Effects of Buckwheat Flour With Fiber, Izumo Soba, on Preventing Liver Steatosis in Mice

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Nutritional characteristics of buckwheat flour and fiber were studied for their effects on obesity and metabolic parameters in mice. Three types of diets with differing fiber content were used for the study. The Gozenko diet consisted of buckwheat flour with 18 wt% fiber. The Izumo Soba diet consisted of buckwheat flour with chaff and contained 15 wt% fiber. The third diet was the Cornstarch diet, consisting of cornstarch flour with 0 wt% fiber. After the mice were fed their respective diets with equal calorie for 10 days 40 kcal / day / mouse the liver weight of the Izumo Soba diet group was significantly less than that of the Cornstarch or Gozenko diet groups. Additionally, the Cornstarch and the Gozenko diet groups had higher values for liver triglyceride contents than did the Izumo Soba diet group. Plasma δ-GTP and GPT for the Izumo Soba diet group were significantly lower than those for the Cornstarch diet groups, and GOT for Izumo Soba diet group was significantly lower than that for the Cornstarch or Gozenko diet groups. We concluded that the Izumo Soba diet, containing large amount of dietary fiber from the buckwheat chaff, has the potential for preventing liver steatosis.

Key words: Izumo Soba, dietary fiber, de novo lipogenesis, liver steatosis.

INTRODUCTION

Over the past decade, attention has focused on the high carbohydrate diets, due to its associate with dyslipidemia and overweight in humans. We have previously reported that high carbohydrate diets contributed to obesity and metabolic syndrome in Japanese. More particularly, we indicated that a decrease in dietary carbohydrate intake was a main contributor to weight loss in overweight Japanese. Dietary carbohydrates are major role-players in postprandial glycemic and insulinemic response. Postprandial hyperglycemia is one leading factor in increased de novo lipogenesis and triglyceride accumulation in the liver and fat tissues. The lowering of postprandial glycemic and insulinemic response is an essential objective in the prevention of those abnormalities.

There is no doubt that the quality and quantity of carbohydrates considerably affect their rate of digestion and absorption. Particularly, the quality of carbohydrate is represented as the Glycemic Index concept, a tool for ranking foods with respect to their postprandial glycemia-raising potential. The use of the GI concept is now widely accepted and various foods have been tested. Low GI diets are those in which carbohydrates are digested and absorbed slowly, while high GI diets are those in which are rapidly digested and absorbed, with resulting different glycemic response. Metabolic advantages of a low GI diet from a long-term perspective have been noted in several studies, particularly for those individuals with diabetes or hyperlipidemia. One nutritional variable frequently linked to low GI properties is fiber. A variety of fiber components, especially soluble fiber including pectin, Oatrim fiber extract, guar gum, gum tragacanth, and methyl cellulose fiber, beneficially lower postprandial glycemic and insulinemic responses, when compared with meals without soluble fiber. Moreover, there
are several indications that metabolites, formed during fermentation of fiber and other indigestible carbohydrates in the large intestine, contribute to the maintenance of colon health. Thus, for most healthy adults, foods with higher fiber likely have beneficial effects.

Of the foods screened for metabolic response and fiber content, little attention has been paid to buckwheat. *Fagopyrum esculentum* Moench. For the Japanese, the buckwheat noodle is represented in a main staple called soba, usually prepared in noodle form. Buckwheat is similarly a staple for some rural populations in Nepal, Bhutan, and China. The Izumo area in Shimane prefecture, Japan, is famous for Izumo Soba, which is unique in that it contains buckwheat chaff, which is added when the buckwheat is ground into flour. In the other area in Japan, it no contains buckwheat chaff in soba flour, such as the famous Gozenko flour. Because the buckwheat chaff contains large amounts of fiber, Izumo Soba is naturally higher in fiber content than are the other types of soba.

Buckwheat flour noodles without the chaff eaten in Japan contain approximately 5% of carbohydrates, with a relatively low fiber content, thus being a potential contributor to postprandial hyperglycemia. Replacement of the lower fiber buckwheat flour with the higher fiber buckwheat flour with chaff added may contribute to the suppression of postprandial hyperglycemia, and prevention followed by *de novo* lipogenesis and triglyceride accumulation in the liver. We conducted an animal study to clarify the beneficial effects of the fiber-rich Izumo Soba flour, as compared to buckwheat flour without chaff.

### MATERIALS AND METHODS

#### Animals and diets

Male ICR mice, aged 8 weeks and weighing 20-22 g, were purchased from Oriental Yeast Co., Ltd, Tokyo, Japan. All mice were allowed to acclimate two weeks before use in the present study. The mice were randomly divided into three groups, each group consisting of 8 mice. Three types of diets, Izumo Soba diet, Gozenko diet and Cornstarch diet, were prepared in pellet form and its compositions are shown in Table 1. The mice were fed their respective diets for four weeks, all at the same calorie rate. All mice were housed on a 12h light (0600-1800)/dark (1800-0600) cycle. Body weight was measured once a day. After four weeks fed, all mice were fasted for 12 hr, and then dissected under ether anesthesia. All procedures in the mice experiments were approved by the Animal Care and Use Committee of Shimane University School of Medicine, Japan.

#### Measurements

After dissection, liver, epididymal fat pad and soleus muscle weights were immediately measured. Blood samples were collected from the abdominal vein, and plasma was separated by centrifugation. Plasma glucose, triglycerides, total cholesterol, HDL cholesterol, and nonesterified free fatty acids were measured by Glucose C-II test, triglyceride E test, cholesterol E test, HDL cholesterol E test, and NEFA C test, respectively. Wako Pure Chemicals, Osaka, Japan. For the liver function test, plasma

<table>
<thead>
<tr>
<th>Table 1 Composition of high carbohydrate diets</th>
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<tr>
<td>Calorie (kcal/100g)</td>
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<tr>
<td>Protein (% kcal)</td>
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<tr>
<td>Fat (% kcal)</td>
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<tr>
<td>Carbohydrate (% kcal)</td>
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<tr>
<td>Fiber (g/100g)</td>
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<tr>
<td>Cornstarch flour (g/100g)</td>
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<tr>
<td>Buckwheat flour (g/100g)</td>
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*: Gozenko flour was used as buckwheat flour.

**: Izumo Soba flour was used as buckwheat flour.
–glutamyltransferase (–GTP) and glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were tested by –GTP C test, and Transaminase CII test, respectively. Wako Pure Chemicals, Osaka, Japan.

The liver lipid contents were extracted according to the method used by Carroll NV. et al. and TG was measured by using the triglyceride E test Wako Pure Chemicals, Osaka, Japan. The liver glycogen was extracted and measured according to Folch, J. et al. using anthrone reagent.

Statistical analyses

Statistical analyses of data were done with SPSS software version 16.0 (SPSS Inc., Tokyo, Japan). In this study, results are expressed as means ± S.D. Comparisons of the three diet groups of mice were performed by one-way analysis of variance (ANOVA) and Bonferroni correction for multiple comparisons, and P-value of less than 0.05 was used to assess significance.

RESULTS

Body weight changes

Fig. 1 show changes in mean body weight gains for each diet group. After days feeding, the mice fed with the Cornstarch diet lowest fiber content, and the Gozenko diet second lowest fiber content, gained body weight dramatically. These two groups had no significant difference body-weight curves and total body weight gain. The body-weight curve of the Izumo Soba diet group leveled off after days feeding in contrast to those of the Cornstarch and Gozenko diet groups, which contrived to show rapid increases after the 13 days mark. Finally after 13 days feeding, though there were no significant differences, the body-weight gain of the Izumo Soba diet group was lower than that of the Cornstarch or Gozenko diet groups 1.2 ± 0.2 g versus 1.6 ± 0.2 g, 1.0 ± 0.1 g, P > 0.05.

Organ weight

Table 2 shows summarized organ weight at 15 days after ingestion. The liver weights for the Cornstarch diet and Gozenko diet groups were significantly heavier than for the Izumo Soba diet group 8.0 ± 0.1 g, 8.2 ± 0.1 g versus 4.6 ± 0.3 g, P = 0.007 and 0.002 respectively. Although there were no significant differences between them, the fat pads weight of the Izumo Soba diet group was slightly less than that for either the Cornstarch or Gozenko diet.
Table 1: Summary of measurement variables

<table>
<thead>
<tr>
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<th>Cornstarch diet group</th>
<th>Gozenko diet group</th>
<th>Izumo Soba diet group</th>
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</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>5.8 ± 1.6</td>
<td>6.4 ± 1.9</td>
<td>4.1 ± 1.2</td>
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<tr>
<td>Organ weight (g)</td>
<td></td>
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<tr>
<td>Liver</td>
<td>2.57 ± 0.28</td>
<td>2.50 ± 0.36</td>
<td>1.93 ± 0.53 *</td>
</tr>
<tr>
<td>Epidydymal fat pad</td>
<td>0.71 ± 0.21</td>
<td>0.85 ± 0.23</td>
<td>0.66 ± 0.37</td>
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<tr>
<td>Soleus muscle</td>
<td>0.40 ± 0.03</td>
<td>0.42 ± 0.07</td>
<td>0.43 ± 0.04</td>
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<tr>
<td>Liver glycogen content</td>
<td></td>
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<tr>
<td>Absolute (mg)</td>
<td>110.9 ± 32.4</td>
<td>73.7 ± 23.9</td>
<td>65.4 ± 32.7</td>
</tr>
<tr>
<td>Relative (mg/g organ wt.)</td>
<td>43.1 ± 12.4</td>
<td>31.4 ± 11.1</td>
<td>33.6 ± 14.7</td>
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<tr>
<td>Liver TG content</td>
<td></td>
<td></td>
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<tr>
<td>Absolute (mg)</td>
<td>24.8 ± 14.0</td>
<td>24.7 ± 14.7</td>
<td>15.6 ± 8.7</td>
</tr>
<tr>
<td>Relative (mg/g organ wt.)</td>
<td>9.9 ± 6.0</td>
<td>10.1 ± 4.9</td>
<td>8.2 ± 4.8</td>
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<tr>
<td>Plasma</td>
<td></td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>140 ± 22</td>
<td>139 ± 42</td>
<td>129 ± 28</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>82 ± 21</td>
<td>94 ± 32</td>
<td>90 ± 37</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>115 ± 17</td>
<td>143 ± 32</td>
<td>126 ± 17</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>80 ± 17</td>
<td>90 ± 20</td>
<td>81 ± 13</td>
</tr>
<tr>
<td>FFA (mEq/dl)</td>
<td>9.2 ± 1.4</td>
<td>9.6 ± 2.2</td>
<td>8.4 ± 1.5</td>
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<tr>
<td>υ-GTP (IU/l)</td>
<td>4.0 ± 0.6</td>
<td>3.4 ± 0.3</td>
<td>2.9 ± 0.5 **</td>
</tr>
<tr>
<td>GOT (IU/l)</td>
<td>115 ± 19</td>
<td>105 ± 13</td>
<td>81 ± 10 **</td>
</tr>
<tr>
<td>GTP (IU/l)</td>
<td>25.0 ± 2.5</td>
<td>22.1 ± 2.2</td>
<td>19.2 ± 3.3 **</td>
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</table>

Values are mean ± SD.
* indicates a significant difference between Izumo Soba diet and the other two diet groups (P < 0.017).
** indicates a significant difference between Izumo Soba diet and Cornstarch diet group (P < 0.017).

groups = g versus = g, P > 0.05. There were no significant differences in soleus muscle weight among all diets groups.

Biochemical analyses of fasting plasma

The results of biochemical analyses of fasting plasma were shown in Table 1. There were no significant differences in fasting plasma glucose and lipids among all diet groups, but the values of fasting plasma glucose and of plasma FFA for the Izumo Soba diet group were slightly lower than those of Cornstarch or Gozenko diet groups. On the liver function test, the values of υ-GTP and GTP for the Izumo Soba diet group were significantly lower than those for Cornstarch diet group (P = 0.000 and > 0.05, respectively). And the value of GOT for the Izumo Soba diet group was significantly lower than that for Cornstarch or Gozenko diet groups (P < 0.000 and = 0.05, respectively).

Liver glycogen and triglyceride contents

Liver glycogen content for the Izumo Soba diet group was > 0.000, although the liver weight of the Gozenko diet group was higher and close to that of the Cornstarch diet group, liver glycogen was lower and comparatively close to the Izumo Soba diet group. The absolute liver triglyceride contents of the Izumo Soba diet group were > 0.05 lower than that of Cornstarch and Gozenko diet groups, though there were no significant differences between them.

DISCUSSION

The highlight of this study was that the Izumo Soba diet, containing large amounts of dietary fiber from buckwheat chaff, suppressed increasing of liver TG contents and liver-derived blood υ-GTP, GOT and GTP, despite all diets used in this study were rated as high carbohydrate diets. And more, though there were slight effects, suppressed body-weight gain and improved blood glucose and lipids metabolism. The other two diets, the Gozenko and Cornstarch diet, had high carbohydrate and low fiber content, seemingly contributing to liver steatosis and slightly body weight gain in the mice, in contrast to the Izumo Soba diet did not, even though all had the
same calories and similar carbohydrate content. These results suggest the unique properties of the Izumo Soba diet. We have previously indicated that high carbohydrate diets were one of main contributing factors to metabolic syndrome and obesity in Japanese. Metabolic syndrome and obesity are frequently accompanied by the liver steatosis. The liver steatosis is believed to be one of causes for abnormal lipid metabolism on metabolic syndrome and obesity. Thus, dietary fiber intake by individuals with healthy or metabolic syndrome might possibly help to prevent or improve metabolic syndrome and obesity by suppressing liver steatosis.

After 22 days feeding, fasting plasma glucose in the Izumo Soba diet group remained lower compared with the other two diet groups. Previously, we demonstrated that a low GI diet used with amylase, a major resistant starch and one of the undigestible components of carbohydrates as well as dietary fiber, strongly suppressed acute postprandial glycemic and insulimemic responses. The cumulative effects of repeated suppression of acute postprandial blood glucose by regular intake of a low GI diet likely lowers fasting plasma glucose levels. Pawlak et al. also reported on the long-term effects of different GI diets on normal rats; in that study, the body fat mass increases for the low GI group were significantly lower than those for the high GI group after 12 weeks of feeding. Oral glucose tolerance tests were performed at the 6th and 12th weeks, with the area under the curves (AUCs) for blood glucose and insulin of the low GI group being significantly lower. Lerer-Metzer et al., also reported that the low GI diet produced favorable effects in controlling postprandial glycemic and insulimemic responses. In these two studies, the high GI diet groups had a much greater proportion of distinctly abnormal islets than did the low GI groups, that showed severely disorganized architecture and extensive fibrosis, and both studies concluded that the proportion of fibrotic islets in each individual strongly correlated to AUC of glycemic response for both high and low GI groups.

It is believed that a main cause of developing liver steatosis is increased de novo lipogenesis and TG accumulation in the liver, which, in turn, are proceeded by postprandial hyperglycemia and hyperinsulinemia. Hudgins and Parks indicated that a feature of de novo lipogenesis was the occurrence of abnormal lipid circulation between the liver and the adipose tissue, mainly through of FFA. Our results indicate that the Izumo Soba diet had an advantageous effect on the limiting of the liver de novo lipogenesis and TG accumulation, with the Izumo Soba diet-fed mice exhibiting lower the liver weight and liver TG content both absolute and relative values. though all three diets used in this study contained large amounts of carbohydrates materials about kcal and all mice fed same calorie rate kcal / day. Further, the plasma FFA in the mice fed the Izumo Soba diet remained low after 22 days ingestion. In addition to these facts, the liver-derived blood -GTP, GOT and GTP for the Izumo Soba diet group, which enzymes were major parameter of liver function, kept lower than that for the Gozenko or Cornstarch diet groups. We concluded that the Izumo Soba diet has the effects of limiting the liver de novo lipogenesis and TG accumulation, and thus the potential for a significant impact on the prevention of liver steatosis.

Finally, we surmise that reducing the postprandial glycemic response is of great importance in maintaining good general health in individuals, as well as in the treatment of metabolic syndrome. While further acute and chronic studies with GI foods, focusing on liver de novo lipogenesis, are needed to examine this presumption more closely, we anticipate that future findings will support our conclusion that the high-fiber diet has the potential for prevention of liver steatosis.

CONCLUSION

This study found that the Izumo Soba diet, which contains large amount of dietary fiber from buckwheat chaff, helped to suppress increasing liver TG, and had a potential to limit liver de novo lipogenesis and for prevention of liver steatosis.

ACKNOWLEDGMENTS

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