Diabetes mellitus is a chronic systemic inflammatory disease. In addition to the muscle, liver, and pancreatic β-cells, adipose tissue (accelerated lipolysis), the gastrointestinal tract (incretin deficiency/resistance), α-cells (hyperglucagonemia), the kidney (increased glucose reabsorption), and the brain (insulin resistance) all play important roles in the pathogenesis of type 2 diabetes mellitus. Accumulating evidence from animals and humans suggests that the gastrointestinal tract is a critical organ in glucose homeostasis.  

Gastrointestinal complications of diabetes includes gastroparesis, intestinal enteropathy, and nonalcoholic fatty liver disease. The gastrointestinal tract may play a key role in glucose homeostasis. Recently, the direct association between gastrointestinal function and hyperglycemia in diabetes has received attention. The gastrointestinal tract is responsible for delivering glucose to the circulatory system. Glucose production from polysaccharides in the small intestine could be lowered in normal rats via protein kinase C-δ-initiated signaling pathway. However, the signaling pathway may be disturbed in rats fed with a high-fat diet or in diabetic rats. The rate of glucose absorption in the duodenum is modulated by the blood glucose concentration within the normal range, but not necessarily under hyperglycemic conditions. Multiple glucoregulatory hormones are secreted in the gastrointestinal tract in concert with insulin and glucagon. These hormones help regulate glucose homeostasis. Therefore, normal intestinal function may be essential in regulating blood glucose. However, current understanding of the pathophysiological alterations in the small intestine in type 2 diabetes mellitus is limited.

ABSTRACT

Objective: Inflammation of the small intestine may occur in type 2 diabetes. This study aimed to investigate whether ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) were altered in chronic inflammation of the small intestine of type 2 diabetic rats.

Methods: Thirty-two male Sprague-Dawley rats were used. Eight rats in the control group were fed with regular chow, and 24 rats were fed a high-fat diet and injected with a single low dose of streptozotocin. All of the control rats and diabetic rats were bred for 10 months. Immunohistochemistry detected ABCA1 and ABCG1 in the small intestine in all the rats.

Results: Hematoxylin-eosin staining showed chronic inflammation in the small intestine of the diabetic rats. Immunohistochemistry staining showed that alteration of ABCA1 and ABCG1 was different in the inflammatory and epithelial cells. Quantitative analysis showed that the overall expression of ABCA1 and ABCG1 increased in the diabetic rats compared to the control rats. Both ABCA1 and ABCG1 were enriched in the inflammatory cells of the small intestine in diabetic rats. In the epithelial cells, ABCA1, but not ABCG1, was detected in significantly more diabetic rats than control rats.

Conclusion: Both ABCA1 and ABCG1 are enriched in chronic inflammation of the small intestine of type 2 diabetic rats. ABCA1, but not ABCG1, is activated in the intestinal epithelial cells of type 2 diabetic rats.

Keywords: ATP-binding cassette transporters, type 2 diabetes mellitus, rat, small intestine

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Abbreviations

ATP, adenosine triphosphate; ABCA1, adenosine triphosphate-binding cassette transporter A1; ABCG1, adenosine triphosphate-binding cassette transporter G1; TBS, tris-buffered saline

1Department of Endocrinology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou; 2Department of Endocrinology, Ningbo Medical Treatment Center, Lihuili Hospital, Ningbo; 3Department of Pathology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou; 4Department of Endocrinology, Jiangshan People’s Hospital, Jiangshan, Zhejiang, People’s Republic of China

*To whom correspondence should be addressed. E-mail: mingzhixu@yeah.net
Adenosine triphosphate-binding cassette transporters are involved in a variety of physiological processes such as lipid metabolism; cellular survival; apoptosis; glucose homeostasis, and insulin secretion. The adenosine triphosphate-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) are the most important transporters in the pathogenesis of type 2 diabetes mellitus. Studies have shown that ABCA1 and ABCG1 are cholesterol transport proteins that help regulate glucose homeostasis and insulin secretion. ABCA1 and ABCG1 may have anti-inflammatory functions independent of their lipid transport activities. The expression of ABCA1 and ABCG1 has been shown to be reduced in peripheral monocytes or monocyte-derived macrophages in type 2 diabetic patients. However, the significance of ABCA1 and ABCG1 activity during chronic inflammation of the small intestine in type 2 diabetes mellitus is not clear. The aim of this study was to investigate the expression of these 2 important cholesterol transporters in the small intestine of type 2 diabetic rats, which might help clarify the pathophysiological alterations of the gastrointestinal tract in diabetes.

Materials and Methods

Establishing the Type 2 Diabetic Rat Model

The type 2 diabetic rat model was established according to Zhang and colleagues, but with some modifications. Thirty-two male Sprague-Dawley rats (250-300 g) were purchased from Zhejiang Academy of Medical Sciences (Hangzhou, China). The rats were housed in standard polypropylene cages (4 rats per cage) and maintained under a controlled room temperature and humidity with a 12/12 h light/dark cycle.

Rats were randomly divided into 2 groups, a control group (8 rats) and experimental group (24 rats). Regular chow (consisting of 11% fat, 65% carbohydrates, and 24% protein) and high-fat chow (consisting of 32% fat, 50% carbohydrates, and 18% protein) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd., (Shanghai, China). Rats in the control group were fed with regular chow and rats in the experimental group were fed the high-fat diet. At the end of the first month, the experimental rats were injected intraperitoneally with a low dose of streptozotocin (30 mg/kg, Sigma, S0130, Beijing, China), while the control rats were given citrate buffer as the vehicle (pH 4.4) in a dose volume of 0.25 mL/kg.

Hematoxylin-eosin and Immunohistochemistry Staining

The rats were euthanized after being anaesthetized by 5% chloral hydrate at the end of the 10 months after fasting overnight. It is difficult to differentiate the duodenum, jejunum, and ileum either from the gross specimen or microscopically. Hence, samples of the small intestine within 2 cm of the pylorus were fixed in 4% formalin and embedded in paraffin. Hematoxylin-eosin staining and immunohistochemistry for ABCA1 and ABCG1 were conducted as previously described. Sections were generally pretreated and incubated with primary antibodies at room temperature for 2 hours. The primary antibodies

After streptozotocin or vehicle injections, blood glucose was monitored. Preliminary experiments showed that the rats with a fasting glucose >25 mmol/L died within 3 days after streptozotocin injection; the deaths may have been due to diabetic ketoacidosis. About half of the rats with a fasting glucose of 18-25 mmol/L survived 1 week after the injection of streptozotocin. However, those rats died without insulin treatment in the following months, which suggested that they were insulin-dependent (type 1 diabetes mellitus).

Regular insulin (Wanbang Biochemical Pharmaceutical Co., Jiangsu, China) was administered if glucose was >18 mmol/L to prevent further islet injury due to induced high glucose. All of the rats fasted for 12 hours prior to blood glucose measurement, which were performed 1 week after the injection of streptozotocin. A fasting glucose ≥ 7.8 mmol/L was considered to be diabetic. Twenty-two of the 24 rats in the experimental group became diabetic. Both the control group and the diabetic rats were fed with the corresponding low- and high-fat diets for at least 9 months. Their body weights were recorded every week and food intake was recorded daily. Body length was measured from the nose to the anus. Lee’s index was used to describe the obesity of the rats and was calculated according to the formula: Lee’s index = the cube root of weight (g) × 1000/length (cm). Biochemical parameters were also monitored monthly. Blood samples were taken after an overnight fast. Total cholesterol and triglyceride, LDL cholesterol, HDL cholesterol, and fasting glucose were determined on the HITACH 7170 autoanalyzer (Tokyo, Japan). Type 2 diabetic rats were diagnosed according to the following 4 items of criteria: 1) fasting glucose ≥ 7.8 mmol/L or non-fasting glucose ≥ 11.1 mmol/L on 3 occasions; 2) survived without insulin treatment; 3) abdominal obesity; and 4) the typical lipid profile of increased triglycerides and decreased HDL.

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of rabbit polyclonal anti-ABCA1 (Novus Biologicals Inc., Littleton, Colo.) and rabbit polyclonal anti-ABCG1 (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) were diluted (1:200 for anti-ABCA1 and 1:100 for anti-ABCG1) with tris-buffered saline (TBS). The tissue sections were then incubated with the anti-rabbit ENVISION System, HRP Labeled Polymer (DAKO, Carpinteria, Calif.) as the secondary antibody at room temperature for 30 minutes. Between each of these steps, tissue sections were rinsed 3 times with TBS. Diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) was used for color development and hematoxylin was used to counterstain the specimen. The specificity of immunohistochemistry was confirmed by omitting the primary antibodies. The expression of ABCA1 and ABCG1 in the epithelial cells and the inflammatory cells of the small intestine was examined by a pathologist who did not know the grouping of the rats. The results were expressed as positive (granular brown in the cytoplasm) or negative. Quantitative analysis was made using Image-Pro Plus 6.0 (OLYMPUS, Tokyo, Japan), and the overall expression of ABCA1 and ABCG1 in the small intestine was expressed as the integrated option density within a unit area.

### Data Analysis and Statistics

Data with normal distribution were expressed as mean and standard error mean. Chi-square analysis was used to evaluate differences in proportions among the groups. The mean differences of continuous variables between 2 independent groups were evaluated using Student’s t-test. Two-sided P value <0.05 was considered to be statistically significant.

### Results

#### General Characteristics of the Rats

The daily intake of the diabetic rats was higher than the control rats (Figure 1A); no stool abnormalities were found. The body weight of the diabetic rats increased more rapidly than that of the control rats (Figure 1B). When sacrificed, the diabetic rats were heavier, longer, and larger in abdominal circumference compared to the control rats (Table 1). As a result, Lee’s index was significantly increased in the diabetic rats (Table 1).

### Table 1. General Characteristics and Clinical Biochemistry Features of Rats When Euthanized

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Diabetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>670.6 ± 43.8</td>
<td>840.2 ± 37.6*</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>27.5 ± 0.6</td>
<td>28.9 ± 0.2*</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>25.1 ± 2.0</td>
<td>28.3 ± 1.1*</td>
</tr>
<tr>
<td>Lee’s index</td>
<td>28.9 ± 0.6</td>
<td>30.7 ± 0.4*</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.12 ± 0.06</td>
<td>0.46 ± 0.17*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.29 ± 0.11</td>
<td>1.66 ± 0.10*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.12 ± 0.08</td>
<td>0.91 ± 0.04*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>0.56 ± 0.08</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>8.69 ± 0.71</td>
<td>18.67 ± 1.66*</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>46.4 ± 5.1</td>
<td>52.6 ± 5.5</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>73.6 ± 8.4</td>
<td>89.9 ± 10.4</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>33.9 ± 4.9</td>
<td>34.1 ± 4.9</td>
</tr>
<tr>
<td>Urea nitrogen (mmol/L)</td>
<td>4.76 ± 0.23</td>
<td>4.27 ± 0.34</td>
</tr>
<tr>
<td>Uric acid (µmol/L)</td>
<td>69.6 ± 12.8</td>
<td>64.2 ± 5.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. Student’s t-test was performed. *P <0.05 compared with control group.
Clinical Biochemistry Features of the Rats

The clinical biochemistry features of the rats are shown in Table 1. As expected, the fasting glucose was significantly higher in the diabetic rats. Plasma triglycerides and total cholesterol also increased, but HDL cholesterol decreased in the diabetic rats. The liver function and renal function of the diabetic rats were normal. Based on abdominal obesity, survival under persistent hyperglycemia for 9 months without insulin supplementation, and increased triglycerides, total cholesterol and decreased HDL cholesterol, the diabetic rat model met the criteria for type 2 diabetes mellitus.

Routine Pathological Findings and ABCA1/ABCG1 Expression in the Small Intestine

Hematoxylin-eosin staining showed that the villous of the small intestine were long and thin with a smooth surface; few inflammatory cells could be seen (Images 1A and 1B). In contrast, the villous became shorter and wider; many lymphocytes infiltrated into the stroma and even into the epithelium of the small intestine in the diabetic rats (Images 1C and 1D).

Immunohistochemistry staining showed that ABCA1 and ABCG1 expression was different between the inflammatory cells.
cells and the epithelial cells. Quantitative analysis showed that the overall expression of ABCA1 and ABCG1 was increased in the small intestine of the diabetic rats compared to control rats (Figure 2).

Immunohistochemistry staining showed that ABCA1 was enriched in the cells surrounding the inflammatory regions in the diabetic rats (Image 2). ABCA1 expression in the epithelial cells of the small intestine could be detected in significantly more diabetic rats than the control rats (77.3% vs. 25.0%, \( P = 0.009 \)). ABCG1 was also enriched in the cells surrounding the inflammatory areas in the diabetic rats (Image 3). ABCG1 expression in the epithelial cells of the small intestine was similar in diabetic and control rats (50.0% vs. 54.5%, \( P = 0.825 \)).

Discussion

Worldwide incidence of diabetes is increasing, with 95% of people with the disease having type 2 diabetes mellitus. Control of blood glucose concentration is crucial to preventing complications of diabetes, and attention has been focused on the role of the gastrointestinal tract in diabetes. Gastrointestinal dysfunction may influence glucose control and therefore aggravate the illness. Experimental evidence suggests that the small intestine helps regulate glucose production and absorption and secretes glucoregulatory hormones. Some hypoglycemic drugs using to treat diabetes work through the small intestine, such as \( \alpha \)-glucosidase inhibitors. Insights into the mechanisms by which the gut contributes to regulation of blood glucose have prompted several strategies designed to lower postprandial blood glucose. Some hypoglycemic drugs have severe gastrointestinal side effects. Current knowledge about the mechanisms of the gastrointestinal changes in diabetes is limited.

Experimental models are essential tools for understanding the molecular basis and pathogenesis of type 2 diabetes. Although several natural or developed models have been used for studying type 2 diabetes, these models do not appear to be consistent with the natural course of type 2 diabetes mellitus. In this study, we tried to set up the diabetic model described by Zhang and colleagues. This model involves the administration of a high-fat diet for 10 months combined with a single low dose of streptozotocin at the end of the first month. The diabetic rats generally displayed the pathophysiological features of type 2 diabetes mellitus. They appeared to have abdominal obesity and they survived with persistent hyperglycemia for 9 months without insulin supplementation. They had increased triglycerides and total cholesterol, and decreased HDL cholesterol. In the diabetic rats, chronic inflammation in the small intestine was histologically evident. This might be the important component of chronic systemic inflammation of type 2 diabetes.

Chronic inflammation in the arterial walls may contribute to diabetes-related atherosclerosis. However, the potential association between gastroparesis or diabetic neuropathy and inflammation in the small intestine is not clear. We considered that the inflammation of the small intestine was a primary result of diabetes rather than a secondary
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change, because the daily intake by the diabetic rats was higher than that of the control rats and no stool abnormalities were found. Therefore, diabetes-related gastrointestinal disorders could be more than functional and targeted therapy warrants further investigation.

In the present study, we aimed to see whether the inflammation of the small intestine in diabetic rats might be related to the alteration of ABCA1 and ABCG1 expression. ABCA1 reduces inflammation in atherosclerotic lesions, and it may activate multiple signaling pathways including Janus kinase 2/signal transducer and activator of transcription 3, protein kinase A, Rho family G protein Cdc42, and protein kinase C. ABCG1 is also implicated in macrophage infiltration and lung homeostasis. We found that ABCA1 and ABCG1 were expressed by inflammatory cells surrounding the areas of chronic inflammation in the small intestine of type 2 diabetic rats, suggesting that these cholesterol transporters might be involved in the process of inflammation. Under normal circumstances, cholesterol homeostasis in the intestine is mediated mainly by ABCG5/ABCG8, and ABCA1 plays

**Image 2**

ABCA1 measured by immunohistochemistry staining. ABCA1 expression was measured in each of the 8 control rats and the 22 diabetic rats. The expression of ABCA1 in the epithelial cells of the small intestine was detected in 2 of the 8 control rats (2A and 2B showed a control rat with negative ABCA1 expression. 2A: magnification 20×; 2B: magnification 40×). However, it was detected in 17 out of 22 diabetic rats (2C and 2D showed a diabetic rat with positive ABCA1 expression. 2C: magnification 20×; 2D: magnification 40×). The percentage of ABCA1 expression in the intestinal epithelial cells was higher in the diabetic rats compared with the control rats (77.3% vs. 25.0%, *P* = 0.009). ABCA1 was also enriched in the inflammatory cells surrounding the chronic inflammation of the small intestine in the diabetic rats (2C and 2D).
a minor role.\textsuperscript{28} We found that ABCA1 expression was activated in the intestinal epithelial cells of type 2 diabetic rats; however, ABCG1 expression was comparable between control rats and type 2 diabetic rats. Whether the alteration of ABCA1 in the intestinal epithelial cells contributes to cholesterol homeostasis under the diabetic condition warrants further investigation. The expression of ABCA1 and ABCG1 is reduced in peripheral monocytes and macrophages in type 2 diabetic patients.\textsuperscript{17-19} Therefore, the expression and function of ABCA1/ABCG1 may be tissue-specific.\textsuperscript{29}

### Image 3

ABCG1 measured by immunohistochemistry staining. ABCG1 expression was measured in each of the 8 control rats and the 22 diabetic rats. The expression of ABCG1 in the epithelial cells of the small intestine was detected in 4 of the 8 control rats (3A showed a control rat with negative ABCG1 expression, magnification 40×; 3B showed a control rat with positive ABCG1 expression, magnification 40×), and it was detected in 12 out of the 22 diabetic rats (3C showed a diabetic rat with negative ABCG1 expression, magnification 40×; 3D showed a diabetic rat with positive ABCG1 expression, magnification 40×). The percentage of ABCG1 expression in the intestinal epithelial cells was comparable between the diabetic rats and the control rats (50.0% vs. 54.5%, \(P=0.825\)). ABCG1 was also enriched in the inflammatory cells surrounding chronic inflammation in the small intestine of the diabetic rats (3C and 3D).

### Conclusion

In summary, our work has provided preliminary evidence that both ABCA1 and ABCG1 are enriched in the cells surrounding chronic inflammation in the small intestine of type 2 diabetic rats. Whether the alteration of ABCA1 and ABCG1 is the cause or the subsequent response of the inflammation in the small intestine of diabetic rats is not known. ABCA1, but not ABCG1, is activated in the intestinal epithelial cells of type 2 diabetic rats. Future
studies concentrating on the upstream and downstream signaling of these molecules in the small intestine of type 2 diabetes mellitus may offer a new perspective on preventing diabetic digestive complications, and suggest novel therapeutic targets. LM

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