Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41

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The distal human intestine harbors trillions of microbes that allow us to extract calories from otherwise indigestible dietary polysaccharides. The products of polysaccharide fermentation include short-chain fatty acids that are ligands for Gpr41, a G protein-coupled receptor expressed by a subset of enteroendocrine cells in the gut epithelium. To examine the contribution of Gpr41 to energy balance, we compared Gpr41−/− and Gpr41+/+ mice that were either conventionally-raised with a complete gut microbiota or were rearmed germ-free and then cocolonized as young adults with two prominent members of the human distal gut microbial community: the saccharolytic bacterium, Bacteroides thetaiotaomicron and the methanogenic archaeon, Methanobrevibacter smithii. Both conventionally-raised and gnotobiotic Gpr41−/− mice colonized with the model fermentative community are significantly leaner and weigh less than their WT (+/+ ) littermates, despite similar levels of chow consumption. These differences are not evident when germ-free WT and germ-free Gpr41 knockout animals are compared. Functional genomic, biochemical, and physiologic studies of germ-free and cocolonized Gpr41−/− and +/+ littermates disclosed that Gpr41-deficiency is associated with reduced expression of PPY, an enteroendocrine cell-derived hormone that normally inhibits gut motility, increased intestinal transit rate, and reduced harvest of energy (short-chain fatty acids) from the diet. These results reveal that Gpr41 is a regulator of host energy balance through effects that are dependent upon the gut microbiota.

Our ability to effectively digest food reflects the combined activities of enzymes encoded in our primate genome and in the genomes of the trillions of microbes that reside in our distal guts. This microbial community, or microbiota, affects both sides of the energy-balance equation, influencing both the harvest of calories and the activity of host genes involved in the metabolism and storage of absorbed energy (e.g., ref. 1).

Our proteome has a very limited repertoire of glycoside hydrolases needed to digest complex dietary plant polysaccharides: the microbiota synthesizes a large arsenal of these enzymes (2), and allows us to process complex dietary carbohydrates to short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate. Host recovery of SCFAs is generally efficient and occurs by both passive diffusion and via mono-carboxylic acid transporters (e.g., MCT1 in the case of butyrate and lactate) (3). Butyrate is the preferred source of energy for colonic epithelial cells. Absorbed acetate and propionate are delivered to hepatocytes, which consume most of the propionate for gluconeogenesis. Although acetate can be used for lipogenesis in colonocytes, hepatocytes and adipocytes are the principal sites for de novo lipogenesis, at least in rodents.

Studies in gnotobiotic mice have emphasized the contributions of the gut microbiota and microbial fermentation of dietary polysaccharides to host energy balance. Adult germ-free (GF) mice are leaner than their age- and gender-matched conventionally raised (CONV-R) counterparts who have acquired a microbiota beginning at birth (1). Transplantation of an unfraccionated gut microbiota from a CONV-R donor to an adult GF recipient results in an increase in adiposity. This increase is greater if the donors are obese because they are homozygous for a null allele in the leptin gene (ob/ob), or if they have diet-induced obesity (4, 5). Comparative metagenomic studies of distal gut (cecal) microbial community DNA prepared from mice with either form of obesity and from lean controls, have shown that the obesity-associated microbiomes have a greater capacity to ferment carbohydrates to SCFAs (4, 5). In addition, colonization of adult GF mice, fed a standard polysaccharide-rich chow diet, with two organisms—Bacteroides thetaiotaomicron, a prominent saccharolytic bacterium in the normal distal human gut microbiota and an adept adaptive forager of polysaccharides (6), plus Methanobrevibacter smithii, a dominant methanogenic archaeon in this community (7) that promotes polysaccharide fermentation by removing the H2 end product—results in higher levels of SCFAs in the colon, and significantly greater host adiposity than colonization of GF animals with either organism alone (8).

SCFAs also act as signaling molecules. Propionate, acetate, and to a lesser extent butyrate and pentanoate, are ligands for at least two G protein-coupled receptors (GPCRs), Gpr41 and Gpr43 (9–11). Both GPCRs are broadly expressed, including in the distal small intestine, colon, and adipocytes (9–11). SCFAs (C1-C6), which are ligands for Gpr41, stimulate expression of leptin, a polypeptide hormone with pleiotropic effects on appetite and energy metabolism, in mouse-cultured adipocytes (11). However, little is known about the regulation of these GPCRs, their mechanism of action, and whether they represent a class of molecules, strategically placed in certain gut epithelial cell lineages that sense the biochemical milieu of the gut and “transduce” information.


The authors declare no conflict of interest.

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about key metabolic activities of the microbiota, such as polysaccharide fermentation, in ways that impact host physiology, including energy balance.

In this report, we compare GF wild-type and Gpr41-deficient mice with and without a model fermentative microbial community composed of B. thetaiotaomicron (Bt) and M. smithii (Ms). The results reveal a pivotal role for Gpr41 in a microbiota-dependent metabolic circuit that regulates the flow of calories between the diet and the host. Our results suggest that inhibition of SCFA activation of Gpr41 is a potential therapeutic target for modulating the efficiency of caloric extraction from a polysaccharide-rich diet.

Results and Discussion

Gpr41 Is Expressed in Enteroendocrine Cells. Analysis of the tissue distribution of Gpr41 mRNA in CONV-R adult mice indicated that highest levels are present in the distal small intestine (ileum) and colon [supporting information (SI) Fig. S1]. Enteroendocrine cells are strategically positioned to transduce information about the nutrient milieu of the gut and the metabolic activity of the microbiota to the host: they produce different sets of peptide hormones, depending upon their location along the length of the gastrointestinal tract (12). These neuroactive and endocrine factors are secreted basolaterally into the portal and systemic circulation where they influence a wide variety of extraintestinal physiological activities.

In situ hybridization studies indicated that Gpr41 is expressed in cells with the morphologic appearance of enteroendocrine cells (Fig. S2A). Cholecystokinin (CCK) is a known biomarker of this gut epithelial-cell lineage. Therefore, we used flow-assisted cell sorting (FACS) to purify CCK-positive cells from the small intestines of CONV-R transgenic mice engineered to express GFP in this enteroendocrine subpopulation (Fig. S2 B and C). qRT-PCR assays of the expression of Gpr41 and seven other known enteroendocrine biomarkers in the crude starting material and in the FACS-purified population confirmed that Gpr41 is expressed in this enteroendocrine subset (Fig. S2 D and E). A similar approach was used in different pedigrees of transgenic mice engineered to express GFP in NeuroD- and Neurogenin3-producing enteroendocrine subpopulations to show that Gpr41 is also localized to these cells (data not shown). Finally, intraepithelial lymphocytes, which have some of the morphologic features of enteroendocrine cells when viewed by light microscopy, were purified using a T-cell antibody plus magnetic bead sorting (see SI Materials and Methods): qRT-PCR established that they do not express appreciable levels of this GPCR (Fig. S2F).

Microbial Suppression of Gpr41 Expression. Ligand-induced down regulation is a hallmark of GPCR activation (13). Therefore, we generated Gpr41−/− mice (Fig. S3 A and B), rederived them as GF, and examined whether colonization of 8- to 10-week-old male GF knockout (Gpr41−/−) mice and their WT (+/+) littersmates (mixed C57Bl6/J:129/Sv background) for 28 days with Bt and Ms (see Methods) affected ileal expression of Gpr41, or other known enteroendocrine biomarkers in the crude starting material and in the FACS-purified population confirmed that Gpr41 is expressed in this enteroendocrine subset (Fig. S2 D and E). A similar approach was used in different pedigrees of transgenic mice engineered to express GFP in NeuroD- and Neurogenin3-producing enteroendocrine subpopulations to show that Gpr41 is also localized to these cells (data not shown). Finally, intraepithelial lymphocytes, which have some of the morphologic features of enteroendocrine cells when viewed by light microscopy, were purified using a T-cell antibody plus magnetic bead sorting (see SI Materials and Methods): qRT-PCR established that they do not express appreciable levels of this GPCR (Fig. S2F).

Quantitative PCR assays established that levels of colonization of the distal gut (cecum) with each microbial species were not significantly affected by the presence or absence of Gpr41 (mean 8.2 ± 4.3 × 10^{12} organisms per gram of luminal contents for B. thetaiotaomicron; 2.4 ± 1.5 × 10^{10} for M. smithii; n, 7–8 mice per genotype). Therefore, any phenotypic differences observed between gnotobiotic WT and knockout animals could not be attributed to differences in their gut microbial ecology.

qRT-PCR assays of ileal RNAs revealed that compared with GF +/+ controls, cocolonization of WT mice produced statistically significant twofold reductions in the steady state levels of Gpr41, Gpr43, and Gpr120 mRNAs (P < 0.05; ANOVA) (Fig. S4). Expression of Gpr40 mRNA in +/+ mice was also reduced by colonization, although the observed change did not quite achieve statistical significance (see Fig. S4). Importantly, the magnitude of the reduction in Gpr40, Gpr43, and Gpr120 gene expression was not affected by the absence of Gpr41 (see Fig. S4).

Together, these findings indicate that Gpr41−/− mice have a specific deficiency affecting only one of these four fatty-acid binding GPCRs and therefore can, in principle, be used to assess the role of Gpr41 in mediating the effects of the microbiota on host energy homeostasis.

Gpr41 Is Needed for Microbiota-Induced Increases in Host Adiposity. Eight to ten-week-old male GF Gpr41−/− mice, maintained on a standard polysaccharide-rich chow diet, exhibited no significant differences in their epididymal fat pad or total body weights compared with +/+ littermates (P > 0.05; n = 8–14 per group) (Fig. 1A and data not shown). In contrast, Gpr41−/− mice cocolonized with Bt/Ms had significantly lower epididymal fat pad weights than +/+ controls (11.4 ± 0.6 versus 14.4 ± 0.9 mg/g body weight, respectively; P < 0.05) (see Fig. 1A), gained significantly less body weight per day (0.05 ± 0.03 versus 0.19 ± 0.02 g/day, respectively; P < 0.05) (Fig. 1B), and weighed significantly less at the end of the 28-day colonization period (24 ± 0.4g versus 26 ± 0.4g; P < 0.05) (n, 13 to 14 animals per group, representing two independent experiments).

These gut microbiota-dependent differences were not a unique feature of the Bt/Ms gnotobiotic model. CONV-R Gpr41−/− animals, maintained on the same polysaccharide-rich, low-fat chow diet as their cocolonized gnotobiotic counterparts, also exhibited statistically significant decreases in weight gain, total body weight, and fat-pad weight compared with age- and gender-matched CONV-R +/+ littermates (P < 0.05) (Fig. 2 A and B plus data not shown). Dual energy X-ray absorptiometry (DEXA) confirmed their reduced adiposity (13 ± 1% versus 19 ± 1% of body weight in +/+ controls; P < 0.005; n, 9 to 13 per group) (Fig. 2C). The differences in body weight and adiposity observed in CONV-R Gpr41-deficient versus WT mice were not attributable to differences in their locomotor activity or body temperature (n, 4 animals per group; P > 0.05) (Fig. S5 A and B).

Fasting serum levels of leptin were similar in GF Gpr41−/−/*
though no statistically significant differences in gastric emptying rates were observed (data not shown), intestinal transit rate was significantly faster in Bt/Ms-colonized Gpr41−/− versus +/+ littermates (P < 0.005; see Fig. 3 B and C). The effect of Gpr41-deficiency on intestinal transit rate was microbiota-dependent: No significant differences were noted among GF animals of either genotype (n, 4–8 per group) (see Fig. 3 B and C). The differences between Bt/Ms-colonized Gpr41−/− and +/+ littermates were not attributable to differences in the length of their small intestines, which were similar (1.8 ± 0.06 versus 1.9 ± 0.07 cm/g body weight, respectively; n, four to eight animals per group; P > 0.05).

Based on the observed increase in intestinal transit rate, we hypothesized that more undigested polysaccharides may reach the distal gut in Gpr41−/− versus +/+ mice. This was confirmed by microanalytic biochemical assays of glucans (glucose-containing polysaccharides) in cecal contents. Bt/Ms-colonized, Gpr41-deficient animals had significantly higher cecal glucan levels than their colonized +/+ littermates (19% increase; 2.2 ± 0.1 versus 1.8 ± 0.1 μmoles/g dry weight of cecal contents; P < 0.05; n, five to six per group; P < 0.05), and higher levels of monomeric glucose (28% increase; 2.7 ± 0.2 versus 2.1 ± 0.2 μmol/g dry weight of cecal contents; P < 0.05). In addition, GC-MS-based analyses of cecal SCFA levels revealed that the concentrations of propionate and acetate were significantly increased in Bt/Ms-colonized Gpr41−/− mice (n, 5–7 animals per group; P < 0.05) (Fig. 4C).

Follow-up whole-genome transcriptional profiling of Bt using (i) microbial RNA isolated from the cecal contents of Bt/Ms-colonized Gpr41-deficient and +/+ littermates, and (ii) custom Bt GeneChips containing probe sets specific for >98% of the organism’s protein-coding genes, failed to reveal statistically significant differences in the expression of bacterial genes involved in fermentation of polysaccharides to SCFA between the two groups of animals (n, 5 per group; P < 0.05; FDR <1%). Follow-up qRT-PCR assays of these RNAs confirmed that...
A 129/SV mouse BAC clone obtained from Children's Hospital Oakland Research Institute was used to construct the

**Materials and Methods**

**Generation of Gpr41 Knockout Mice.** A 129/SV mouse BAC clone obtained from Children's Hospital Oakland Research Institute was used to construct the
targeting vector shown in Fig. S3A. SM-1 ES cells (18), cultured on irradiated LIF-producing STO feeder layers, were electroporated with the linearized targeting vector and selected for resistance to G418 (18). Resistant ES cell clones were screened by Southern blotting using a flanking 3' genomic fragment external to the targeting vector (see Fig. S3B). Two of these ES cell clones were microinjected into C57BL/6 blastocysts to produce germline transmitting chimeric mice. PCR genotyping used the primer set 5'-CACAAGTGCCTGATCCGGAAACCTT and 5'-GAGAACTGTCTGGAAAACGCTCAC to identify the mutant Gpr41 allele, and 5'-GATCCGGAACCCTT and 5'-/H11032 CAGTGGATAGGCCACGC to detect the WT allele. This PCR genotyping protocol was validated by Southern blotting (see Fig. S3B).

Mice were provided with food and water ad libitum and maintained on a strict 12 h light–dark cycle. All procedures involving genetically engineered mice used in this study were approved by the Institutional Review Board for Animal Research of the University of Texas Southwestern Medical Center at Dallas.

Husbandry of Gnotobiotic Mice. Gpr41–/– mice and their +/+ littermates (mixed C57BL/6J:129Sv background) were rederived as GF and housed in flexible film plastic gnotobiotic isolators (19), where they were maintained on a strict 12 h light cycle (lights on at 0600 h) and fed a standard autoclaved polysaccharide-rich chow diet (B and K Universal) ad libitum. on a strict 12-h light cycle (lights on at 0600 h) and fed a standard flexible film plastic gnotobiotic isolators (19), where they were maintained

**Statistical Analysis.** Unless otherwise noted, the significance of differences noted among different groups of mice was defined using ANOVA and Tukey’s posthoc test.

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Analysis of Host Adiposity and Energy Harvest. All mice were fasted (4 h) before being killed. Epididymal fat pads, livers, and segments of the distal intestine (ileum) and colon were removed and flash-frozen in liquid nitrogen. Epididymal fat pad and liver weights were recorded before freezing.

Before killing, total body fat content was measured by DEXA (Lunar PIXimus Mouse, GE Medical Systems), 5 min after mice had been anesthetized with an intraperitoneal injection of ketamine (10 mg/kg body weight) and xylazine (10 mg/kg). Weight gain and chow consumption were monitored weekly in mice who were individually caged for the duration of the experiment. The energy content of fecal samples (freeze-dried immediately after collection) was defined using a bomb-calorimeter (Parr Instruments) and previously established methods (8).

**Measurement of Physiological Parameters.** Locomotor activity and body temperature were assessed for 5 d using a telemetry device (minimitter PDT-4000; Mini Mitter) beginning 7 d after implantation (20). Locomotor activity data were processed using VitalView software (Mini Mitter).

*G*astric emptying and gastrointestinal transit time was measured in GF and Bt/Ms-colonized *Gpr41*–/– and +/+ littermates using established methods, after an 18-h overnight fast (21, 22). FITC-labeled dextran (70,000 MW; Molecular Probes) was administered by gavage (100 μl of a 5-mg/ml solution prepared in PBS). Sixty minutes later, the entire GI tract from stomach to rectum was removed and placed in ice-cold PBS for 30 s to extract the fluorescence signal. The stomach small intestine (divided into 10 equal length segments), cecum, and colon (subdivided into two equal-length segments) were each placed in a separate tube containing 1 ml of PBS (5 ml for stomach and cecum). The segments were coarsely chopped with scissors, and luminal contents suspended using a combination of vigorous washing and vortexing. A dilution series was completed for each sample (1:10 to 1:1000 in PBS) and the fluorescent signal quantified in a multiwell fluorescence plate reader (Stratagene Mx3000; excitation at 485 nm; emission at 530 nm). A histogram of the fluorescence signal distributed along the gastrointestinal tract was then plotted and the geometric center determined (SUM (% of total fluorescence per segment × segment number))/100) (23). Gastric emptying was calculated based on the amount of FITC-dextran left in the stomach compared with the total amount of fluorescence in the intestine.

The methods used for measurement of serum proteins, cecal, and fecal SCFAs, plus triglycerides are described in the SI Materials and Methods.


Supporting Information

Samuel et al. 10.1073/pnas.0808567105

SI Materials and Methods

Transgenic Mice That Produce GFP in CCK-NeuroD- and Neurogenin3-Expressing Enteroendocrine Subpopulations. CCK-GFP and NeuroD-GFP mice, generated using a recombinant BAC clone in which GFP replaced the first coding exon of Cck and NeuroD, respectively, were produced by the Genesat project (http://gensat.org) and were obtained from Mutant Mouse Regional Resource Centers (MMRRC) by resuscitation of cryopreserved embryos. Ngn3–/– mice (1) obtained from MMRRC had GFP knocked into the Ngn3 locus.

For in situ hybridization, a mouse Gpr41 cDNA was used to generate labeled sense (control) and antisense riboprobes using SP6 and T7 polymerases (respectively), the Maxiscript kit (Ambion), and 35S-CTP (Amersham). Paraffin-embedded sections were prepared from the ileum and colon of adult CONV-R 129/SvEv animals. Following prehybridization, sections were hybridized at 55°C with sense and antisense riboprobes [7 × 105 cpm per slide; (2)]. Following overnight incubation, unhybridized probe was removed with stringent washes and treatment with RNase A. Slides were subsequently coated with K5 nuclear emulsion, exposed at 4°C for 21 days, developed, counterstained with hematoxylin, and examined using bright and dark field optics.

FACS Analysis. The small intestine and colon were harvested from 3- to 4-month-old CONV-R mice, opened, rinsed with PBS, cut into 2- to 3-cm long fragments, and washed three times in a 10-cm dish containing RPMI medium 1640/5% FCS (FCS). The fragments were then placed in a 50-ml conical tube containing RPMI1640 medium with 5% FCS, 0.5 mM DTT, and 1 mM EDTA. The conical tube was then shaken at 225 rpm (37°C) to dislodge epithelial cells. Residual intestinal fragments were discarded and the dislodged cells pelleted, washed with RPMI containing 5% FCS, passed through a 40-μm pore-diameter cell strainer, and resuspended (5 × 106 cells/ml) in RPMI 1640/5% FCS. One to 1.5 × 108 cells were then sorted by FACS (MoFlo, Cyanometry) into RLT buffer (Qiagen), and total RNA purified using SuperScript II (Invitrogen). Cells were then reverse transcribed and resulting cDNA quantified by qRT PCR as described below.

Biochemical Analyses. A portion of the liver was assayed for triglyceride content using a standard method described in ref (3). Serum was collected from fasted (4 h) animals by retro-orbital phlebotomy, aliquoted, and stored at −80°C until analysis. Standard biochemical methods were used to assay sera for lactate (4), glucose (5), triglycerides (3), and nonesterified fatty acids (3). Insulin and leptin levels were defined using ELISA (Crystal Chemical). A luminox bead-based assay (Millipore) was used to quantify levels of PYY.

Cecal glucans were measured using a microanalytic assay (6). Cecal samples were collected with a 10-μl inoculation loop just before killing, freeze-dried at −35°C for 4 d, weighed, and stored under vacuum at −80°C until use (stable for at least 1 month). Samples (10–15 mg) were then homogenized at 1°C in 0.25 ml of 1% oxalic acid (prepared in H2O) and divided into two equal-sized aliquots: one aliquot was heated to 100°C for 30 min (acid hydrolysis sample); the other was maintained at 1°C (control sample). A 10-μl aliquot of each sample was subsequently added to 1 ml solution containing 50-mM Tris-HCl (pH 8.1), 1-mM MgCl2, 0.02% BSA, 0.5-mM ATP, 0.1-mM NADP+, 2 μg/ml Leuconostoc mesenteroides glucose-6 phosphate dehydrogenase (253 U/mg protein; Calbiochem), 10 μg/ml yeast hexokinase (50 U/mg protein; Sigma) and 10 μg/ml yeast phosphoglucoisomerase (500 U/mg protein; Sigma). The mixture was incubated for 30 min at 24°C and the resulting NADPH product was detected using a fluorimeter. Glucose standards (5–10 nmol) were carried through all steps.

SCFAs in cecal and fecal samples were assayed by GC-MS using a modification of the method of Moreau et al. (7). Frozen fecal or cecal contents (100–200 mg) were transferred to a 4-ml glass vial fitted with a septum cap PTFE liner (National Scientific) containing 10 μl of a stock solution of internal standards (Isotec; each of the following components at 20 mM: [1-13C]acetate, [1-13C]pyruvate, and [1-13C]butyrate). Following acidification with 10 μl of 37% HCl, SCFAs were extracted (2 ml diethyl ether/extraction; two cycles). An aliquot of each sample was then derivatized with N-tetra-ethyl(dimethyl)silyl-N,methyltrifluoracetamide (MTBSTFA; Sigma). SCFAs were subsequently quantified using a gas chromatograph (Hewlett Packard 6890) coupled to a mass spectrometer detector (Agilent 5973) as described (8).

RNA Isolation and qRT-PCR Analysis. Host RNA was extracted from liver, epididymal fat pads, the ileum (segment 14 of a small intestine that had been divided into 16 equal sized segments), and proximal half of the colon by homogenizing each sample in 2 ml of Buffer RLT, followed by isolation on QIAGen RNaseasy mini columns (Qiagen). Oligo(dT)-primed cDNA synthesis was performed using SuperScript II (Invitrogen).

For isolation of microbial RNA, 100–300 mg of frozen cecal contents from each gnotobiotic mouse was added to 2-ml tubes containing 250 μl of 212–300 μm-diameter acid-washed glass beads (Sigma), 500 μl of Buffer A (200-mM NaCl, 20-mM EDTA), 210 μl of 20% SDS, and 500 μl of a mixture of phenol:chloroform:isoamyl alcohol (125:24:1; pH 4.5; Ambion). Samples were lysed using a bead beater (BioSpec; high setting for 5 min at room temperature). Cellular debris were pelleted by centrifugation (10,000 × g at 4°C for 3 min). The extraction was repeated by adding another 500 μl of phenol:chloroform:isoamyl alcohol to the aqueous supernatant. RNA was precipitated, resuspended in 100-μl nuclease-free water (Ambion), 350 μl Buffer RLT (Qiagen) was added, and RNA further purified using QIAgen RNeasy mini kit. cDNA synthesis was completed using SuperScript II (Invitrogen) and random hexamer primers.

Methods for target synthesis, GeneChip hybridization, and data analysis are described in an earlier publication (8). qRT-PCR analyses were performed using a Mx3000 real-time PCR system (Stratagene). Reactions of 25 μl contained SYBRGreen Supermix (Bio-Rad), 300 nM of gene-specific primers, uracil-DNA glycosidase (0.01 U/μl), and 10 ng of cDNA. Data were normalized to either 16S rRNA (microbial transcripts) or L32 (control sample). A 10-μl aliquot of each sample was subsequently added to a 1 ml solution containing 50-mM Tris-HCl (pH 8.1), 1-mM MgCl2, 0.02% BSA, 0.5-mM ATP, 0.1-mM NADP+, 2 μg/ml Leuconostoc mesenteroides glucose-6 phosphate dehydrogenase (253 U/mg protein; Calbiochem), 10 μg/ml yeast hexokinase (50 U/mg protein; Sigma) and 10 μg/ml yeast phosphoglucoisomerase (500 U/mg protein; Sigma). The mixture was incubated for 30 min at 24°C and the resulting NAPD product was detected using a fluorimeter. Glucose standards (5–10 nmol) were carried through all steps.

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Fig. S1.  qRT-PCR analysis of RNA isolated from different tissues of CONV-R adult WT 129/Sv mice. Levels of Gpr41 expression are highest in the intestine. Levels of Gpr41 expression were normalized to GAPDH expression. Mean values ± SD are plotted (n, three to four animals, each assayed in triplicate).
Fig. S2. Gpr41 is expressed in small intestinal enteroendocrine cells. (A) In situ hybridization, using Gpr4135S labeled RNA probes, of mouse ileal sections reveal localization of this mRNA to cells with the morphologic appearance of enteroendocrine cells or intraepithelial T-lymphocytes (IEL). The upper two left images show results obtained using an antisense probe, while the upper two right images represent results generated with the control sense probe. The hematoxylin and eosin-stained images shown in the bottom of the panel are from the boxed regions in the middle row of images. (Scale bar, 25 μm.) (B) Fluorescence images of sections obtained from the distal small intestine (Upper) and colon (Lower) of CCK-GFP transgenic mice. (Scale bar, 25 μm.) (C) FACS analysis of the mechanically dispersed intestinal cells from CCK-GFP transgenic mice. The boxed area shows the designated GFP-positive cell population. (D) qRT-PCR analysis of RNA isolated from FACS sorted, GFP-positive cells from GFP-CCK mice. Note the enrichment in levels of enteroendocrine biomarkers in sorted cells versus the starting material or the GFP-negative fraction. Abbreviations: Glc, glucagon; NTS, neurotensin; TAC, tachykinin; SECR, secretin; GIP, gastric inhibitory polypeptide; PYY, pancreatic polypeptide Y. (E) qRT-PCR assays of RNA isolated from IELs recovered from the small intestine by magnetic immuno-affinity cell sorting (MACS) using CD8 antibody, reveal enrichment of T-cell specific markers (TCRδ/γ, CD103, and CD8) but not Gpr41.
Fig. S3. Targeted deletion of the mouse Gpr41 gene. (A) A partial map of a mouse genomic DNA fragment containing the complete Gpr41 gene and Gpr40 is shown. A 4.5-kb insert consisting of a LacZ expression cassette, followed by a neomycin resistance cassette and the mouse phosphoglycerol kinase (PGK) promoter, was substituted for a 1.2-kb segment of mouse DNA containing the exon coding for Gpr41. Restriction enzymes: B; BamHI, H; HindIII, RI; EcoRI, S; SalI, Xba; Xbal. Probes used for the Southern blot shown in (B) are indicated below the map of the targeted allele. The lengths of the EcoRI and Xbal restriction fragments hybridized to probes outside the long and short arm of the targeting vector are shown on the maps of the WT and targeted alleles. (B) Southern blot analysis of tail DNA prepared from Gpr41−/− mice. DNA was extracted from F2 littermates of a heterozygote cross, digested completely with EcoRI and Xbal, and Southern blots of the digest probed with the random-primed 32P-labeled probes shown in (A).
Fig. S4. Loss of Gpr41 does not affect microbial suppression of expression of other fatty-acid binding GPCRs in the distal small intestine. qRT-PCR analysis of ileal RNA isolated from GF or Bt/Ms cocolonized Gpr41−/− and +/+ mice. Levels of mRNAs encoding short-chain fatty-acid-responsive Gpr41 and Gpr43, and long-chain fatty-acid-responsive Gpr40 and Gpr120 are expressed relative to their expression in WT GF controls (n, 5–7 animals assayed per group; mean values ± SEM are plotted; samples assayed in triplicate). ND, not detected. *, P < 0.05, **, P < 0.01.
Fig. S5. Energy intake, locomotor activity, and body temperature are similar in CONV-R Gpr41−/− and +/+ mice. (A and B) Telemetry-based assessment of body temperature (A) and locomotor activity (B) (plots average counts where each interval equals 10 min, for 5 days) (n, 4 animals per group). (C) Calories of chow consumed/day (n, 11–16 males per group). Mean values are plotted ± SEM.
Fig. S6. The colonization-mediated increase in hepatic de novo lipogenesis is attenuated in Gpr41−/− mice. (A) Biochemical analyses of liver triglyceride levels in GF and Bt/Ms cocolonized, Gpr41−/− and +/+ mice (n, 7–8 per group). (B) qRT-PCR assays of fatty-acid synthase (Fas) expression (n, 5–7 per group). Mean values ± SEM are plotted. *, P < 0.05, **, P < 0.01.
Table S1. Biochemical analysis of fasting sera obtained from GF and Bt/Ms colonized Gpr41−/− and +/+ littermates

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<td>+/+</td>
<td>−/−</td>
<td>+/+</td>
</tr>
<tr>
<td>Glucose mM</td>
<td>9.4 ± 0.5</td>
<td>9.7 ± 0.8</td>
<td>11.6 ± 0.4</td>
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<tr>
<td>Lactate mM</td>
<td>5.8 ± 0.4</td>
<td>6.4 ± 0.6</td>
<td>4.1 ± 0.4</td>
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<tr>
<td>Triglycerides mg/dl</td>
<td>63.6 ± 7.1</td>
<td>71 ± 4.3</td>
<td>96.8 ± 13.7</td>
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<tr>
<td>Free fatty acids mM</td>
<td>0.56 ± 0.04</td>
<td>0.6 ± 0.03</td>
<td>0.82 ± 0.1</td>
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<tr>
<td>Leptin ng/ml</td>
<td>0.54 ± 0.1</td>
<td>0.43 ± 0.1</td>
<td>1.61 ± 0.17</td>
</tr>
<tr>
<td>Insulin ng/ml</td>
<td>4.9 ± 0.3</td>
<td>4.64 ± 0.4</td>
<td>8.12 ± 0.4</td>
</tr>
</tbody>
</table>

Mean values ± SEM are presented; n = 4–14 animals/group; P-values were obtained by ANOVA followed by Tukey’s posthoc test.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5' -&gt; 3')</th>
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<tbody>
<tr>
<td>GPR41</td>
<td>Gpr41.F</td>
<td>TTCTTGAGCCAACACATGCTC</td>
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<td>Gpr41.R</td>
<td>GCCCAACACATGGAACATAT</td>
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<td>GPR43</td>
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<td>TGGTTGACACGTGAAGACTG</td>
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<td>GPR40</td>
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<td>GPR120</td>
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<td>mGPR120.R</td>
<td>GAGTTGCAAAAGCTGAAGGC</td>
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<td>Peptide YY (PYY)</td>
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<td>mPYY.R</td>
<td>TCCAAACCTCTGCTGAAA</td>
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<tr>
<td>Interleukin 2 Receptor (IL-2R)</td>
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<td>mTAC.R</td>
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<td>Neurotensin (NTS)</td>
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<td>Gastric inhibitory polypeptide (GIP)</td>
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<td>mGIP.R</td>
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<td>Pancreatic polypeptide Y (PPY)</td>
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<td>Monocarboxylate transporter (Mct1)</td>
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<td>Mct1.R</td>
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<td>Lipid-binding lipoprotein (Fat/Cd36)</td>
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<td>Liver fatty-acid binding protein (Fabp-pm)</td>
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<td>mFABP.R</td>
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