Hypoglycemic effects of colestimide on type 2 diabetic patients with obesity

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Abstract. Recent studies have shown colestimide, a bile acid-binding resin, to also exert a glucose-lowering effect via amelioration of insulin resistance. To evaluate the effects of colestimide on glucose metabolism and to elucidate the underlying mechanism, we conducted a 6-month, open-label pilot study on 43 type 2 diabetic patients with obesity (BMI ≥ 25). The subjects were randomized to either treatment with colestimide 4g/day (T group, n=23) or continuation of their current therapy (C group, n=20). In the T group patients, mean hemoglobin A1c (HbA1c) and fasting glucose improved markedly (from 7.71 ± 0.32% to 6.97 ± 0.20%; from 147.4 ± 7.3mg/dL to 127.0 ± 5.0mg/dL, respectively), while obesity-related parameters, i.e. body weight, waist circumference, and visceral fat and subcutaneous fat as determined by umbilical slice abdominal CT, showed no significant changes. Fractionation analyses of serum bile acids revealed significantly increased cholic acids (CA) and decreased chenodeoxycholic acids (CDCA) in the T group patients. However, no correlation was observed between these changes and ΔHbA1c. According to logistic regression analysis, baseline HbA1c was the only variable predicting the decrease of HbA1c (>0.5%) among sex, age, BMI, total cholesterol, ΔCA and ΔCDCA. The index of insulin resistance, i.e. homeostasis model assessment of insulin resistance (HOMA-R), did not improve, and the index of β cell function, i.e. homeostasis model assessment of β-cell function (HOMA-β), actually increased significantly. These results suggests that, in obese patients with type 2 diabetes, the mechanism underlying improved glycemic control with colestimide treatment involves enhanced β cell activity rather than improved insulin resistance.

Key words: Colestimide, Cholic acids, Obesity, Insulin resistance, Homeostasis model assessment of β-cell function (HOMA-β)

TYPE 2 DIABETES is a common metabolic disease, and the worldwide prevalence is rising rapidly. Management of type 2 diabetes usually requires multiple oral hypoglycemic agents (OHA) to maintain glycemic control. In obese diabetic patients, metformin and/or thiazolidinediones (TZD) are frequently used as initial therapy [1, 2]. However, when diabetic patients show marked insulin resistance, as with severe obesity, even the maximum doses of OHA including insulin secretagogues are often inadequate to achieve recommended treatment goals. These patients must thus be treated with insulin. However, insulin treatment can lead to marked weight gain and more hypoglycemic episodes, despite fairly good glycemic control [3]. Thus, anti-hyperglycemic agents, which are effective in obese type 2 diabetic patients, are a focus of current research.

Bile acid binding resins are useful for improving lipid profiles. Thus, one of these agents, colestimide, is clinically used for Japanese patients with hyperlipidemia [4]. These agents bind bile acids in the intestine and reduce enterohepatic circulation of bile acids, resulting in enhanced conversion of cholesterol into bile acids, thereby lowering serum cholesterol levels in patients with hyperlipidemia [5]. Recently, a number of clinical studies have shown colestimide to also exert a glucose-lowering effect in type 2 diabetic patients with hypercholesterolemia [6-10]. However, the precise mechanisms underlying the glucose lowering actions of these agents remain unknown. Basic molecular research, using model mice with diet-induced diabetes, showed colestimide to prevent obesity and reduce insulin resistance via diverse metabolic pathways, such as those for fatty acid synthesis and gluconeogenesis [11]. In
the present study, to evaluate the metabolic effects of colestimide and also to clarify the mechanism by which colestimide improves glycemic control, 43 obese type 2 diabetic patients were randomized to either treatment with colestimide or continuation of their current therapy. Surprisingly, colestimide had a marked glucose-lowering effect without affecting insulin resistance, as evaluated by homeostasis model assessment of insulin resistance (HOMA-R), body weight waist circumference and body fat accumulation. In addition, we examined changes in bile acids, by fractionation analysis using gas chromatography-mass spectrometry (GC-MS). Though a number of studies have focused on the glucose-lowering effects of colestimide, this is the first to precisely investigate bile acid compositions before and after colestimide treatment. Our results indicate the glucose-lowering effects of colestimide in type 2 diabetic patients to be complex, and to differ from the current theory which is based on rodent data.

Materials and Methods

Study design

In total, 43 Japanese type 2 diabetic patients with obesity (body mass index (BMI) ≥ 25), maintained on conventional OHA therapy, were enrolled in a 6-month, open-label pilot study. Patients with impaired hepatic function (serum AST/ALT >40) or renal function (serum creatinine >1.5), or on insulin, were excluded. At baseline, the subjects were randomized to either treatment with colestimide 4g/day (T group, n=23) or continuation of their current therapy (C group, n=20). Treatments with other drugs including OHA remained unchanged throughout the study period. The following were measured by standard laboratory techniques, with commercially available procedures, at baseline, and at 2, 4 and 6 months after the initiation of treatment: serum total cholesterol, triglycerides, fasting plasma glucose (FPG), fasting insulin (IRI) and hemoglobin A1c (HbA1c). In addition, indexes that are considered to reflect insulin resistance, HOMA-R=FPG × IRI/405, and β cell activity, homeostasis model assessment of β-cell function (HOMA-β) =360 × IRI/(FPG –63), were calculated. At baseline and at the end of this study, body weights, waist circumferences, serum concentrations of adiponectin, measured by enzyme-linked immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan), visceral fat area (VFA) and subcutaneous fat area (SFA), as determined by umbilical slice abdominal CT, were measured. All patients gave written informed consent prior to participation in this study, which met the Declaration of Helsinki principles and was approved by our institution’s Ethics Committee.

Fractionation analysis by gas chromatography-mass spectrometry (GC-MS)

The fractionation analysis for serum bile acids was performed using GC-MS [12]. In brief, GC-MS was performed on a JEOL JMS-AM 150 instrument (JEOL Co., Tokyo, Japan) using a gas chromatographic column DB-1 with the column temperature programmed from 170 to 230°C at 10°C/min and 230 to 310°C at 3°C/min. Helium was used as the carrier gas with a flow rate of 45 cm/s. The mass spectra were recorded at an ionization energy of 70 eV with an anion source temperature of 290°C.

Statistical analysis

Data are presented as means ± SEs. Log transformation of continuous variables was used when needed to satisfy distributional requirements for parametric tests. Differences in clinical characteristics were assessed using the paired Student’s t test and a P value <0.05 was considered statistically significant. Statistical analyses were performed using Stat View software (Version 5.01; SAS Institute, Cary, NC, USA).

Results

Table 1 shows changes in metabolic parameters in the two groups. Before treatment, there were no significant differences between the two groups in sex, age, BMI, waist circumference, glycemic control, lipid profiles, serum adiponectin concentration or body fat distribution. Colestimide was well tolerated in this study, as no T group subjects experienced serious adverse reactions. Unexpectedly, obesity-related parameters, i.e. body weight, waist circumference and visceral fat and subcutaneous fat, as determined by umbilical slice abdominal CT, did not significantly change in either group. Serum total and high molecular weight (HMW) adiponectin concentrations were also unchanged. Total cholesterol was significantly reduced only in the T group, while serum triglyceride levels showed no significant change in either the T or the C group. Thus, the lipid changes observed were attributed solely to the pharmacological effects of colestimide treatment [13].
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Regarding glycemic profiles, mean HbA1c and fasting plasma glucose improved markedly (from 7.71 ± 0.32% to 6.97 ± 0.20% and from 147.4 ± 7.3 mg/dL to 127.0 ± 5.0 mg/dL, respectively) only in the T group patients, with no significant changes being observed in the C group. In particular, these reductions were clearly observed in only 2 months after the initiation of the therapy, then gradually decreased more as shown in Fig. 1A and 1B. To investigate the characteristics most likely to change with colestimide treatment, we further analyzed the data by dividing the subjects into subgroups based on HbA1c. Interestingly, when subjects were divided by HbA1c, i.e., into a poor glycemic control (HbA1c ≥ 7.5%) and a fairly good glycemic control (HbA1c < 7.5%) group, only the poor glycemic control group patients showed significant improvements in HbA1c and fasting plasma glucose (Fig. 1C and 1D). These results suggest that colestimide exerts a more marked glucose lowering effect in type 2 diabetic patients with higher HbA1c.

### Table 1 Changes in metabolic parameters in groups C and T

<table>
<thead>
<tr>
<th>Table 1 Changes in metabolic parameters in groups C and T</th>
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<tbody>
<tr>
<td>C Group (n=20)</td>
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<tr>
<td>At baseline</td>
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<td>Male/Female</td>
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<td>Age (years)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist (cm)</td>
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<td>FPG (mg/dL)</td>
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<tr>
<td>HbA1c (%)</td>
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<tr>
<td>Fasting IRI (IU/mL)</td>
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<tr>
<td>HOMA-R</td>
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<td>HOMA-β</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
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<tr>
<td>T-Chol (mg/dL)</td>
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<tr>
<td>Adiponectin (mg/mL)</td>
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<td>HMW adiponectin (mg/mL)</td>
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<td>Visceral fat area (cm²)</td>
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<td>Subcutaneous fat area (cm²)</td>
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Data are presented as means + SE. *P value <0.05  HbA1c, hemoglobin A1c; HOMA-R, Homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function.

![Fig. 1](image)

**Fig. 1** (A, B) Changes in HbA1c (A) and fasting plasma glucose (FPG)(B) during the 6-month study period (dashed lines; C group, solid lines; T group), *p<0.05, compared with baseline. (C, D) Changes in HbA1c (C) and fasting plasma glucose (FPG)(D) during the 6-month study period (dashed lines; fairly good glycemic control (HbA1c<7.5%), solid lines; poor glycemic control (HbA1c ≥ 7.5%), *p<0.05, compared with baseline.
involves enhanced β cell function.

As the changes in bile acid profiles appear to be involved in the glucose-lowering effects of colestimide, we investigated bile acid compositions employing GC-MS analysis [12]. Fig. 2 shows the fractionation analyses for serum bile acids at baseline and at the end of the study, for both groups. In the T group patients, cholic acids (CA) were significantly increased, while the serum total bile acid concentration was decreased, observations probably attributable to a significant reduction in chenodeoxycholic acids (CDCA).

To evaluate whether these changes in bile acids composition affect the HbA1c, we analyzed the correlation between ΔHbA1c and ΔCA, ΔCDCA. However, no correlations were observed, while a significant correlation ($p=0.025$) between ΔHbA1c and ΔHOMA-β was observed (Fig. 3). In addition, according to logistic regression analysis, baseline HbA1c was the only variable predicting the decrease of HbA1c (>0.5%) among sex, age, BMI, total cholesterol, ΔCA and ΔCDCA (Table 2). These results suggest that the changes in the serum concentrations of CA and CDCA are not capable of explaining the underlying mechanism of colestimide to improve the glycemic control.

**Discussion**

The coexistence of four metabolic features, i.e. hypertension, dyslipidemia, hyperglycemia and visceral obesity, is defined as metabolic syndrome, which is associated with increased risk of stroke and other cardiovascular events [14]. In addition to the cardiovascular risk, metabolic syndrome is associated with lifestyle habits, such as smoking and exercise, which are also related to macrovascular disease events [15]. Among a number of risk factors, dyslipidemia and hyperglycemia, in particular, were shown to be the major factors raising the risk of atherosclerotic cardiovascular diseases in a prospective epidemiologic study [16]. In the present study, colestimide significantly improved hyperglycemia as compared with conventional therapy and also reduced serum cholesterol levels. As no
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These results suggest bile acid binding resins to be highly beneficial for type 2 diabetic patients, beyond their cholesterol-lowering effects, for the prevention of cardiovascular diseases. Though a number of studies have focused on the glucose-lowering effects of colestimide, our study is the first attempt to clarify this mechanism by precisely analyzing bile acid compositions using gas chromatography-mass spectrometry (GC-MS) before and after colestimide treatment. As expected, CDCA levels were significantly decreased while CA levels were increased in the colestimide-treated subjects. The mechanisms by which bile acid binding resins affect glycemic parameters are not fully understood. The most widely accepted hypothesis involves interactions of bile acids with nuclear receptors. Bile acids, as endogenous legends, were demonstrated to activate the farnesoid X receptor (FXR) [20], which leads to the inhibition of gluconeogenesis via inhibiting the PEPCK expression [21].

Most notably, FXR was reported to be mostly activated by the hydrophobic bile acid CDCA but not by cholic acids [18, 19]. These results suggest bile acid binding resins to be highly beneficial for type 2 diabetic patients, beyond their cholesterol-lowering effects, for the prevention of cardiovascular diseases.

Table 2 Logistic regression analysis for reduction of HbA1c level by >0.5%

<table>
<thead>
<tr>
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<th>Odds ratio (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>Sex</td>
<td>0.530 (0.021-46.43)</td>
<td>0.6975</td>
</tr>
<tr>
<td>Age</td>
<td>1.049 (0.893-1.232)</td>
<td>0.5635</td>
</tr>
<tr>
<td>BMI</td>
<td>1.080 (0.766-1.522)</td>
<td>0.6607</td>
</tr>
<tr>
<td>HbA1c</td>
<td>9.065 (1.128-88.41)</td>
<td>0.0378</td>
</tr>
<tr>
<td>T-Chol</td>
<td>1.019 (0.969-1.069)</td>
<td>0.6338</td>
</tr>
<tr>
<td>Δ-CA</td>
<td>0.514 (0.010-11.89)</td>
<td>0.9691</td>
</tr>
<tr>
<td>Δ-CDCA</td>
<td>0.350 (0.052-1.645)</td>
<td>0.5595</td>
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Δ-CA, delta cholic acids, Δ-CDCA, delta chenodeoxy cholic acids

clinically meaningful threshold level of cholesterol has been established for patients with type 2 diabetes, according to a previous investigation [17], colestimide, as used in the present study, is likely to be effective in diabetic patients regardless of whether or not hypercholesterolemia is present. In fact, coleveselam, another bile acid binding resin, is also used clinically as an anti-diabetic agent, based on the results of previous studies which demonstrated its glucose-lowering effect [18, 19].
In our study, bile acid binding resins disrupted the bile acid enterohepatic circulation, thereby decreasing serum levels of bile acids CDCA. Thus, the decrease of CDCA is not likely to affect the HbA1c reduction, or rather HbA1c increase. Plausible explanation for the glucose-lowering effect of bile acid binding resins is cholic acid (CA)-induced energy expenditure, which is promoted via intracellular thyroid hormone activation [23]. Bile acid binding resins inhibit the absorption of CDCA, but not that of CA. In addition, bile acid binding resins actually stimulate CA production. Thus, the up-regulation of CA, observed in our study, is expected to be related to the reduction of HbA1c or body weight. However, no correlations were observed between the HbA1c reduction and changes in CA levels. Moreover, we detected no changes in insulin sensitivity, i.e., BMI, waist circumference, VFA and SFA, and HOMA-R, despite the up-regulation of CA, in colestimide-treated subjects. These results suggest that changes in bile acid components can not explain the glucose-lowering effect of bile acid binding resins. We can raise the two reasons for no correlation between the CA and HbA1c changes in our study; 1) Comparing with rodents, human has smaller amounts of brown adipose tissue, which is the main target for CA-induced energy expenditure [23]. 2) In the experiments using various animal models [23], the serum concentration is much higher (over than 10 folds of physiological conditions) than the serum CA levels observed in our study. Thus, these two conditions resulted in our results that the up-regulation of CA could not contribute to the HbA1c reduction.

Another proposed mechanism accounting for the glucose-lowering effects of bile acid binding resins is related to incretins such as glucagon-like peptide 1 (GLP-1). Recently, bile acids demonstrated to stimulate GLP-1 secretion via TGR5 activation [24]. Except for bile acids-mediated mechanism, there are other possibilities for colestimide to enhance GLP-1 excretion via binding activities with other substances than bile acids. In fact, the GLP-1 increasing effect of colestimide was recently reported [6]. GLP-1 enhances β cell function by stimulating insulin secretion [25], which may explain that the HOMA-β increase contributed to the HbA1c reduction in the colestimide-treated group. In addition, our results indicate that colestimide exerts a stronger glucose lowering effect in type 2 diabetic patients with higher HbA1c. This feature was also observed in the recent report, which showed the glucose-lowering effects of colestimide [26]. Exenatide, a GLP-1 receptor agonist, which is widely used for treating type 2 diabetic patients, also has a stronger glucose lowering effect in type 2 diabetic patients with higher HbA1c [27]. Thus, the GLP-1-increasing activity of colestimide explains, at least in part, the mechanism of its glucose-lowering activity in type 2 diabetic patients. Though no GLP-1 related data were obtained in the present study, our results support this hypothesis.

In conclusion, we have demonstrated dual effects, i.e. both glucose and lipid lowering actions, of colestimide administered to type 2 diabetic patients with obesity. Unexpectedly, none of the insulin sensitivity-related parameters were significantly altered by colestimide treatment, and one, HOMA-β, actually increased significantly. The analysis of bile acid compositions before and after the treatment could not explain the glucose-lowering effect of colestimide. These results suggest that the mechanism underlying improved glycemic control with colestimide treatment involves enhancing β cell activity rather than ameliorating insulin resistance in obese type 2 diabetic patients.

**Disclosure Statement**

None of the authors have any potential conflicts of interest associated with this research.

**Acknowledgment**

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**References**


