose tolerance test; this increase in insulin secretion was associated with a fivefold increase in plasma gastrin concentration (Fig. 5a, b and Extended Data Fig. 9c–h).

All of these effects were prevented by vagotomy. Consistent with the hypothesis that chronic postprandial hyperinsulinaemia leads to increased weight gain, rats that received chronic intragastric acetate infusions exhibited more than a doubling in daily caloric intake and in weight gain over the ten-day infusion, which may be attributable, at least in part, to a threefold increase in plasma ghrelin concentrations (Fig. 5c, d and Extended Data Fig. 9i, j). These effects were also prevented by vagotomy, demonstrating that parasympathetic activation is necessary to mediate the effects of chronic acetate on GSIS in awake, unrestrained rats. Finally, acetate-infused rats exhibited insulin resistance, as indicated by impaired glucose disposal and impaired insulin suppression of hepatic glucose production during a hyperinsulinaemic–euglycaemic clamp, and increases in plasma, liver, and skeletal muscle triglyceride content without any changes in plasma glucagon concentrations (Fig. 5e–h and Extended Data Fig. 9k–p). Vagotomized rats exhibited none of these consequences of acetate infusion.

Together, these findings strongly suggest that the gut microbiota are responsible for generating increased acetate turnover and driving obesity in HFD-fed rats, although we cannot rule out the possibility that the microbiota also modulate acetate absorption. To conclusively test the hypothesis that the gut microbiota are primarily responsible for increasing acetate turnover in HFD-fed rodents, we measured plasma and colonic acetate content in germ-free mice lacking gut microbes and ex-germ-free mice 4 weeks after colonization with normal mouse faeces (conventionalized; CONV-D) fed either a regular Chow diet or HFD. Demonstrating the role of the gut microbiota as the main producer of acetate in vivo, germ-free mice had negligible plasma, colonic lumen, and tissue acetate concentrations as compared to CONV-D mice; only conventionalized mice exhibited an increase in acetate concentrations on HFD (Fig. 6a–c). Germ-free mice fed [13C]bicarbonate also exhibited strikingly lower plasma and tissue [13C] enrichment than CONV-D mice. Furthermore, in CONV-D mice but not germ-free mice, [13C] acetate was doubled in HFD-fed relative to chow-fed animals (Fig. 6d).

As rodent do not possess the enzymes necessary to convert bicarbonate to acetate, these results demonstrate that the gut microbiota are responsible for the increased acetate turnover in HFD-fed animals, a conclusion corroborated by the fact that colonic [13C]acetate enrichment in CONV-D mice was more than double the enrichment in plasma or in any other tissue (Extended Data Fig. 10a). In contrast, propionate and butyrate concentration and enrichment were minimal in plasma and tissues in all mice (Extended Data Fig. 10b–g). Finally, because of the role of increased acetate in promoting parasympathetic activation, we measured plasma gastrin and ghrelin concentrations in the germ-free and CONV-D mice, and found that CONV-D mice exhibited two- and tenfold increases in gastrin and ghrelin, respectively, which were associated with two- and fivefold increases in liver and skeletal muscle triglyceride content (Fig. 6e–h), compared with the germ-free mice. Together, these data clearly implicate the gut microbiota as being responsible for the majority of the whole-body plasma acetate turnover in vivo and for the increase in acetate turnover observed in HFD-fed rats.

Conclusions

In summary, we show here that increased acetate production due to a gut microbiota–nutrient interaction in HFD-fed rodents leads
to activation of the parasympathetic nervous system and results in increased ghrelin secretion and GSIS. This generates a positive feedback loop, resulting in hyperphagia, hypertriglyceridaemia, ectopic lipid deposition in liver and skeletal muscle, and liver and muscle insulin resistance (Extended Data Fig. 1). The increased acetyl acid production that occurs when the gut microbiota are exposed to caloricically dense nutrients may mediate an important positive feedback loop between the gut microbiota and the CNS that promotes hyperphagia (due to increased ghrelin secretion) and increased energy storage as fat (due to increased GSIS) in foraging animals when they stumble across calorically dense foodstuffs in the wilderness. However, in the setting of chronic exposure to calorically dense, abundant food, this gut microbiota–brain–

#### Figure 6 | Gut bacteria are responsible for the majority of acetate production in vivo, and for the increase in HFD-fed rodents.

**Figure 6** Panel a: Plasma and caecal/colon luminal acetate concentrations in germ-free (GF) and CONV-D mice. Panel b: Tissue acetate concentrations. Panel c: Plasma [13C]acetate enrichment in mice fed water containing [13C]bicarbonate for 3 days. Panel d: Plasma gastrin and ghrelin concentrations. Panel e: Plasma acetate and caecal/colon lumen acetate concentrations in germ-free and control mice. Panel f: Plasma ghrelin concentrations. Panel g: Liver and skeletal muscle triglyceride content. Panel h: Liver and skeletal muscle acetate content. Panel i: Total caecal and colon lumen acetate concentration. 

**Methods**

Methods, along with any additional Extended Data display items and Supplementary Information are available in the online version of the paper. References unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Information** Sequence data are deposited to the European Nucleotide Archive with accession code PRUEB13505. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to G.I.S. (gerald.shulman@yale.edu).

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Extended Data Figure 1 | Mechanism by which a diet–microbiota interaction drives obesity and the metabolic syndrome.
Extended Data Figure 2  |  HFD-fed rats exhibit increased gut acetate production. a, Plasma triglycerides. b, HOMA-IR. c, Dietary acetate concentrations. n = 2 replicates per diet. d, Faecal acetate normalized to dry weight. e–g, Plasma propionate, whole-body propionate turnover, and faecal propionate concentrations. h–j, Plasma butyrate, whole-body butyrate turnover, and faecal butyrate concentrations. k, [13C]acetate enrichment in plasma of rats fed [13C]bicarbonate-labelled food and water. l, [13C]acetate from faeces incubated in [U-13C]glucose or fatty acids. m, In vitro acetate production rate from faeces incubated in [U-13C]glucose or fatty acids. n, In vitro acetate production rate in control, boiled, and UV-irradiated faecal samples. ****P < 0.0001 versus control. o, In vitro faecal acetate production following treatment with antibiotics. Unless otherwise specified, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus chow-fed rats; §§P < 0.01 versus 3-day HFD-fed rats by one-way ANOVA with Bonferroni’s multiple comparisons test. In k, data were compared by two-tailed unpaired Student’s t-test. In l–o, data are the mean ± s.e.m. of n = 4 per group, with comparisons to controls via two-tailed unpaired Student’s t-test (n). Unless otherwise specified, n = 6 replicates per group.
Extended Data Figure 3 | HFD-fed rats exhibit increased GSIS driven by increased acetate turnover. a, b, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus chow-fed rats. c, Plasma insulin area under the curve (AUC) during the hyperglycaemic clamp. d, Plasma acetate. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus 2 μmol kg⁻¹ min⁻¹ acetate; §§§§P < 0.0001 versus 8 μmol kg⁻¹ min⁻¹ acetate. e, f, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. g, Plasma insulin AUC during the clamp. h, i, Plasma butyrate and whole-body butyrate turnover. *P < 0.05, ****P < 0.0001. j, k, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. l, m, Plasma insulin concentrations during the hyperglycaemic clamp, and plasma insulin AUC. In all panels, data are the mean ± s.e.m. of n = 6 animals per group, with comparisons by one-way ANOVA with Bonferroni’s multiple comparisons test (a–g) or two-tailed unpaired Student’s t-test (h–m).
Extended Data Figure 4 | Increasing total caloric intake leads to increased acetate turnover and GSIS via the microbiota in rats.

a, b, Plasma acetate and whole-body acetate turnover. c, d, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. e, f, Plasma insulin and insulin AUC during the clamp. g, Caloric intake from protein, fat, and carbohydrate. In g–m, each group was compared to pair-fed, high-carbohydrate-fed rats. h, i, Plasma glucose and glucose infusion rate in the hyperglycaemic clamp. j, k, Plasma acetate and whole-body acetate turnover. l, m, Plasma insulin and insulin AUC during the hyperglycaemic clamp. n, Linear regression: whole-body acetate turnover versus total caloric intake in each diet group. o, p, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp in 4-week HFD-fed rats treated with broad-spectrum non-absorbable antibiotics. q, Plasma acetate. r, Plasma [13C]acetate enrichment following three days of feeding [13C]bicarbonate food and water. Data were compared using the two-tailed unpaired Student's t-test. s, Insulin AUC during a hyperglycaemic clamp. In all panels, data are the mean ± s.e.m. of n = 6 rats per group, with groups compared by one-way ANOVA with Bonferroni's multiple comparisons test, unless otherwise stated. In a–f, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus 12-h starved rats; §P < 0.05, §§P < 0.01, §§§P < 0.001, §§§§P < 0.0001 versus 48-h starved rats. In h–m, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus pair-fed rats given the high-carbohydrate diet. In o–s, ***P < 0.001, ****P < 0.0001 versus HFD-fed rats; §§§P < 0.001, §§§§P < 0.0001 versus antibiotics-treated rats.
Faecal transplantation alters recipient microbiomes to resemble their donors as revealed by culture-independent 16S rRNA sequencing of faecal microbiomes from donors and recipients. 

**a.** Relative abundance at the phylum level. Only phyla with relative abundance $\geq 0.1\%$ in at least one group are shown. Data are the mean ± s.e.m. of $n = 7–8$ replicates per group; *$P < 0.05$ by 2-tailed unpaired Student's $t$-test.

**b–f.** Beta diversity analysis of faecal microbiomes before and after transplantation. The largest component of variation (captured by principal coordinate (PC1)) is shown in b and PC1–PC3 are shown in c–f. Rats from independent litters were randomized before diet administration or faecal transplantation. Beta diversity reflects principal coordinates analysis based on Hellinger distances; the results from unweighted, non-phylogenetic distance metrics and from phylogenetic metrics (weighted and unweighted UniFrac) are similar.
Extended Data Figure 6 | The gut microbiota drive increased acetate turnover and GSIS. a, b, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp in rats following faecal transplant replicates acetate turnover and GSIS in the donor group. c, Plasma acetate. d, Faecal acetate concentration. e, Plasma insulin AUC. f, Glucose-stimulated insulin release in isolated islets incubated with 400 μM acetate in a physiological buffer. n = 7 (HFD to Chow) or 8 (chow to Chow, Chow to HFD) per group. g, Plasma C2 acetylcarnitine content. h, Glucose-stimulated insulin release in isolated islets incubated with 100 μM acetylcarnitine. i–m, Plasma alanine, leucine, arginine, glucagon, and GLP-1 concentrations. n, o, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp in acetate-infused rats treated with a GLP-1 inhibitor. p, q, Plasma acetate and whole-body acetate turnover. r, s, Plasma insulin and insulin AUC during the clamp. In all panels, data are mean ± s.e.m. of n = 6 per group. In a–e, ****P < 0.0001 versus chow-fed donor to chow-fed recipient transplants; §§§§P < 0.0001 versus chow-fed donor to HFD-fed recipient transplants by one-way ANOVA with Bonferroni’s multiple comparisons test. Data are the mean ± s.e.m. of n = 6 (HFD to Chow) or 7 (Chow to Chow, Chow to HFD) per group. In g–s, data are the mean ± s.e.m. of n = 6 (unless otherwise specified) per group. In n–s, no significant differences were measured by the two-tailed unpaired Student’s t-test.
Extended Data Figure 7 | Acetate drives GSIS via a CNS mechanism. a, Body weight before and after vagotomy. b, c, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. d, e, Plasma acetate and whole-body acetate turnover. f, Insulin AUC during the clamp. g, Plasma gastrin during the clamp. h, Plasma glucagon after 120 min of the clamp. h, i, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp in acetate-infused, atropine-treated rats. k, l, Plasma acetate and whole-body acetate turnover. m, Plasma insulin AUC during the clamp. n, Plasma glucagon. In all panels, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by the two-tailed unpaired Student’s t-test; data represent the mean ± s.e.m. of n = 6 replicates per group.
Extended Data Figure 8 | Acetate drives GSIS via parasympathetic activation. 

**a, b**, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp in rats treated with ICV acetate. **c**, Plasma insulin AUC. **d, e, f**, Plasma acetate and whole-body acetate turnover in rats treated with systemic intra-arterial acetate and ICV methylatropine. **g, h**, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. **i**, Plasma insulin AUC during the clamp. **j, k**, Plasma and brain tissue acetate in rats given an injection of acetate into the nucleus tractus solitarius. **l, m**, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. **n**, Plasma insulin AUC during the clamp. **o**, Plasma glucagon. In all panels, data are the mean ± s.e.m. of n = 6 animals per group, with comparisons by one-way ANOVA with Bonferroni’s multiple comparisons test (a–i) or two-tailed unpaired Student’s t-test (j–o). In b–d, **P < 0.01, ***P < 0.001 versus controls; §§P < 0.01, §§§P < 0.001 versus ICV acetate-treated rats by one-way ANOVA with Bonferroni’s multiple comparisons test. In e–i, ***P < 0.001, ****P < 0.0001 versus controls; §§§P < 0.001, §§§§P < 0.0001 versus acetate-infused rats.
Chronic intragastric acetate infusion causes hyperphagia and metabolic syndrome through parasympathetic activation. a, b, Plasma acetate and whole-body acetate turnover. c, d, Plasma glucose and insulin concentrations during an intraperitoneal glucose tolerance test. e, Insulin AUC during the glucose tolerance test. f, g, Plasma glucose and glucose infusion rate during a hyperglycaemic clamp. h, Insulin AUC during the hyperglycaemic clamp. i, Body weight before and after the infusion study (n = 16 controls, 16 acetate-infused, and 12 acetate-infused and vagotomised rats). j, Caloric intake during the 10-day acetate infusion study. k, Homeostatic model assessment of insulin resistance (HOMA-IR). l, Plasma triglyceride concentrations. m, Plasma insulin at the 120-min time point of a hyperinsulinaemic–euglycaemic clamp. n, o, Plasma glucose and glucose infusion rate during the hyperinsulinaemic–euglycaemic clamp. p, Plasma glucagon. Unless otherwise specified, data are mean ± s.e.m. of n = 6 rats per group, with comparisons by one-way ANOVA with Bonferroni’s multiple comparisons test. In all panels, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus controls; §P < 0.05, §§P < 0.01, §§§P < 0.001, §§§§P < 0.0001 versus intragastric acetate-infused rats.
Extended Data Figure 10 | Germ-free mice have negligible endogenous short-chain fatty acid production. 

a, Ratio of tissue:plasma [13C]acetate in mice fed [13C]bicarbonate. 
b, c. Plasma and tissue propionate concentrations. 
d. Plasma [13C]propionate enrichment. 
e. f. Plasma and tissue butyrate. 
g. Plasma [13C]butyrate enrichment. 
h, i, Liver and muscle diacylglycerol concentrations. 
In all panels, data are the mean ± s.e.m. of n = 9 (GF) or n = 10 (CONV-D) mice per group, with comparisons by two-tailed unpaired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus CONV-D mice on the same diet.