Cholestyramine Reverses Hyperglycemia and Enhances Glucose-Stimulated Glucagon-Like Peptide 1 Release in Zucker Diabetic Fatty Rats

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ABSTRACT

Bile acid sequestrants (BAS) have shown antidiabetic effects in both humans and animals but the underlying mechanism is not clear. In the present study, we evaluated cholestyramine in Zucker diabetic fatty (ZDF) rats. Although control ZDF rats had continuous increases in blood glucose and hemoglobin A1c (HbA1c) and serum glucose and a decrease in serum insulin throughout a 5-week study, the cholestyramine-treated ZDF rats showed a dose-dependent decrease and normalization in serum glucose and HbA1c. An oral glucose tolerance test showed a significant increase in glucose-stimulated glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and insulin release in rats treated with cholestyramine. Quantitative analysis of gene expression indicated that cholestyramine treatment decreased farnesoid X receptor (FXR) activity in the liver and the intestine without liver X receptor (LXR) activation in the liver. Moreover, a combination of an FXR agonist with cholestyramine did not reduce the antihyperglycemic effect over cholestyramine alone, suggesting that the FXR-small heterodimer partner-LXR pathway was not required for the glycemic effects of cholestyramine. In summary, our results demonstrated that cholestyramine could completely reverse hyperglycemia in ZDF rats through improvements in insulin sensitivity and pancreatic β-cell function. Enhancement in GLP-1 and PYY secretion is an important mechanism for BAS-mediated antidiabetic efficacy.

In the last decade, the traditional view of bile acids as essential players in dietary lipid absorption and cholesterol catabolism has changed. The discovery of bile acids as endogenous ligands for the nuclear receptor farnesoid X receptor (FXR; NR1H4) and the G-protein-coupled bile acid receptor (TGR5; GPBAR1) transformed bile acids from lipid absorptive facilitator into autocrine, paracrine, and endocrine factors (Parks et al., 1999; Kawamata et al., 2003). In addition to negative feedback regulation of bile acid synthesis in the liver (Makishima et al., 1999), FXR influences many pathways involved in lipid and glucose metabolism (Lefebvre et al., 2005; Stayrook et al., 2005; Cariou et al., 2006; Ma et al., 2009). FXR agonists have been shown to lower circulating cholesterol, triglycerides, and glucose and improve insulin sensitivity in multiple preclinical models (Duran-Sandoval et al., 2005; Stayrook et al., 2005; Cariou et al., 2006; Ma et al., 2006; Zhang et al., 2006). Similar to FXR, TGR5 mediates some hormonal actions of bile acids, including glucose metabolism. It has been demonstrated that bile acids promote glucagon-like-peptide 1 (GLP-1) secretion through TGR5 in STC-1, a murine enteroendocrine cell line (Katsuma et al., 2006). A part of the manuscript has been published as an abstract at the 69th Scientific Sessions of the American Diabetes Association. Chen L, McNulty J, Anderson D, Liu Y, Nystrom C, Collins J, Handlon T, Klein R, Grimes A, Murray D, et al. (2009) Cholestyramine reverses hyperglycemia and enhances GLP-1 release in Zucker Diabetic Fatty rats. Diabetes 58 (Suppl. 1) A713. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.110.166892.

ABBREVIATIONS: FXR, farnesoid X receptor; BAS, bile acid sequestrant; ZDF, Zucker diabetic fatty; OGTT, oral glucose tolerance test; GLP-1, glucagon-like peptide 1; PYY, peptide YY; LXR, liver X receptor; SHP, small heterodimer partner; T2DM, type 2 diabetes mellitus; HbA1c, blood glucose and hemoglobin A1c; IVITT, intravenous insulin tolerance test; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphate dehydrogenase; TGR5 and GPBAR1, G protein-coupled bile acid receptor 1; NR1H4, nuclear receptor subfamily 1, group H, member 4; GW4064, an FXR agonist.

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S The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.
lestyramine could completely reverse hyperglycemia in male rat pancreas and islet cells (Nakatani et al., 2009; Patti et al., 2007). The stimulatory effect of bile acids on intestinal GLP-1 and PYY secretion has also been demonstrated in preclinical animal models (Plaisancié et al., 1995; Dumoulin et al., 1998) and human (Adrian et al., 1993). Similar effects have been seen with TGR5 agonists (unpublished data). In addition, bile acids may increase energy expenditure in high-fat diet fed mice via TGR5 activation (Watanabe et al., 2006).

Although both FXR and TGR5 agonists are being developed for treatment of type 2 diabetes mellitus (T2DM), there has been no clinical report on their efficacy in humans. On the other hand, an old class of bile acid-related agents, bile acid sequestrants (BAS), has demonstrated good efficacy on reducing blood glucose and hemoglobin A1c (HbA1c) in patients with T2DM (Bays and Goldberg, 2007; Staels and Kuipers, 2007). Bile acid sequestrants are resins that bind to bile acids in the intestinal tract, resulting in a decrease in bile acid reabsorption from the gut and disruption of the enterohepatic circulation of bile acids. BASs were initially approved as hypolipidemic agents. Since the 1990s, several controlled clinical trials demonstrated glucose and HbA1c-lowering effects of BAS (Bays and Goldberg, 2007; Staels and Kuipers, 2007), and the Food and Drug Administration recently approved a BAS, colestelam HCl, for reducing glycemia in patients with T2DM. Given their known effects on low-density lipoprotein cholesterol, BASs may represent a new approach of multi-targeted therapy to improve both cholesterol and glucose management in patients with T2DM (Goldfine, 2008).

Besides its clinical efficacy, little is known about mechanisms by which BAS may alter glucose homeostasis, particularly when compared to the abundance of knowledge on its mechanism of action on lipid metabolism. Although both FXR and TGR5 pathways have been hypothesized in several review articles (Bays and Goldberg, 2007; Staels and Kuipers, 2007; Brinton, 2008; Goldfine, 2008; Staels, 2009), there has been no report of a carefully designed preclinical or clinical study that investigated those hypotheses specifically. In addition, the importance of the gastrointestinal tract in regulating glucose homeostasis has been highlighted by the data that bariatric surgery rapidly corrects diabetes before weight loss (Dar and Pories, 2009), which also changes serum bile acid levels and composition (Nakatani et al., 2009; Patti et al., 2009). In the current study, we demonstrated that cholestyramine could completely reverse hyperglycemia in male ZDF rats via improvements in insulin sensitivity and β-cell function. The cholestyramine treatment also improved glucose-stimulated GLP-1 and insulin secretion in ZDF rats. In addition, our results suggested that the FXR-SHP-LXR pathway was not necessary for the BAS-mediated glucose-lowering effect.

Materials and Methods

Animal Preparation. Male Zucker diabetic fatty (ZDF/GmiCrl-fa/fa) and lean Zucker control rats from Charles River (Raleigh, NC) were housed under controlled conditions (12/12-h light/dark cycle, 24°C and 50% relative humidity), with free access to standard rodent chow (Purina 5008; Harlan Teklad, Indianapolis, IN). All rats arrived at 6 weeks of age (±3 days). After a 1-week acclimatization period, rats were anesthetized with isoflurane (Abbott Laboratories, Abbott Park, IL), and tail-vein blood samples were collected at 9:00 AM without fasting. Blood glucose levels were measured using a glucometer (Bayer, Leverkusen, Germany). ZDF rats were then randomized based on blood glucose and assigned to three treatment groups: powdered Purina 5008 (Purina, St. Louis, MO) diet only; powdered Purina 5008 diet mixed with 1.5% (w/w) cholestyramine; and powdered Purina 5008 diet mixed with 4.5% (w/w) cholestyramine. All animals were followed for 5 weeks, and blood samples were collected via tail vein from isoflurane-anesthetized rats by the end of weeks 1, 2, and 4 at 9:00 AM without fasting. All animals were cannulated (jugular vein) at the beginning of the 3rd week, and an oral glucose tolerance test (OGTT) was performed 5 days later. All animals were euthanized at 9:00 AM without fasting by the end of the 5th week. Blood and tissue samples were collected for further analysis. Two-day food consumption data and 24-h fecal samples were collected during the 2nd week of treatment.

In a separate study, 8-week-old male ZDF rats were randomized based on blood glucose and assigned to one of the three treatment groups: powdered Purina 5008 diet only; powdered Purina 5008 diet mixed with 4.5% (w/w) cholestyramine; and powdered Purina 5008 diet mixed with 4.5% (w/w) cholestyramine and GW4064 (30 mg/100 g food), an FXR agonist (Maloney et al., 2000), for 3 weeks. Serum glucose, insulin, and blood HbA1c were monitored once per week.

In another study, 7-week-old jugular vein-cannulated male ZDF rats were ordered from Charles River and randomized based on blood glucose at the age of 8 weeks. Rats were assigned to one of two groups: powdered Purina 5008 diet only or powdered Purina 5008 diet mixed with 4.5% (w/w) cholestyramine. Six days after the initial treatment, an intravenous insulin tolerance test (IVITT) was performed without fasting. All procedures performed were in compliance with the Animal Welfare Act and United States Department of Agriculture regulations and were approved by the GlaxoSmithKline Animal Care and Use Committee.

Oral Glucose Tolerance Test. All rats were fasted overnight (16 h) and dosed with sitagliptin (10 mg/kg), a dipeptidyl peptidase IV inhibitor, followed by oral administration of dextrose (1 g/kg) 30 min later. Blood samples were collected via jugular vein cannulation immediately before and 5, 10, 20, 30, 60, 90, and 120 min after the administration of dextrose. Blood glucose levels were measured with glucometers (Bayer, Leverkusen, Germany), and plasma samples were collected for further analysis.

Intravenous Insulin Tolerance Test. Nonfasted rats were given a bolus of regular human insulin (Humulin R, 2 U/kg i.v.; Eli Lilly, Indianapolis, IN). Blood samples were collected via jugular vein cannulation immediately before and 5, 10, 20, 30, 45, and 60 min after the administration of insulin. Blood glucose levels were measured using glucometers (Bayer, Leverkusen, Germany).

Measurement of Clinical Chemistry Parameters. Serum glucose, total cholesterol, nonesterified fatty acids, triglyceride, glycerol, bile acids and β-hydroxybutyrate, and lipids and bile acids in fecal extracts were measured with the Instrumentation Laboratory Lia600TM clinical chemistry analyzer (Instrumentation Laboratory, Boston, MA). GLP-1 (active) was measured with an enzyme-linked immunosorbent assay kit (Millipore, Billerica, MA). Total plasma PYY was determined using a Milliplex multi-analyte profiling kit (Millipore). Plasma immunoreactive insulin was determined with an insulin enzyme-linked immunosorbent assay kit using rat insulin as a standard (Crystal Chem, Downers Grove, IL).

Tissue Gene Expression. All tissue samples were collected as described above and immediately frozen in liquid nitrogen and then stored at ~80°C. Total RNA was isolated using an RNeasy Mini Kit (QIAGEN, Valencia, CA). The RNAase Free DNase set (QIAGEN) was used to remove DNA contamination. Cleaned total RNA was reverse-transcribed using a High Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Quantitative PCR was performed and analyzed using a 384-format ABI 7900HT Sequence Detection System (Applied Biosystems), with gene-specific primers and probes (Supplemental Table S-1). The expression level was normalized for a group of house-keeping genes.

Data Analysis. All values are given as mean ± S.E. The data were analyzed in JMP 7.0.0 (SAS Institute, Cary, NC) using one-way analysis of variance (ANOVA) and Dunnet multiple comparison test (GraphPad PRISM 5.00, GraphPad Software Inc., La Jolla, CA). A p value <0.05 was considered statistically significant.
analysis of variance with prespecified contrasts to compare each group with the appropriate control group. \( P \) values <0.05 were considered to indicate a significant difference between treatment groups.

**Results**

**Effects of Cholestyramine on Serum Glucose, HbA1c, and Insulin.** ZDF rats started receiving treatment at the age of 7 weeks when their serum glucose levels (269 ± 13 mg/dL) were significantly higher than those of lean Zucker controls (152 ± 3 mg/dL, \( p < 0.001 \)). After randomization, all three groups of ZDF rats had comparable levels of serum glucose, insulin, and blood HbA1c (Fig. 1, A and B) before the treatment. The ZDF control group showed progressive hyperglycemia and hyperinsulinemia during the study with a gradual decrease in serum insulin and increase in serum glucose (Fig. 1, A and B). After 1 week of treatment, both 1.5 and 4.5% cholestyramine-treated groups had significantly lower serum glucose compared with the ZDF control group, and the glucose level in the 4.5% cholestyramine-treated group was similar to that of the lean controls (Fig. 1A). Whereas the 4.5% cholestyramine treatment group main-

![Figure 1](https://jpet.aspetjournals.org/)

**Fig. 1.** The effects of cholestyramine on serum glucose (A) and insulin (B) and blood HbA1c (C). Seven-week-old male ZDF rats were treated with 1.5 or 4.5% cholestyramine for 5 weeks. Blood and serum samples were collected weekly before and after treatment without fasting. *, \( p < 0.05 \), **, \( p < 0.01 \), ***, \( p < 0.001 \) versus ZDF control group. Inset: baseline insulin.

...tained normal glycemia until the end of the study, serum glucose of the 1.5% cholestyramine-treated group started to rise after the 2nd week (Fig. 1A). Serum insulin showed a similar pattern. Although both treatment groups had a significant drop in serum insulin in the 1st week of treatment, the insulin levels of 1.5% cholestyramine-treated rats started to rise later in the study (Fig. 1B). The HbA1c results were consistent with the serum glucose data. Both the 1.5 and 4.5% treatment groups had significantly lower HbA1c compared with the ZDF control group for the first 2 weeks. HbA1c of the 4.5% cholestyramine group continued to drop at week 4, but the 1.5% treatment group had an increase of HbA1c, which was indistinguishable from that of the ZDF control group (Fig. 1C).

**Responses of GLP-1, PYY, and Insulin during OGTT.** To determine the potential mechanisms for cholestyramine-mediated improvement in glucose metabolism, we performed an OGTT in three treatment groups during the 3rd week of treatment: Zucker lean control, ZDF control, and 4.5% cholestyramine. All rats were pretreated with sitagliptin 30 min before glucose challenge to prevent the degradation of active GLP-1. Although the 4.5% cholestyramine group had normalized fed glucose (Fig. 1A), there was no difference in fasting glucose between the ZDF control and 4.5% cholestyramine groups where the fasting serum glucose levels were higher than those in the Zucker lean controls (Zucker lean: 83 ± 4 mg/dL; ZDF control: 130 ± 13 mg/dL; 4.5% cholestyramine: 128 ± 26 mg/dL; \( p < 0.05 \)). Both ZDF groups had similar glucose response curves in OGTT (Fig. 2A). However, cholestyramine-treated animals had significantly enhanced insulin secretion in both the early and late phases (Fig. 2B). The 4.5% cholestyramine-treated group also had an enhanced glucose-stimulated GLP-1 release (Fig. 2C), which suggested a potential incretin mechanism for cholestyramine-mediated hypoglycemic effects. Although there was no difference at baseline (ZDF control, 101.7 ± 7.4 mg/dL; 4.5% cholestyramine, 110.5 ± 8.9 mg/dL), there was a highly significant difference in glucose-stimulated PYY release in ZDF rats with or without cholestyramine treatment. All animals in the ZDF control group had decreased serum PYY 5 min after glucose challenge, and all cholestyramine-treated rats had increased serum PYY (\( 5' \)-ΔPYY. ZDF control, −15.8 ± 6.0 mg/dL; 4.5% cholestyramine, 20.0 ± 9.3 mg/dL; \( p < 0.001 \)).

**Glucose Response during IVITT.** To assess the effect of cholestyramine on insulin sensitivity, we performed an IVITT in ZDF rats treated with 4.5% cholestyramine for 6 days. As shown in Fig. 3, there was a significant separation between the ZDF control and the cholestyramine group. Insulin induced a bigger glucose-lowering response in the cholestyramine-treated rats.

**Changes in Intestinal and Hepatic Gene Expression.** To further explore potential mechanisms for cholestyramine-mediated anti-diabetic effects, we examined the expression of a group of intestinal and hepatic genes with quantitative reverse transcription-polymerase chain reaction. There was no change in intestinal FXR expression in ZDF rats compared with the lean controls. Cholestyramine treatment did not change FXR expression, with the exception that 1.5% cholestyramine slightly increased FXR mRNA in descending colon (Fig. 4A). Cholestyramine significantly decreased the expression of SHP (Fig. 4A), whose expression has been dem-
onstrated previously to be regulated by FXR (Goodwin et al., 2000). *SHP* was up-regulated in the descending colon where FXR expression was also induced in the 1.5% cholestyramine group (Fig. 4A). Control ZDF rats had decreased *TGR5* expression in duodenum, jejunum, and descending colon. In contrast, cholestyramine treatment increased *TGR5* mRNA expression in those segments of the intestine (Fig. 4A). Since cholestyramine treatment significantly enhanced GLP-1 and PYY secretion in OGTT, we analyzed the expression of GLP-1 and PYY in different regions of the intestine. The current data suggested that this effect of cholestyramine might not be associated with an elevated gene expression of those gastrointestinal hormones (data not shown). It is important to point out that we did not find good dose responses of cholestyramine in intestinal gene expression. In fact, for some genes, the 1.5% cholestyramine seemed to have larger effects, suggesting that additional factors other than bile acids may also play an important role in the current study. Similar to its effect in the intestine, cholestyramine treatment also significantly decreased hepatic FXR activation suggested by reduced *SHP* expression (Table 1). The expression of *ABCA1* and *ABCG5* can be up-regulated by the activation of LXR (Jamroz-Wisniewska et al., 2007; Wojcicka et al., 2007). The expression of both *ABCA1* and *ABCG5* was down-regulated in ZDF rats. When *ABCA1* expression was elevated in the 4.5% cholestyramine group, ABCG5 expression tended to be suppressed (Table 1). The expression of *SREBP1c*, another LXR-regulated gene, was elevated in ZDF rats. Cholestyramine treatment decreased SREBP1c mRNA levels (Table 1), which is consistent with observed changes in serum and hepatic triglycerides (Supplemental Fig. S-2). In addition, cholestyramine treatment was also associated with a clear trend of reduced expression of gluconeogenesis genes, such as *PEPCK* and *G6Pase* (Table 1).

Assessment of the Role of FXR Inactivation in the Antidiabetic Efficacy of Cholestyramine. To determine the potential role of the decreased FXR activity in the antidiabetic efficacy of cholestyramine in ZDF rats, we repeated the experiment with 4.5% cholestyramine with or without GW4064, a potent FXR agonist, in 8-week-old male ZDF rats. GW4064 had no effects on cholestyramine-mediated efficacy on serum glucose, insulin, and blood HbA1c (Fig. 4B).

**Discussion**

The effectiveness of BASs for glycemic control has been reported in several clinical trials (Bays and Goldberg, 2007; Staels and Kuipers, 2007). In the current study, we attempted to determine underlying mechanisms in a diabetic rat model. Insulin resistance and β-cell failure are two major interactive pathophysiological defects in T2DM. In this study, we demonstrated that cholestyramine had significant glucose-lowering effects attributable to its effects on both insulin sensitivity and β-cell function. After 1 week of treatment, both 1.5 and 4.5% treatment groups had significantly reduced fed serum glucose and insulin (Fig. 1, A and B), which strongly suggested a delayed whole-body insulin sensitivity (Supplemental Fig. S-4). This was also supported by the IVITT results (Fig. 3). Future studies such as hyperinsulinemic euglycemic clamp will be needed to evaluate insulin sensitivity in individual metabolic tissues. The 1.5% cholestyramine group gradually lost its efficacy on glycemic control late in the study (Fig. 1A). However, serum insulin of the 1.5% treatment group remained significantly higher than that of the ZDF control group, suggesting an improvement or at least a delay in β-cell failure (Fig. 1B). An improvement in
after cholestyramine treatment (Supplemental Fig. S-3).

A small but clear trend of increased proliferation (Ki67-positive cells) was detected in insulin-positive cells when cholestyramine significantly increased first- and second-phases (Fig. 2B), which are consistent with a previous report in which patients with diet-induced obesity rats treated with colesevelam. Eight-week-old male ZDF rats were treated with 4.5% cholestyramine with or without FXR agonist GW4064 for 3 weeks. Blood and serum samples were collected weekly before and after treatment without fasting. *p < 0.05, **p < 0.01, ***p < 0.001 versus ZDF control group.

Multiple mechanisms for the antidiabetic effect of BAS have been proposed in literature mainly based on existing knowledge of bile acid biology (Bays and Goldberg, 2007; Staels and Kuipers, 2007; Brinton and Kuipers, 2007; Brinton, 2008; Goldfine, 2008; Staels, 2009). Bile acids are generated in the liver via a biosynthetic pathway with cholesterol as the substrate. After being released into the duodenum, bile acids facilitate the digestion and absorption of dietary fat and fat-soluble vitamins. Approximately 90% of the bile acids are reabsorbed in the terminal ileum via an active transporter and return to the liver completing an enterohepatic recirculation (Kullak-Ubl et al., 2004). In addition to digestion, the enterohepatic recirculation of bile acids also has important autocrine, paracrine, and endocrine effects (Keitel et al., 2008). BASs decrease reabsorption of bile acids along the entire intestine, modulate the enterohepatic recirculation, and alter bile acid composition. Physical retention of more bile acids in the lumen of the intestine may modulate the binding and activation of the G-protein-coupled bile acid receptor TGR5 (Kawamata et al., 2003). TGR5 activation may subsequently regulate the release of incretins such as GLP-1 (Katsuma et al., 2003). In the current study, the GLP-1 response during OGTT was associated with an increase in PYY secretion, another peptide hormone from L-cells (Ballantyne, 2006). Similar responses were seen with specific TGR5 agonists (Evans et al., 2009). During the preparation of this article, Shang et al. (2010) reported similar GLP-1 responses during OGTT in diet-induced obesity rats treated with colesevelam. We also noticed that cholestyramine-treated ZDF rats had significantly higher serum GLP-1 levels at fed states compared with untreated ZDF controls (data not shown). This is consistent with a previous report in which patients with

β-cell function was further supported by the improved insulin response in OGTT. Although the ZDF control group had a reduced and right-shifted insulin response to glucose challenge compared with the lean control group, the 4.5% cholestyramine group showed a significant increase in glucose-stimulated insulin secretion in both the first and second phases (Fig. 2B), which are consistent with a previous report where cholestyramine significantly increased first- and second-phase insulin secretion in isolated perfused pancreas from normal rats (Kogire et al., 1992). Furthermore, the improvement in β-cell function occurred without a significant change in islet morphology (Supplemental Fig. S-3). Although no caspase 3 staining in pancreatic islets of ZDF rats was observed, a small but clear trend of increased proliferation (Ki67-positive cells) was detected in insulin-positive cells after cholestyramine treatment (Supplemental Fig. S-3).

**TABLE 1**

Gene expression in the liver (arbitrary units)

Zucker lean controls or ZDF rats with or without cholestyramine treatment for 5 weeks were taken down without fasting at the end of the study. Liver samples were collected for RNA extraction, and qPCR was performed using gene-specific primers and probes.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Zucker Lean Control</th>
<th>ZDF Control</th>
<th>ZDF + 1.5% Cholestyramine</th>
<th>ZDF + 4.5% Cholestyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>73 ± 6***</td>
<td>41 ± 5</td>
<td>45 ± 2</td>
<td>58 ± 5*</td>
</tr>
<tr>
<td>ABCG5</td>
<td>88 ± 8***</td>
<td>19 ± 6</td>
<td>16 ± 5</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>G6Pase</td>
<td>34 ± 1</td>
<td>35 ± 7</td>
<td>29 ± 7</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>SHP</td>
<td>41 ± 6</td>
<td>42 ± 7</td>
<td>28 ± 4</td>
<td>20 ± 4**</td>
</tr>
<tr>
<td>FXR</td>
<td>22 ± 1**</td>
<td>17 ± 1</td>
<td>21 ± 1*</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>50 ± 7***</td>
<td>114 ± 4</td>
<td>77 ± 5***</td>
<td>53 ± 6***</td>
</tr>
<tr>
<td>LXRα</td>
<td>54 ± 2</td>
<td>48 ± 1</td>
<td>50 ± 2</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>LXRβ</td>
<td>98 ± 8</td>
<td>89 ± 5</td>
<td>77 ± 4</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>PEPCK</td>
<td>110 ± 14</td>
<td>86 ± 15</td>
<td>70 ± 10</td>
<td>51 ± 6</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 vs. ZDF control group.
T2DM treated with colestimide had increased GLP-1 levels in 2 h. (Suzuki et al., 2007). Our results also indicated a reduction of TGR5 mRNA expression in duodenum, jejunum, and descending colon of control ZDF rats versus the lean controls (Fig. 4). Cholestyramine treatment tended to increase TGR5 expression at those locations (Fig. 4). It is important to note that TGR5-mediated activation of L-cells may not be the only mechanism for BAS-modulated secretion of GLP-1 and PYY. Our preliminary data also suggested that cholestyramine had significant effects on the expression of genes for various functional pathways that might also regulate secretion of GLP-1 and PYY from enteroendocrine L-cells (data not shown).

In addition to TGR5 signaling, bile acids also have hormonal effects via various nuclear receptors, including the FXR and LXR. Both nuclear receptors play important roles in bile acid and triglyceride homeostasis and glucose metabolism (Hansen and Connolly, 2008; Sugden and Holness, 2008). Bile acids are endogenous ligands for FXR (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Upon FXR activation, the expression of SHP is up-regulated. SHP then inhibits the activation of LXR (Macchiarulo et al., 2006; Kim et al., 2009). The FXR-SHP-LXR pathway has been proposed as a major potential mechanism for the glycemic effect of BAS in several reviews (Bays and Goldberg, 2007; Goldfine, 2008). Although one report demonstrated that activation of FXR induced the expression of hepatic gluconeogenesis genes and in vivo glucose production (Stayrook et al., 2005), most studies demonstrated that selective FXR agonists decreased serum glucose and the expression of hepatic gluconeogenesis genes with reduced hepatic lipogenesis in multiple animal models (Ma et al., 2006; Zhang et al., 2006). Meanwhile, LXR also has profound effects on hepatic glucose and triglyceride metabolism (Jamroz-Wisniewska et al., 2007; Wojcicka et al., 2007). Activation of LXR with synthetic agents inhibited gluconeogenesis by down-regulating PEPCK and G6Pase expression, it significantly stimulated biosynthesis of triglycerides in liver by up-regulating lipogenesis genes such as SREBP1c. In Zucker fatty rats, the dose of a LXR agonist that normalized plasma glucose also significantly increased plasma and liver triglycerides (Cao et al., 2003). In our study, cholestyramine treatment reduced both serum glucose (Fig. 1A) and triglyceride levels and liver triglyceride contents (Supplemental Fig. S-2) in ZDF rats. To further assess the role of FXR-SHP-LXR pathway in cholestyramine-mediated glycemic effects, we determined the expression of several genes regulated by either FXR or LXR in the intestine and the liver. There was an increase in the expression of SHP in the upper regions of the small intestine of ZDF rats (Fig. 4A). Cholestyramine treatment significantly decreased SHP expression from the duodenum to the ascending colon without changing FXR expression levels (Fig. 4A). The hepatic expression of SHP was also significantly suppressed in the treatment groups (Table 1). These data indicated a reduction of FXR activity in both the intestinal tract and the liver with cholestyramine treatment. It is noteworthy that we did not find any increase in hepatic LXRα, LXRβ, and ABCG5 expression in cholestyramine-treated groups (Table 1). Although ABCA1 was slightly up-regulated in the 4.5% treatment group, SREBP1c expression was significantly down-regulated (Table 1). This was consistent with the lower hepatic triglyceride levels found in cholestyramine-treated groups (Supplemental Fig. S-2). Meanwhile, although not statistically significant, there was a clear trend of suppression of PEPCK and G6Pase expression in the liver (Table 1). Overall, the gene expression data did not support the hypothesis that increasing hepatic LXR activity mediated the hypoglycemic effects of cholestyramine. To further explore the role of the FXR-SHP-LXR pathway in the whole-body glycemic effects of cholestyramine, we repeated the experiment with or without GW4064, a potent FXR agonist, in ZDF rats. As shown in Fig. 4B, the addition of GW4064 did not change the effects of cholestyramine on glucose, insulin, and HbA1c. Taken together, our results suggested that the FXR-SHP-LXR pathway was not required for the antidiabetic efficacy of cholestyramine in ZDF rats.

Changes in diet, food intake, and body weight may also affect the diabetic condition of obese ZDF. In a previous report, colestimide significantly reduced blood glucose in high-fat diet induced T2DM mice, which was associated with a significant decrease in body weight and fat absorption (Kobayashi et al., 2007). Although cholestyramine treatment did increase fecal lipid contents, there was no difference in body weight and food consumption between the control and cholestyramine groups (Supplemental Fig. S-1). Decreases in circulating or tissue triglyceride levels with cholestyramine treatment might contribute to the increase in insulin sensitivity. However, compared with the lean Zucker control group, both cholestyramine treatment groups were still severely hypertriglyceridemic (Supplemental Fig. S-2).

In summary, the present study showed that cholestyramine improved glycemic control and β-cell function in male ZDF rats by enhancing nutrient-regulated incretin responses, such as GLP-1 and PYY. Our data also suggested that the FXR-SHP-LXR pathway was not required for cholestyramine-mediated glycemic effects. Understanding the mechanisms for bile acid-related metabolic effects within and outside of the intestinal tract may provide additional insights into new drug development for diabetes.

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References


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