Effects of High and Low Molecular Weight Chitosan on Plasma Cholesterol, Glucose and Adipocytokines in Diabetic Rats Induced by Streptozotocin and Nicotinamide

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ABSTRACT

Chitosan is known for its lipid-lowering effect. To investigate the comparative effects of low (LCS) and high molecular weight chitosan (HCS) on plasma glucose, cholesterol and adipocytokines, male Sprague-Dawley rats were divided into four groups: normal control, diabetic, and diabetic fed the HCS or LCS diet for seven weeks. Diabetes was induced by subcutaneous injection of nicotinamide and streptozotocin. Results showed that diabetic rats fed the HCS diet had reduced liver weight and perirenal adipose weight, compared to those that were fed the control diet. Higher fecal triglyceride and cholesterol excretion and lower hepatic cholesterol and triglyceride contents were found in diabetic rats fed with both chitosan diets. These observations were more significant in the HCS group than in the LCS group. HCS, but not LCS diet, significantly reduced plasma fructosamine, leptin, total cholesterol and lowered the insulin resistance index (homeostasis model assessment; HOMA) in diabetic rats. A lower intestinal disaccharidase activity including sucrase and lactase was found in diabetic rats fed the HCS diet. Moreover, plasma tumor necrosis factor-alpha and plasminogen activator inhibitor-1 concentrations were significantly reduced by both chitosan supplementations. The results suggested that HCS feeding may reduce insulin resistance by the suppression of lipid accumulation in liver and adipose tissue and amelioration of chronic inflammation in diabetic rats. HCS may improve glucose and lipid metabolism more significantly than LCS in diabetic rats.

Key words: chitosan, molecular weight, diabetes, insulin resistance, adipocytokines

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease. Statistical analysis has shown that more than 90% of diabetic patients suffer from type 2 DM. Type 2 DM is mostly caused by obesity and insulin resistance, and increases the risk of cardiovascular diseases(1,2). Moreover, obesity refers to an excessive amount of adipose tissue, which is an important endocrine organ that secretes adipocytokines such as leptin that may influence energy metabolism and affect insulin actions(3). In addition, some cytokines such as tumor necrosis factor-alpha (TNF-α) and plasminogen activator inhibitor-1 (PAI-1) can also be secreted by adipose tissue(4). PAI-1 is a key element in the inhibition of fibrinolysis and plays a role in the development of atherosclerosis in diabetic patients(5). It can be stimulated with various inflammatory cytokines, such as TNF-α. Indeed, increased TNF-α and PAI-1 have been shown to enhance insulin resistance and atherosclerosis in diabetic subjects(5,6).

Many studies reported that dietary fibers are suggested to have beneficial effects on the plasma lipids of patients with diabetes and atherosclerosis. Chitosan is a dietary fiber produced by the deacetylation of chitin. Its chemical structure is similar to cellulose, linked by β (1→4) glycosidic linkage, and is not digested by mammalian digestive enzymes(7). Chitosan is generally known for its plasma cholesterol-lowering effect due to an increase of fecal fat excretion(8,9). Many investigators demonstrated that chitosan may have beneficial effects on the reduction of adipose tissue in rodents(10,11). Recent studies have also demonstrated that chitosan decreased plasma glucose concentrations in type 1 diabetic rats and the action mechanism probably involved a decrease in liver gluconeogenesis, an increase in skeletal muscle glucose uptake and use(12) and lower intestinal disaccharidase activities(10). A few studies have shown that chitosans with different molecular weights (MW) have different hypocholesterolemic and hypoglycemic effects. Yao et al.(10) reported that high molecular weight (MW) chitosan...
(average MW: 1.0 × 10^6 dalton) had greater ability to lower plasma glucose and cholesterol concentrations than low MW chitosan (average MW: 1.4 × 10^4 dalton) in STZ-induced diabetic rats. LeHoux and Grondin\(^{(13)}\), however, reported that a chitosan sample with average MW greater than 7.5 × 10^5 dalton, had less hypocholesterolemic effect than that of chitosan with low average MW (average MW: 7 × 10^4 dalton). Other studies show that low MW chitosan (average MW of about 2 × 10^4 dalton)\(^{(14,15)}\) and chitosan oligosaccharides\(^{(16)}\) can also reduce plasma glucose level in diabetic animals. These results indicate that the effects of chitosan with low or high molecular weight on plasma glucose and cholesterol concentrations are still inconclusive.

Streptozotocin (STZ) is an antibiotic from *Streptomyces achromogenes*. It can selectively damage the pancreatic β-cell and develop hypoinsulinemia and high blood glucose similar to type 1 DM animals\(^{(17)}\). Pretreatment of nicotinamide can scavenge free radicals and recruit the consumption of nicotinamide adenine dinucleotide (NAD\(^+\)) in pancreatic β-cell after STZ injection. Hence the injection of nicotinamide can partially protect the pancreatic β-cell after STZ injection and then develop mild hyperglycemia\(^{(17)}\). Chitosan has been shown to reduce plasma cholesterol and adipose weight\(^{(11,18)}\) and improve glucose metabolism in type 1 diabetic rats\(^{(12)}\). The effect of chitosan on type 2 DM is less discussed. The present study was to investigate the comparative effects of high MW chitosan (average MW: 8.6 × 10^5 dalton) and low MW chitosan (average MW: 1.8 × 10^5 dalton) on plasma glucose, lipids and adipocytokines (e.g. adiponectin, TNF-α, leptin and PAI-1) in a diabetic rat model induced by STZ and nicotinamide.

**MATERIALS AND METHODS**

I. **High Molecular Weight Chitosan (HCS) and Cellulose**

HCS from shrimp shell was generously supplied by Taiwan Tanabe Seiyaku Co., Ltd (Taipei, Taiwan). Cellulose was purchased from Sigma Chemical Co. (USA).

II. **Preparation of Low Molecular Weight Chitosan (LCS)**

LCS was prepared from HCS using the method of Värum *et al.*\(^{(19)}\) with slight modification. HCS powder was dissolved in 8 N HCl with stirring and hydrolyzed at 55°C for 3 h. Subsequently, 8 N NaOH was used to stop the reaction in an ice bath and a pH of 9-10 was obtained. After the precipitation of LCS, the supernatant was removed and the LCS was washed with distilled water (pH 7). The fluid was then centrifuged at 12,000 ×g for 30 min (4°C). The LCS powder was obtained by freeze-drying the precipitate.

III. **Determination of Average Molecular Weight (MW), Degree of Deacetylation (DD) and Viscosity of Chitosan Samples**

The average MW was determined by the Mark-Houwink equation\(^{(20)}\). Five concentrations of chitosan solution (0.01, 0.025, 0.05, 0.075 and 0.1%) were prepared. The intrinsic viscosity (\(\eta\)) was measured in a 0.2 M CH\(_3\)COOH/0.1 M CH\(_3\)COONa solution using Cannon Fenske, No. 100 capillary viscometer at 30°C\(^{(21)}\). The viscosity MW (M) was calculated by the Mark-Houwink equation\(^{(20)}\): \(\eta = KM^a\).

The constants, K and a, were dependent on DD, where DD was expressed as the percentage: \(K = 1.64 \times 10^{-30} \times DD^{14} + 1.82 \times 10^{-2} \times DD + 1.82\).

DD was assayed by Fourier transform infrared spectroscopy (FTIR)\(^{(22)}\). Chitosan powder was mixed with KBr (1:100) and pressed into a pellet. The absorbance values of amide I (1655 cm\(^{-1}\)) and the hydroxyl band (3450 cm\(^{-1}\)) were measured by a Bio-Rad FTS-155 infrared spectrophotometer. The percentage of the amine group’s acetylation in a sample is given by \((A_{1655}/A_{3450}) \times 115. A_{1655} \times A_{3450}\) are the absorbance values at 1650 and 3450 cm\(^{-1}\), respectively.

IV. **Animals and Treatment**

Six-week-old male Sprague-Dawley rats were purchased from BioLASCO Taiwan Co., Ltd. (Taiwan). The Rats were fed a chow diet (Rodent Laboratory Chow, Ralston Purina, St. Louis, MO, USA) for 3 weeks and diabetic rats were induced by the intraperitoneal injection of nicotinamide (230 mg/kg body weight in water) 15 min prior to the intraperitoneal injection of STZ (60 mg/kg body weight in 0.05 M citrate buffer; pH 4.5)\(^{(17)}\). The non-diabetic group (cellulose control group) also received nicotinamide prior to the injection of citrate buffer alone. Seven days after the STZ+nicotinamide injection, the diabetic rats were checked for plasma glucose concentration after a 2-h oral glucose tolerance test (OGTT; 2 g glucose/kg body weight). Except for fasting glucose level, the plasma glucose concentration was more than 200 mg/dL within 2 h after glucose challenge to confirm the diabetic status. The diabetic rats were then divided into three groups with 7 to 9 rats in each group. One group was treated with a 5% cellulose control diet (the same diet as the control group), while the other two groups were treated with 5% LCS and 5% HCS diets, respectively. The rats were fed the experimental diets for seven weeks. The physiochemical properties of chitosan samples and composition of the experimental diet given to test animals are shown in Table 1. The rats were housed in individual stainless steel cages in a room kept at 23 ± 1°C and 60 ± 5% relative humidity with a 12-h light and dark cycle. Food and drinking water were available *ad libitum* and measured daily. Body weight was measured every week. Feces and urine were collected during the final 3 days of the experiment. The feces samples were then dried and weighed. This study was approved by the Animal House Management Committee of the National Taiwan Ocean University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council.

V. **Collection of Plasma and Tissue Samples**
At the end of the experiment, the rats were fasted overnight and then sacrificed by exsanguinations via the abdominal aorta while under diethyl ether anesthesia. Heparin was used as the anticoagulant. Plasma was separated by centrifugation at 1,570 ×g for 20 min (4°C) from the blood. Liver and adipose tissues (perirenal and epididymal) were excised from each rat and weighed. All the tissue samples were immediately frozen and were stored at -80°C until further analysis.

VI. Determination of Plasma Glucose, Fructosamine, Insulin, Adiponectin, Leptin, TNF-α, PAI-1 and Insulin Resistance Measurement

Plasma concentrations of glucose (Kyokuto Pharmaceutical Industrial Co., Ltd., Japan) and fructosamine (Hospitex Diagnostics, Italy) were assayed by commercial kits. Plasma insulin concentration was determined by rat insulin ELISA kit (Mercodia AB, Sweden). Plasma leptin concentration was measured by rat leptin ELISA kit (Assay Designs, Inc., USA). Plasma tumor necrosis factor-alpha (TNF-α) concentration was determined by rat TNF-α ELISA kit (R&D systems, Inc., USA). Plasma adiponectin concentration was assayed by rat adiponectin ELISA kit (Chemicon International, Inc., USA). The homeostasis model assessment (HOMA) was expressed as an index of insulin resistance (23). HOMA = fasting glucose concentration (mmol/L) × fasting insulin concentration (mU/L) / 22.5

<table>
<thead>
<tr>
<th>Table 1. Composition of experimental diets (%)</th>
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<tr>
<td>Dieta</td>
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<tr>
<td>Casein</td>
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<td>Corn oil</td>
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<tr>
<td>Vitamin mixtureb</td>
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<td>Mineral mixtureb</td>
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<tr>
<td>Cholesterol</td>
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<tr>
<td>Choline chloride</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Low molecular weight chitosan (LCS)b</td>
</tr>
<tr>
<td>High molecular weight chitosan (HCS)d</td>
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<tr>
<td>Corn starch</td>
</tr>
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</table>

aControl: Nondiabetic with cellulose diet; DM: Diabetic with cellulose diet; LCS: Diabetic with low molecule weight chitosan diet; HCS: Diabetic with high molecule weight chitosan diet.
bAIN 76 vitamin and mineral mixtures: procured from ICN Biochemicals (Costa Mesa, CA, USA)
cThe average MW and viscosity of chitosan were about 1.8 × 10⁴ dalton and 27 cps, respectively. The degree of deacetylation was about 95%.
dThe average MW and viscosity of chitosan were about 8.6 × 10⁵ dalton and 538 cps, respectively. The degree of deacetylation was about 93%.

The preparation of mucosa samples from small intestine was described previously (10). Mucosa from each rat was homogenized in saline over ice and the homogenate was then centrifuged at 1,570 ×g for 10 min. The resulting supernatant was used to determine the specific activities of sucrase, maltase and lactase, according to the amount of glucose released from sucrose, maltose and lactose, respectively, as described by Dahlqvist (27). The glucose concentration was determined with a test kit purchased from Audit Diagnostics Co. (Cork, Ireland).
RESULTS

As shown in Table 2, there was no difference in body weight among the animals in the four groups. Diabetic rats fed the LCS or HCS diet had a statistically significant reduction in relative liver weight compared with diabetic rats fed the cellulose diet after 7 weeks of feeding period. Moreover, HCS significantly decreased the relative perirenal adipose weight. There was no difference in food intake, volume of drinking water and urine excretion among the four groups (data not shown).

The effects of chitosan on plasma concentrations of glucose, insulin, fructosamine, insulin and HOMA value in diabetic rats are reported in Table 3. Diabetic rats fed the HCS diet tended to have lower plasma concentrations of glucose and insulin, but this difference was not significant. Lower plasma fructosamine concentration and HOMA value were observed in diabetic rats fed the HCS, but not rats fed the LCS diet, indicating that insulin resistance was improved by HCS. These results indicated that HCS was more effective than LCS in reducing insulin resistance in diabetic rats.

Plasma adiponectin, leptin, TNF-α and PAI-1 in diabetic rats are reported in Table 3. Diabetic rats had higher plasma adiponectin levels than non-diabetic rats. There was, however, no significant difference in the plasma adiponectin concentrations of the cellulose and the chitosan-receiving groups in diabetic rats. Moreover, it is noteworthy that the plasma concentrations of TNF-α and PAI-1 were significantly decreased in diabetic rats receiving HCS and LCS diets. Lower plasma leptin level was observed in diabetic rats receiving HCS diet.

As shown in Table 4, diabetic rats fed with HCS diet had lower plasma total cholesterol and LDL-C + VLDL-C concentrations. LCS feeding also decreased plasma LDL-C + VLDL-C concentration, but the hypocholesterolemic ability was lower than that of HCS in diabetic rats. The hepatic total cholesterol and triglyceride contents are also shown in Table 4. Chitosan feeding significantly reduced the hepatic contents of cholesterol and triglycerides in diabetic rats, and HCS tended to lower hepatic cholesterol and triglyceride contents more significantly than LCS. This result indicated that HCS was more effective than LCS in reducing hepatic total cholesterol and triglycerides in diabetic rats.

Intestinal disaccharidase activities including sucrase, maltase and lactase of diabetic rats are reported in Figure 1. Diabetic rats fed the cellulose diet had no effect on intestinal disaccharidase activities when compared with non-diabetic rats. Lower lactase activity was found in diabetic rats receiving HCS diet.

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Table 2. Body weights, tissue weights and fecal weights of diabetic rats fed the chitosan diets for 7 weeks

<table>
<thead>
<tr>
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<th>Control</th>
<th>DM</th>
<th>LCS</th>
<th>HCS</th>
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<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>358.3 ± 22.9</td>
<td>348.0 ± 34.1</td>
<td>342.6 ± 24.5</td>
<td>345.1 ± 27.5</td>
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<tr>
<td>Final body weight (g)</td>
<td>511.2 ± 35.2</td>
<td>514.1 ± 29.8</td>
<td>486.2 ± 39.4</td>
<td>480.0 ± 42.9</td>
</tr>
<tr>
<td>Relative liver weight (g/100 g BW)</td>
<td>4.47 ± 0.65</td>
<td>4.52 ± 0.33</td>
<td>3.76 ± 0.33 *</td>
<td>3.02 ± 0.49 *#</td>
</tr>
<tr>
<td>Relative perirenal adipose weight (g/100 g BW)</td>
<td>2.77 ± 0.89</td>
<td>2.93 ± 0.65</td>
<td>2.67 ± 0.58 *</td>
<td>2.14 ± 0.62 *#</td>
</tr>
<tr>
<td>Relative epididymal adipose weight (g/100 g BW)</td>
<td>1.92 ± 0.55</td>
<td>1.82 ± 0.21</td>
<td>1.72 ± 0.52</td>
<td>1.51 ± 0.48</td>
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<tr>
<td>Fecal wet weight (g/100 g BW)</td>
<td>2.91 ± 0.45</td>
<td>3.19 ± 0.46</td>
<td>3.30 ± 0.45</td>
<td>4.25 ± 0.92 *#</td>
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1 Data are mean ± SD values (n = 7-9). * p < 0.05, DM versus LCS or HCS; # p < 0.05, LCS versus HCS.

Table 3. Plasma concentrations of glucose, fructosamine, insulin, TNF-α, leptin, PAI-1 and insulin resistance index (HOMA) of diabetic rats fed the chitosan diets for 7 weeks

<table>
<thead>
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<th>Control</th>
<th>DM</th>
<th>LCS</th>
<th>HCS</th>
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<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>174.5 ± 22.7 *</td>
<td>190.6 ± 15.2</td>
<td>194.5 ± 16.8</td>
<td>179.5 ± 31.4</td>
</tr>
<tr>
<td>Fructosamine (μmol/L)</td>
<td>191.9 ± 35.8</td>
<td>206.1 ± 31.3</td>
<td>196.9 ± 26.9</td>
<td>165.5 ± 20.9 *#</td>
</tr>
<tr>
<td>Insulin (μg/L)</td>
<td>1.03 ± 0.67</td>
<td>0.83 ± 0.35</td>
<td>0.79 ± 0.15</td>
<td>0.67 ± 0.21</td>
</tr>
<tr>
<td>HOMA2</td>
<td>9.06 ± 5.30</td>
<td>9.40 ± 1.97</td>
<td>7.35 ± 2.39</td>
<td>5.87 ± 2.37 *#</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>55.3 ± 19.6 *</td>
<td>99.4 ± 41.7</td>
<td>44.2 ± 1.23 *</td>
<td>43.2 ± 1.78</td>
</tr>
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</table>
| Leptin (ng/mL)   | 7.28 ± 0.93 | 7.51 ± 0.66 | 7.65 ± 0.49 | 6.44 ± 1.43 *#
| PAI-1 (ng/mL)    | 2.15 ± 1.11 * | 4.12 ± 2.31 | 1.00 ± 0.53 * | 1.01 ± 0.33 *# |
| Adiponectin (μg/mL) | 9.87 ± 3.44 * | 14.0 ± 2.8 | 15.4 ± 4.5 | 14.7 ± 4.9 |

1 Data are mean ± SD values (n = 7-9). 2 Homeostasis model assessment (HOMA) equation for insulin resistance = (fasting plasma glucose concentration (mmol/L) × fasting plasma insulin concentration (μU/L)) / 22.5

** p < 0.05, Control versus DM; * p < 0.05, DM versus LCS or HCS; # p < 0.05, LCS versus HCS.
diabetic rats fed with both chitosan diets. HCS, but not LCS, reduced intestinal sucrase activity in diabetic rats.

**DISCUSSION**

According to our data, diabetic rats fed the HCS diet for 7 weeks exhibited significant reduction in lipid accumulation in liver and adipose tissues and reduced plasma concentrations of adipocytokines including leptin, TNF-α and PAI-1. Lower plasma fructosamine concentrations and insulin resistance (HOMA value) were observed in diabetic rats fed the HCS diet. As fructosamine is a parameter that can present short-term glucose metabolic status and reflect the average plasma glucose concentration over the last 1-2 weeks, it is suggested that HCS feeding may have beneficial effects on glucose control and reduce insulin resistance in diabetic rats. LCS, however, caused relatively less effect on these alterations in diabetic rats.

Chitosan has been shown to improve insulin sensitivity in obese subjects\(^{28}\). Studies indicate that visceral fat accumulation due to adipocyte hypertrophy induces the secretion of leptin and TNF-α in adipocytes and thus increase insulin resistance\(^{29-31}\). The present study showed that lower perirenal adipose tissue weight occurred in diabetic rats fed HCS diets, but not LCS diet. HCS significantly decreased plasma leptin and TNF-α levels, accompanied with a lower adipose tissue weight in diabetic rats. Therefore, lower plasma leptin and TNF-α level caused by HCS might be partly due to lower adipose tissue weight in diabetic rats. In addition, lower fructosamine and HOMA levels were observed in animals fed the HCS diets. Thus, HCS may have the ability to lower insulin resistance by reducing fat accumulation in adipocytes and lowering plasma adipocytokines (e.g. leptin and TNF-α) levels in diabetic rats. Furthermore, PAI-1 is a key element in the inhibition of fibrinolysis and plays a role in the development of atherosclerosis in diabetic patients\(^{5,32}\). The source of PAI-1 included endothelial cells, liver and adipose tissue\(^{33-35}\), which can be regulated by TNF-α\(^{36}\). In the present study, plasma TNF-α and PAI-1 levels were increased in diabetic rats. However, this elevation could be effectively reversed by both chitosan treatments. Therefore, the reduction of plasma PAI-1 after chitosan feeding may be related to the reduction of plasma TNF-α. Our results indicated that chitosan feeding may reduce chronic inflammation in diabetic rats, at least in part, through the reduction of plasma TNF-α and PAI-1 levels. These observations are consistent with the previous report that chitosan counteracts some inflammatory disorders that occur in high-fat diet induced obese mice\(^{12}\). Taken together, it is suggested that chitosan may reduce chronic inflammation and thus contribute to improving insulin resistance in diabetic rats. HCS appeared to have greater effect on improving insulin resistance than LCS in diabetic rats.

Liver can regulate the metabolic pathway of gluconeogenesis and glycolysis\(^{37}\). Many studies demonstrated that hepatic fat accumulation may reduce insulin sensitivity and impaired glucose oxidation of liver cells\(^{38,39}\). Our present study revealed that hepatic cholesterol and triglyceride

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<tr>
<th>Plasma</th>
<th>Control</th>
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<th>HCS</th>
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<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>59.9 ± 17.9**</td>
<td>72.4 ± 18.6</td>
<td>63.0 ± 10.1</td>
<td>50.9 ± 19.9*</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>24.8 ± 7.8**</td>
<td>28.5 ± 6.3</td>
<td>31.2 ± 5.2</td>
<td>34.3 ± 9.5</td>
</tr>
<tr>
<td>LDL-C + VLDL-C (mg/dL)</td>
<td>35.1 ± 14.3</td>
<td>43.9 ± 13.3</td>
<td>29.1 ± 5.9*</td>
<td>16.6 ± 11.2**</td>
</tr>
</tbody>
</table>

**Liver**

| Total cholesterol (mg/g liver) | 61.5 ± 11.1 | 72.4 ± 14.8 | 30.4 ± 11.2* | 16.4 ± 6.18** |
| Triglyceride (mg/g liver) | 71.6 ± 25.5 | 92.2 ± 16.5 | 51.2 ± 9.16* | 44.3 ± 15.7* |

**Feces**

| Total cholesterol (mg/g dry feces) | 16.0 ± 3.0 | 16.3 ± 2.1 | 26.9 ± 5.4* | 25.5 ± 3.0* |
| Triglyceride (mg/g dry feces) | 1.53 ± 0.56 | 1.25 ± 0.24 | 2.65 ± 0.42* | 3.50 ± 0.84* |

\(^{1}\)Data are mean ± SD values (n = 7-9). **p < 0.05, Control versus DM; *p < 0.05, DM versus LCS or HCS; #p < 0.05, LCS versus HCS.

Figure 1. The changes of intestinal disaccharidases activities in diabetic rats fed the chitosan diets for 7 weeks. Data are mean ± SD values (n = 7-9). **p < 0.05, Control versus DM; *p < 0.05, DM versus LCS or HCS; #p < 0.05, LCS versus HCS.
contents were significantly reduced by chitosan in diabetic rats. The reduced liver weight may be attributed to the lower hepatic lipid accumulation (\textsuperscript{10,13}). HCS reduced hepatic lipids more significantly than LCS. The reason for this observation might be related to higher fecal lipids excretion caused by HCS (Table 4). Therefore, the reduction of insulin resistance by HCS treatment was not only due to a decrease in plasma leptin and TNF-α levels but also lower hepatic lipid accumulation in diabetic rats.

Chitosan can be dissolved in acidic fluid and, therefore, can become a highly viscous solution and act as viscous dietary fiber in the stomach. Many viscous soluble dietary fibers are capable of reducing the activity of intestinal disaccharidase and delaying gastric emptying. This will then slow down the absorption of glucose and increase the insulin sensitivity of the peripheral tissues and hence reduce plasma glucose (\textsuperscript{40,41}). In our present study, the viscosity of HCS (538 cps) in 0.1 M HCl solution. Hence, HCS may be more capable than LCS in delaying gastric emptying and slowing down the absorption of glucose. Furthermore, Yao et al. (\textsuperscript{10}) demonstrated that high MW chitosan (about 1.0 × 10^6 dalton) had more ability in lowering intestinal disaccharidase activities than low MW chitosan (about 1.4 × 10^4 dalton) in type 1 STZ-induced diabetic rats. Our present study also found that lower intestinal disaccharidase (sucrase and lactase) activities were observed in diabetic rats after HCS treatment. These results suggested that the glucose absorption in the small intestines of diabetic rats might be slowed down after feeding with diet containing HCS, which, in turn, may help to control plasma glucose level after the meal. Additionally, the present study demonstrated that only HCS had plasma cholesterol-lowering effect in diabetic rats. LCS, however, had no effect on plasma cholesterol level despite its ability to reduce hepatic cholesterol content and increase fecal cholesterol excretion. These results are similar to the results of our previous study that the hypocholesterolemic ability of chitosan in STZ-induced type 1 diabetic rats was dependent on their molecular weight (\textsuperscript{10}). HCS may entrap more lipids and reduce fat digestion more significantly than LCS. Therefore, the different influences of high and low MW chitosans on plasma cholesterol lowering effect appear to be related to the differences in hepatic cholesterol contents, since high and low MW chitosans have different inhibitory potentials on the intestinal absorption of dietary cholesterol and triglycerides.

In conclusion, HCS feeding may improve insulin resistance and chronic inflammation in STZ+nicotinamide induced diabetic rats. The possible mechanisms may be due to the decreased absorption of dietary lipids and slower glucose absorption in the small intestines after the meal, which may result in a decrease in hepatic lipids and adipose tissue weight, and lowering of plasma levels of adipocytokine including leptin, TNF-α and PAI-1. HCS may improve glucose and lipid metabolism more significantly than LCS in diabetic rats.

ACKNOWLEDGMENTS

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REFERENCES