Elucidating the mechanisms of action of short-chain fatty acids

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Protection against the metabolic syndrome by guar gum-derived short-chain fatty acids depends on peroxisome proliferator-activated receptor γ and glucagon-like peptide-1

Abstract

Dietary supplementation with the fiber guar gum decreases hypercholesterolemia, hyperglycemia and obesity in both rodents and humans. Short-chain fatty acids (SCFAs), the main products of intestinal bacterial fermentation of dietary fiber, have been suggested to play a key role. Previously, we showed that SCFAs protect against metabolic syndrome via a signaling cascade that involves peroxisome proliferator-activated receptor (PPAR) γ repression and AMPK activation. In this study we investigated the molecular mechanism via which the dietary fiber guar gum protects against the metabolic syndrome. C57Bl/6J mice were fed a high-fat diet supplemented with 0% or 10% of the fiber guar gum for 12 weeks and effects on lipid and glucose metabolism were studied. We demonstrate that, like SCFAs, also guar gum protects against high-fat diet-induced metabolic abnormalities by PPARγ repression, subsequently increasing mitochondrial uncoupling protein 2 expression and AMP/ATP ratio, leading to the activation of AMP-activated protein kinase and culminating in enhanced oxidative metabolism in both liver and adipose tissue. Furthermore, guar gum markedly increased peripheral glucose clearance, possibly mediated by the SCFA-induced colonic hormone glucagon-like peptide-1. Overall, this study provides novel molecular insights into the beneficial effects of guar gum on the metabolic syndrome and strengthens the potential role of guar gum as a dietary-fiber intervention.
Introduction

The shift in diet in Western and developing countries from a traditional high-fiber, low-fat, low-calorie diet towards a low-fiber, high-fat, high-calorie diet is accompanied with a growing prevalence of obesity and insulin resistance (167, 168). Epidemiological and large observational studies reported an inverse correlation between dietary fiber intake and body weight, insulin resistance, hypertension and dyslipidemia (169) suggesting that fiber supplementation to the diet may be beneficial. Indeed, a variety of studies have shown that dietary fiber intervention decreased obesity and insulin resistance in both healthy and metabolic syndrome patients (reviewed by Galisteo et al. (167)). The dietary fiber guar gum is especially promising, as it has been shown to decrease hypercholesterolemia, hyperglycemia and obesity. In randomized controlled clinical trials a 6-week supplementation of guar gum reduced fasting plasma glucose and insulin levels, and increased insulin sensitivity in healthy and type 2 diabetes humans (171, 178). Prolonging the guar gum supplementation to 8 weeks also led to a decrease in fat mass and body weight in overweight subjects (179, 280). The beneficial effects of dietary fibers work through the intestinal microbiota (281). Short-chain fatty acids (SCFAs), of which acetate, propionate and butyrate are the most abundant, are the main products of intestinal bacterial fermentation of dietary fiber and have been suggested to play a key role in the dietary fiber-induced beneficial effects (Chapter 1). We showed that dose-dependent effects of guar gum-supplementation on body weight and insulin sensitivity correlate with the rate of SCFA uptake by the host, but not with their cecal concentration (Chapter 2), suggesting that fiber-derived SCFAs need to be taken up to exert their full physiological effect.

Also when SCFAs are supplied via the diet, they are highly efficacious in protection against high-fat diet-induced obesity and insulin resistance (17, 122, Chapter 5). We showed that all three individual SCFAs repress peroxisome proliferator-activated receptor (PPAR) γ expression, subsequently increasing mitochondrial uncoupling protein (UCP) 2 expression and AMP/ATP ratio, leading to the activation of AMP-activated protein kinase (AMPK) and culminating in enhanced oxidative metabolism in both liver and adipose tissue (Chapter 5).

In this study we investigate directly whether guar gum exerts its effects on body weight and insulin sensitivity via the same signaling cascade as the SCFAs, as should be expected when it acts through the SCFAs that are taken up by the host. In addition, we reveal a role for the SCFA-induced colonic hormone glucagon-like peptide-1.

Materials and Methods

Animals and Experimental Design

Male C57Bl/6J mice (Charles River, L’Arbresle Cedex, France), 2 months of age, were housed in a light- and temperature-controlled facility (lights on 6:30 a.m. to 6:30 p.m., 21 °C) and had free access to water and diet. They were fed a high-fat semi-synthetic diet (D12451, Research Diet Services, Wijk Bij Duurstede, The Netherlands) in which 0 or 10% (w/w) guar gum (Viscogum™ MP 41230, Cargill, United States) replaced an equivalent amount of corn starch. Experimental procedures were approved by the Ethics Committees for Animal Experiments of the University of Groningen.
Plasma and tissue sampling

The mice were fasted from 6-10 a.m. Blood glucose concentrations were measured using a EuroFlash meter (Lifescan Benelux, Belgium). Mice were subsequently sacrificed by cardiac puncture under isoflurane anesthesia. DPP IV inhibitor (DPP4, Millipore) was added, blood was centrifuged (4000 x g for 10 min at 4 °C) and plasma was stored at -80 °C. Liver was quickly removed, snap-frozen in liquid nitrogen and stored at -80 °C. Epididymal fat pads were weighed. Cecal content and cecum were quickly removed, snap-frozen in liquid nitrogen and stored at -80 °C. Plasma NEFA concentrations were determined using a commercially available kit (Roche Diagnostics, Germany). Plasma insulin (ALPCO Diagnostics, United States), PYY (ALPCO Diagnostics, United States) and GLP-1 levels (Merck Millipore, United States) were determined using ELISA and HOMA-IR was calculated (IR = fasting insulin mU/L x fasting glucose mM ÷ 22.5). Hepatic TG content was determined using a commercially available kit (Roche) after lipid extraction (223).

Lipogenesis was determined from the incorporation of [1-13C]-acetate into palmitate by providing 2% (w/v) [1-13C]-acetate in drinking water for 24h as described previously (194). Fatty acid ß-oxidation capacity was measured in fresh liver and adipose homogenates according to Hirschey et al. (255). Briefly, tissue was homogenized in sucrose/Tris/EDTA buffer, incubated for 30 min in the reaction mixture (pH 8.0) containing [1-14C]palmitic acid, and trapped [14C]CO2 was measured. Adenosine concentrations were determined by HPLC according to Miller et al. (251).

Indirect calorimetry

Oxygen consumption, energy expenditure, respiratory exchange ratio and food intake were measured simultaneously for each mouse using the Comprehensive Laboratory Animal Monitoring System and Software (TSE Systems GmbH, Germany). The energy balance was determined by measuring the energy content (290) of diet and dried, homogenized feces using a bomb calorimeter (CBB 330, standard benzoic acid 6320 cal g⁻¹, BCS-CRM no.90N).

Glucose and insulin tolerance

Intraperitoneal glucose tolerance testing was conducted by intraperitoneal injection of 2 g glucose per kg body weight after an overnight fast of 9h. Intraperitoneal insulin tolerance testing was conducted by intraperitoneal injection of insulin (NovoRapid) at 0.75 units/kg body weight after a 4h fast.

Oxygen consumption rates in liver mitochondria

Mitochondria were isolated from fresh liver tissue according to Mildaziene et al. (255). The rates of oxygen consumption in isolated liver mitochondria were measured at 37 °C using a two-channel high-resolution Oroboros oxygraph-2k (Oroboros, Innsbruck, Austria) with palmitoyl CoA and malate as substrates in MiR05 buffer (282). Maximal ADP-stimulated oxygen consumption (state 3) was achieved by adding 4.8 U ml⁻¹ hexokinase, 12.5 mM glucose and 1 mM ATP. The resting state oxygen consumption rate (state 4) was determined after blocking ADP phosphorylation with 1.25 μM carboxyatractyloside. The respiratory control
ratio (RCR) was calculated by dividing the oxygen consumption rate in state 3 by the oxygen consumption rate in state 4.

**In vivo hepatic carbohydrate flux measurements**

Mice were operated for jugular vein catheterization after 11 weeks on diet and *in vivo* hepatic carbohydrate flux measurements were determined as prescribed before (283). In brief, mice were fasted for 9h and were infused for a 3h basal period with a solution containing [U-\(^{13}\)C]glucose (1.25 mg/ml), [2-\(^{13}\)C]glycerol (7.5 mg/ml), [1-\(^{2}\)H]galactose (3 mg/ml) (Cambridge Isotope Laboratories, Andover, MA), and paracetamol (1 mg/ml) at an infusion rate of 0.54 ml/h. For the hyperinsulinemic condition, solutions were changed after 3h, and mice were subjected to a 3h hyperinsulinemic period by infusing a solution containing insulin (44 mU/ml, Actrapid; Novo Nordisk, Denmark), somatostatin (40 μg/ml; UCB, The Netherlands), [2-\(^{13}\)C]glycerol (30 mg/ml), [1-\(^{2}\)H]galactose (12 mg/ml), paracetamol (4 mg/ml), and 1% BSA (Sigma, St. Louis, MO) at a constant rate of 0.135 ml/h. During hyperinsulinemia, euglycemia was kept by infusion of a second solution that contained 27% glucose (291 mg/ml) and 3% [U-\(^{13}\)C]glucose (9 mg/ml) at an adjustable rate to maintain plasma glucose levels. During the experiment, blood glucose levels were measured every 15 min in blood drops collected by tail tip bleeding. Every 30 min, blood spots for GC-MS analysis were taken by tail tip bleeding on filter paper, air dried, and stored at room temperature until further analysis. Urine samples were collected on filter paper at hourly intervals. Hepatic carbohydrate fluxes were calculated using mass isotopomer distribution analysis (MIDA) as previously described (190).

**Cecal SCFA infusion experiments**

Cecal SCFA infusions were performed as described in Chapter 3. Briefly, mice were equipped with a permanent cecum catheter and allowed a recovery period of at least 5 days. On the day of the experiment, the mice were individually housed and fasted from 6:00 to 10:00 a.m. All infusion experiments were performed in conscious, unrestrained mice. Four different groups of each 8 mice received a 140 mM phosphate-buffered saline (140 mM NaCl, 10 mM sodium phosphate at pH 5.8), a 140 mM sodium acetate (S2889; Sigma), a 140 mM sodium propionate (P1880; Sigma) or a 140 mM sodium butyrate (303410; Sigma) solution infused via the cecum catheter at a rate of 0.2 ml/h for 6h. The infusion rate of SCFA was based on the recommended intake of dietary fiber for humans of 38 g/day/human, which results in approximately 380 mmol SCFAs/day/human (31, 224). When converted to mice, this corresponds to 170 μmol SCFAs/day/mouse. By infusing 140 mM SCFAs directly into the cecum at a rate of 0.2 ml/h for 6h, a total amount of 168 μmol SCFA was given per mouse. After 6h of infusion, animals were terminated by cardiac puncture under isoflurane anesthesia. Cecum was removed quickly, freeze-clamped, and stored at -80°C. Blood was centrifuged (4000 x g for 10 min at 4 °C) and plasma was stored at -80°C.

**Gene expression levels and immunoblot analysis**

RNA was extracted from livers using Tri reagent (Sigma-Aldrich, St. Louis, MO) and converted into cDNA by a reverse transcription procedure using M-MLV and random primers according
to the manufacturer’s protocol (Sigma-Aldrich). For quantitative PCR (qPCR), cDNA was amplified using the appropriate primers and probes. Taqman RT-PCR primer and probe were used to determine mRNA for MCT-1 (Mm01315398_m1), SMCT-1 (Mm00520629_m1), Ffar2 (Mm02620654_s1), Ffar3 (Mm02621638_s1), PYY (Mm00520716_g1) and GLP-1 (Mm01269055_m1). mRNA levels were calculated relative to 36b4 (Mm00725448_s1) expression and normalized for expression levels of mice fed the control diet.

For immunoblot analysis, whole-cell lysate was prepared in lysis buffer and the protein concentrations were determined using the BCA Protein Assay kit (Pierce). Individual samples were mixed with loading buffer, heated for 5 min at 96 °C and subjected to SDS-PAGE. Antibodies and their sources are phosphorylated AMP kinase (pAMPK Thr172, no. 2531; Cell Signaling), phosphorylated acetyl CoA carboxylase (pACC S79, no. 31931; Abcam), uncoupling protein 2 (UCP2, no. 6525; Santa Cruz), peroxisome proliferator-activated receptor γ (PPARγ, no. 2435; Cell Signaling) and fatty acid synthase (FASN, no. 3180; Cell Signaling). As loading control, β-actin (no. 2066; Sigma) was used for liver and adipose tissue. Finally, horseradish peroxidase-conjugated anti-rabbit from donkey (Amersham Pharmacia Bioscience) or horseradish peroxidase-conjugated anti-goat from donkey (Dako, Glostrup, Denmark) and SuperSignal West Pico Chemiluminescent Substrate System (Pierce) were used. The immunoblots were analyzed by densitometry using Image Lab software (Bio-Rad).

Statistics
All data are presented as mean values ± SEM. Statistical analysis was assessed by Mann-Whitney U-test or one-way ANOVA using the Tukey test for post-hoc analysis. Statistical significance was reached at a \( p \) value below 0.05.

Results

Guar gum protects against high-fat diet-induced obesity and insulin resistance
To examine the effects of guar gum during the development of obesity and insulin resistance, wild-type C57Bl/6J mice were fed a high-fat diet (HFD) supplemented with 0 or 10% guar gum for 12 weeks. As expected, HFD induced body weight (BW) gain, while guar gum supplementation attenuated the increase in BW (Figure 1A) and concomitantly reduced the mass of white adipose tissue (WAT) (Figure 1B). Guar gum increased the cecal SCFA concentrations as well as the mRNA expression of the cecal SCFA transporters (Figure 1C and 1D, respectively). These results correspond with those of a previous 6-week guar gum-supplementation (Chapter 2). To investigate the whole-body effect of guar gum on energy metabolism, the mice were subjected to indirect calorimetry. The reduced BW gain of guar gum-fed mice was not due to reduced food intake, as guar gum-fed mice actually had increased energy intake and uptake when normalized for BW (Figure 1E). Furthermore, guar gum did not affect the activity pattern of mice (Figure 1F), indicating that the lower BW on HFD was not due to different physical activity either. Rather, the phenotype was explained by enhanced energy expenditure (Figure 1G). Mice fed guar gum displayed a shift towards increased fatty acid oxidation, as indicated by higher \( O_2 \) consumption rates and lower respiratory exchange
Guar gum protects against dietary-induced obesity. (A) Body weight evolution was monitored for 12 weeks. (B) White adipose tissue to body weight ratio. (C) Cecal SCFA concentrations were determined by GC/MS. (D) Cecal mRNA expression of genes involved in SCFA transport was assessed via qPCR. (E) Energy balance was determined by measuring the energy content of the diet and dried homogenized feces. Uptake is defined as the difference between intake and output. (F-I) Total activity, energy expenditure, VO₂, and RER were evaluated using indirect calorimetry data after 10 weeks on high-fat diet. All values throughout the figure are presented as mean ± SEM for n=7-8; *p<0.05, **p<0.001.

ratio (RER) values (Figures 1H and 1I, respectively).

Guar gum enhances oxidative metabolism via the same signaling cascade as SCFAs

Next we wondered if guar gum acts through the same signaling cascade as SCFAs when the latter are supplied via the diet (Chapter 5). SCFA supplementation resulted in repression of PPARγ expression, subsequently increasing mitochondrial UCP2 expression and AMP/ATP ratio, leading to the activation of AMPK and culminating in enhanced oxidative metabolism in both liver and adipose tissue (Chapter 5).

Indeed also guar gum supplementation decreased expression of PPARγ and increased the expression of UCP2 in both liver and adipose tissue (Figure 2A). Increased UCP2 expression by guar gum enhanced uncoupling of mitochondrial oxidative phosphorylation, as demonstrated
Figure 2. Guar gum decreases lipogenesis and increases mitochondrial fatty-acid oxidation in liver and adipose tissue. (A) PPARγ and UCP2 expression in liver and adipose tissue were analyzed by western blot of mice 12 weeks on diet. Quantification is shown in the lower panel. (B-C) Liver mitochondria were isolated and maximal ADP-stimulated oxygen consumption (i.e. state 3) and resting state oxygen consumption (i.e. state 4) were determined using palmitoyl CoA and malate as substrates. (D) AMP to ATP ratios in liver and adipose tissue were determined by HPLC. (E) pAMPK, pACC and FASN expression in liver and adipose tissue were analyzed by western blot of mice 12 weeks on diet. Quantification is shown in the lower panel. (F-G) Lipogenesis and β-oxidation in liver and adipose tissue after 12 weeks of diet. (H-I) Liver triglycerides and plasma NEFA concentrations after 12 weeks of diet. All values throughout the figure are presented as mean ± SEM for n=6-8; *p<0.05, **p<0.01, ***p<0.001.

by a significant increase of the respiration rate in state 4 and a 1.5-fold decrease of the respiratory control ratio in isolated liver mitochondria (Figures 2B-C). Unfortunately, the low amount of mitochondria in WAT precluded direct measurements of oxygen consumption in the latter. Consistently with the increased mitochondrial uncoupling, the AMP/ATP ratio was increased in both liver and adipose tissue upon guar gum feeding (Figure 2D). The AMP/ATP ratio is a sensitive reflection of the metabolic state of the cell and a direct activator of AMPK (260). Indeed, guar gum-supplementation resulted in increased AMPK phosphorylation and increased phosphorylation and decreased expression of the downstream AMPK targets acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FASN), respectively (Figure 2E). We observed a consistent decrease in lipogenesis and increase in lipid oxidation capacity in the guar gum group-fed mice (Figures 2F-G). Together this resulted in decreased liver
Guar gum protects against the metabolic syndrome via PPARγ and GLP-1

Guar gum improves peripheral glucose and insulin handling

In conclusion, dietary guar gum induced the same signaling cascade in liver and adipose tissue as previously described for dietary SCFAs (Chapter 5), as should be expected if SCFAs are the main molecular mediators of the guar gum-induced effects.

triglycerides and plasma NEFA concentrations (Figures 2H-I).

In conclusion, dietary guar gum induced the same signaling cascade in liver and adipose tissue as previously described for dietary SCFAs (Chapter 5), as should be expected if SCFAs are the main molecular mediators of the guar gum-induced effects.

Guar gum improves peripheral glucose and insulin handling

In agreement with our results in Chapter 2, fasting plasma glucose and insulin concentrations, and thereby HOMA-IR levels, were significantly lower in guar gum-supplemented mice (Figures 3A-C). Glucose disposal upon insulin delivery was slightly increased but glucose tolerance was markedly enhanced in guar gum-fed mice (Figures 3D-E). To further investigate the adaptations in glucose metabolism, we determined in vivo carbohydrate fluxes during basal and hyperinsulinemic conditions as previously described (283). Under basal conditions, guar gum supplementation resulted in decreased glucose levels (Figure 4A). During hyperinsulinemic conditions, the glucose-infusion rate was adjusted to keep the same euglycemic state as under basal conditions (Figure 4A). The glucose-infusion rate required for maintaining euglycemia (a measure of whole-body insulin sensitivity) was approximately 2.5-fold higher in guar gum-supplemented mice compared to control mice (Figure 4B). Although guar gum remodeled the hepatic carbohydrate fluxes by increasing glucose 6-phosphate cycling (Table 1), the decreased glucose levels were not caused by a decreased hepatic glucose production (Figure 4C). Instead, guar gum-fed mice significantly enhanced the glucose clearance by increasing the glucose disposal rate (Figure 4D). Moreover, hyperinsulinemia stimulated the uptake of glucose into the peripheral tissue much more in guar gum-fed mice than in control-fed mice (Figure 4D).
Altogether, these results clearly indicate that guar gum-fed mice have improved glucose handling which is mainly mediated by an enhanced peripheral glucose disposal.

**Guar gum and cecal SCFAs increase plasma GLP-1 concentrations**

In contrast to dietary SCFA-supplementation (Chapter 5), guar gum did not only enhance insulin-stimulated glucose metabolism, but it also decreased basal plasma glucose concentrations and enhanced glucose tolerance (Figures 3 and 4). Together this suggests that there might be an additional factor that improved glucose metabolism in guar gum-fed mice. The gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) both regulate peripheral glucose metabolism (140, 148). PYY reinforces the insulin action on glucose disposal in muscle and adipose tissue (140) and GLP-1 increases peripheral glucose-mediated glucose uptake independently of hyperinsulinemia (148, 285). Guar gum feeding increased the cecal mRNA expression and concentration of GLP-1 whereas no effect was observed on PYY (Figures 5A-C).

Colonic SCFAs stimulate GLP-1 secretion and mice lacking the free fatty acid receptor (Ffar) 2 exhibited reduced SCFA-triggered GLP-1 secretion and a parallel impairment of glucose tolerance (145, 151). Guar gum also increased cecal mRNA expression of the SCFA receptor Ffar2 (Figure 5D). Therefore, we wondered if cecally supplemented SCFAs were capable of inducing GLP-1 expression. To investigate this, we infused a physiological amount of SCFAs (see materials and methods) directly into the cecum of conscious, unrestrained mice...
Table 1. Hepatic carbohydrate fluxes under basal and hyperinsulinemic conditions. Values are presented as mean ± SEM for n=6-8; *p<0.05 Guar gum vs. control, #p<0.05 hyperinsulinemic vs. basal period within diet.

<table>
<thead>
<tr>
<th>Fluxes (µmol/kg BW/min)</th>
<th>Basal condition</th>
<th>Hyperinsulinemic condition</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Guar Gum</td>
</tr>
<tr>
<td>Glucose 6-phosphatase</td>
<td>125.0 ± 7.1</td>
<td>134.1 ± 4.7</td>
</tr>
<tr>
<td>Glucose kinase</td>
<td>35.7 ± 3.3</td>
<td>25.4 ± 1.4*</td>
</tr>
<tr>
<td>Glycogen synthase</td>
<td>29.6 ± 0.9</td>
<td>50.0 ± 3.6*</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>60.8 ± 2.7</td>
<td>74.4 ± 4.0*</td>
</tr>
<tr>
<td>Gluconeogenesis</td>
<td>58.1 ± 2.2</td>
<td>84.2 ± 2.1*</td>
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that were fed a semi-synthetic diet without guar gum. Infusing SCFAs resulted in an increase in cecal concentration of the infused SCFA without altering the concentrations of the other SCFAs (Figure 6A). All three SCFAs increased the cecal mRNA expression of Ffar2 (Figure 6B). However, only acetate and propionate infusion increased cecal GLP-1 mRNA expression and concentration, whereas cecal PYY mRNA expression and concentration did not respond to any of the three SCFA infusions (Figures 6B-D). Together, these data suggest that guar gum increases peripheral glucose disposal at least partially by enhancing GLP-1 secretion, mediated through the cecal SCFAs acetate and propionate.

Discussion

Above we demonstrated that guar gum protects against HFD-induced obesity and insulin resistance through the same signaling cascade in liver and adipose tissue as supplemented SCFAs, indicating that the absorbed SCFAs are the molecular mediators of the guar gum-induced effect. In addition, guar gum exerts improved peripheral glucose handling, which is at least partially mediated by the SCFA-induced colonic hormone GLP-1.

That guar gum-supplementation protects against HFD-induced obesity, is in agreement with earlier studies that showed that guar gum can protect and reverse body weight gain in rodents and humans (179, 280, 286, Chapter 2). Recently, we showed that the dose-dependent amelioration of the metabolic syndrome by guar gum correlates with in vivo SCFA uptake fluxes by the host, strongly suggesting that the physiological effects of guar gum are primarily mediated by SCFAs that are taken up by the host (Chapter 2). The protection of guar-gum supplementation against HFD-induced obesity and insulin resistance works via repressing PPARγ expression, subsequently increasing mitochondrial UCP2 expression and AMP/ATP ratio, leading to the activation of AMPK and culminating in enhanced oxidative metabolism in both liver and adipose tissue, like previously found for supplemented SCFAs (17, 122, Chapter 5). The finding that guar gum activates the same signaling cascade and downstream metabolic response as SCFAs, further corroborates the notion that absorbed SCFAs are the main molecular mediators of the beneficial effects of guar gum on the metabolic syndrome.

Guar gum supplementation decreases fasting plasma glucose levels in healthy, type 1 and type 2 diabetic humans but a clear mechanism has been lacking so far (171, 178, 287, 288).
Figure 5. Guar gum increases colonic GLP-1 expression. (A) Cecal mRNA expression of PYY and GLP-1 were assessed via qPCR after 12 weeks of diet. (B-C) Plasma PYY and GLP-1 concentrations after 12 weeks of diet. (D) Cecal mRNA expression of Ffar2 was assessed via qPCR after 12 weeks of diet. All values throughout the figure are presented as mean ± SEM for n=6-8; ***p<0.001.

Here we demonstrate that guar gum decreases plasma glucose levels by increasing the rate of glucose clearance by peripheral tissues. Dietary SCFA supplementation elicited no effect on basal plasma glucose levels and glucose tolerance (Chapter 5), while guar gum did. This may be explained by the fact that SCFAs produced by cecal bacterial fermentation of dietary fibers appear in the cecum, while orally ingested SCFAs are absorbed in the small intestine and do not reach the cecum (Supplemental Figures 1A-B). This is likely to affect their metabolic effects, since different hormonal and regulatory responses are triggered in the different places in the gastrointestinal tract (289). Consistently, we found increased plasma levels of GLP-1 in mice supplemented with guar gum or cecally infused acetate or propionate (Figures 5 and 6), but not with orally ingested SCFAs (Supplemental Figure 1C). GLP-1 has been shown to increase peripheral glucose metabolism independently of hyperinsulinemia (290-292), which is consistent with our results that show that guar gum mainly acts on peripheral tissues and that orally ingested SCFAs did not affect basal glucose metabolism (Chapter 5). In addition, we show that unlike acetate and propionate, butyrate does not induce GLP-1 expression when infused directly into the cecum. This selective effect on GLP-1 is in agreement with the result that SCFAs increase GLP-1 through Ffar2 activation (145), which has a preference for acetate and propionate (109, 110).

Although we conclude here that the guar gum-induced effects are primarily mediated by the SCFAs, it is possible that additional metabolites are involved in the response to other fibers. The gut microbiota produces a wide range of other metabolites that have potential biological
functions in host energy metabolism (5). Various dietary modulations alter the composition of the gut microbiota and, subsequently, the host metabolic phenotype and disease risk (293, 294). Overall, this study provides novel molecular insights into the beneficial effects of guar gum on the metabolic syndrome and strengthens the potential role of guar gum as a dietary-fiber intervention.
Supplemental figures

Figure S1. Dietary SCFA-supplementation does not affect GLP-1 expression. (A) Cecal SCFA concentrations after 12 weeks of SCFA feeding. (B) Cecal mRNA expression of genes involved in SCFA transport (MCT-1 and SMCT-1), SCFA signaling (Ffar2 and Ffar3) and PYY and GLP-1 were assessed via qPCR after 12 weeks of SCFA feeding. (C) Plasma GLP-1 concentration after 12 weeks of SCFA feeding. All values throughout the figure are presented as mean ± SEM for n=6-8.
Guar gum protects against the metabolic syndrome via PPARγ and GLP-1