Randomized, Double-Blind, Crossover Clinical Trial of the Effect of Calcium Alginate in Noodles on Postprandial Blood Glucose Level

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We conducted a prospective, randomized, double-blind, 3-group, 3-phase crossover study to evaluate the effect of calcium alginate (Ca-Alg) on the postprandial increase of blood glucose in 15 healthy adult subjects who were given udon noodles containing or not containing Ca-Alg (5 or 8%). The value of ΔC max (difference between the maximum (C max) and pre-feeding (C 0) blood glucose levels) was significantly reduced in both Ca-Alg groups, and the area under the blood glucose level–time curve over 120 min (AUC, with C 0 as the baseline) was also significantly reduced. Thus, supplementation of noodles with Ca-Alg significantly suppressed both the peak postprandial blood glucose level and the total amount of glucose absorption. Blood calcium (Ca) concentration was significantly increased at 120 min after ingestion, but there was no marked change of other parameter values. A questionnaire indicated that addition of Ca-Alg did not affect the acceptability of the noodles. These results indicate that Ca-Alg might a useful food additive for helping to prevent lifestyle-related diseases without adversely affecting individual eating habits.

Key words calcium alginate; alginic acid; blood glucose level; starch; noodle; clinical study

Lifestyle-related diseases are major causes of death in Japan,1–10 and among them, diabetes increases the risk of developing gangrene, angina pectoris, dementia, cancer, myocardial infarction and cerebral infarction.11,12 Type 2 diabetes, which is more common in Japan, is often triggered by dysregulation of blood glucose levels due to impaired insulin production.13,14 Therefore, measures that may prevent excessive increase of blood glucose levels, such as improvement of dietary habits and moderate exercise, are extremely important.15 In this connection, sodium alginate (Na-Alg), a polysaccharide derived from brown seaweed, is already widely used as a food additive (thickening stablizer), as a gastric mucosa-protective agent in pharmaceuticals, and as a health food, since it lowers blood cholesterol.16–19 However, high levels of sodium are a risk factor for hypertension,20 so it may be preferable to use the calcium salt of alginic acid (Ca-Alg), if it has the same efficacy as Na-Alg.

We have shown that Ca-Alg promotes excretion of strontium and cesium in the body in rats.21,22 We also reported that Ca-Alg has a blood cholesterol-lowering effect in rats22 by suppressing bile acid reabsorption in the gastrointestinal tract and promoting catabolism of cholesterol to bile acid in the liver.22 Moreover, we have investigated the mechanism of this effect, and established that Ca-Alg inhibits the degradation of starch into glucose in the gastrointestinal tract by inhibiting α-glucosidase activity.23 In that report, it was established that the α-glucosidase-inhibitory effect of Ca-Alg is greater than that of Na-Alg. It has been reported elsewhere that the rise in blood glucose level after ingestion of curry and rice was suppressed by Ca-Alg in adult males,24 and some clinical reports have mentioned the use of Ca-Alg.

In the present work, in order to confirm the efficacy of Ca-Alg, we conducted a prospective, randomized, double-blind, 3-group, 3-phase crossover study to examine the effect of Ca-Alg on the postprandial increase of blood glucose in healthy Japanese adult subjects. We focused on udon, a traditional Japanese noodle, and measured blood glucose levels after ingestion of udon alone, and udon containing 5 or 8% Ca-Alg (supplementation levels were chosen based on our previous study of the optimum amount and particle size of Ca-Alg in rats25). We also examined the effect of Ca-Alg on other chemical parameters in plasma or serum such as insulin (IRI), total cholesterol (T-Chol), high density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), triglyceride (TG), uric acid (UA), urea nitrogen (BUN) and calcium (Ca) levels.

METHODS

Compliance and Guidelines The protocol for this study was reviewed and approved by the Research Ethics Committee at Takasaki University of Health and Welfare (approval No. #2908). The study was pre-registered in the University Hospital Medical Information Network (UMIN) clinical trial system (UMIN000028124). We also complied fully with the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare,
According to the Japan Diabetes Association, fasting blood glucose in the range of 110 to 126 represents borderline diabetes mellitus. The Japan Obesity Society considers a BMI of 25 or more as pathological obesity. Therefore, in this study, subjects with fasting blood glucose level–time curve over 120 min with $\Delta AUC_{max}$ was significantly reduced in male rats given 5% Ca-Alg-containing starch feed ($25.0 \pm 19.9 \text{ mg/dL}$ vs. $53.8 \pm 20.3 \text{ mg/dL}$). If the efficacy of Ca-Alg is maintained in humans, we calculated that the minimum number of subjects required to detect a difference between Ca-Alg and non-Ca-Alg groups at significance levels of 5 and 1% would be 5. In addition, the minimum number of cases required to afford a detectability of 80% or more was calculated as $7$. Also, the area under the blood glucose level–time curve over 120 min with $C_{max}$ as the baseline ($\Delta C$) was significantly reduced by 5% Ca-Alg in the rat study ($561.3 \pm 2303.4 \text{ mg min/dL}$ vs. $3550.6 \pm 2047.8 \text{ mg min/dL}$). and, assuming again that the efficacy is maintained in humans, we calculated that a detectability of 80% or more at a significance level of 5% or less would require 7 subjects. Finally, the number of subjects was set at 15, since we considered that 1) individual differences are more likely to occur in a clinical study than in experiments using animals, 2) not only male but also female subjects were included, so that gender difference could be important, 3) it was possible that some subjects might withdraw from the study.

Subjects were healthy men and women of 20 years of age or older (healthy volunteers). Invitations to participate were posted on the Takasaki University of Health and Welfare website and on a bulletin board at the university. The purpose, procedures and safety aspects were fully described to volunteers at the time of registration, and each of them signed a consent form to participate. The age, sex, height, weight, current/past medical history, regular medication/supplements, allergies (drugs, foods, others), and smoking history of all subjects were recorded on a questionnaire form. All personal information was anonymized. Fasting blood glucose was measured at screening and body mass index (BMI) was calculated and their BMI was calculated after making inquiries to confirm their health. Subjects were asked to ingest one of three types of test noodles, the weight was about 180 g, for each predetermined group. The temperature of the noodles was 50 to 70°C, the weight was about 230 g after boiling, and no additional ingredients were provided. Subjects had 5 min to eat the noodles at a consistent feeding speed, and with no intake of soup. The subjects took 100 mL of water afterwards and did not take any other liquids or foods until the end of the test. For several tests, blood was collected from the veins of the brachial artery (cephalic vein, median midline cranial vein, or ulnar cephalic vein) before ingestion, and afterwards and did not take any other liquids or foods until the end of the test. For several tests, blood was collected from the veins of the brachial artery (cephalic vein, median midline cranial vein, or ulnar cephalic vein) before ingestion, and 30 and 120 min after ingestion, and sent to Gunma Clinical Laboratory Test Center (http://www.gunrin.com) for testing. In addition, blood was collected by fingertip puncture for blood glucose measurement prior to feeding, and at 15, 30, 45, 60, 90 and 120 min after ingestion (7 points in total). Blood glucose level was measured twice at each point using a simple blood glucose meter (Freestyle FS Precision blood glucose measuring system), and the average value was calculated as follows:

$$\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height (m)}]^2}$$

Table 1. Components of Tested Noodles

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Ca-Alg 5%&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ca-Alg 8%&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour (g)</td>
<td>57.4</td>
<td>59.7</td>
<td>58.0</td>
</tr>
<tr>
<td>Salt (g)</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Modified starch (g)</td>
<td>10.9</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Acidulant (g)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Ca-Alg (g)</td>
<td>0.0</td>
<td>3.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Water (g)</td>
<td>110.9</td>
<td>111.0</td>
<td>111.1</td>
</tr>
<tr>
<td><strong>Total (g)</strong></td>
<td>180</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>239</td>
<td>236</td>
<td>234</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.5</td>
<td>5.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>52.0</td>
<td>51.1</td>
<td>50.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Weight % of Ca-Alg to flour and modified starch.
the measured value. When the difference between the 2 measurements was 10 mg/dL or more, blood was collected again, the glucose level was measured, and the average value of the 2 points closest to the mean value was taken as the measured value. The test was conducted three times at the same time in the same facility at intervals of 7 to 14 d. After eating the noodles, subjects were given a tasting questionnaire to evaluate “chewiness,” “thickness” and “favorability” of the noodles in 5-point grades.

**Statistical Analysis** The change of blood glucose level at $n$ minutes after ingestion ($\Delta C_n$) was calculated from the pre-feeding blood glucose level $C_0$ and the blood glucose level at $n$ minutes ($C_n$) as follows:

$$\Delta C_n = C_n - C_0$$

Further, we determined $T_{max}$ as the postprandial elapsed time showing the highest blood glucose level, $C_{max}$, and the difference between $C_{max}$ and $C_0$ was calculated as $\Delta C_{max}$. In addition, the area under the blood glucose level–time curve $\Delta AUC$ during 0 to 120 min was calculated based on $\Delta C_n$. The obtained data were analyzed using a random chunk method, and the significance level was set to 5 or 1%.

**RESULTS**

**Subject Eligibility** No subjects were ineligible based on the results of the blood test. Moreover, no subjects withdrew from the study. The subjects’ characteristics are summarized in Supplementary Table 1.

**Blood Glucose Level** The blood glucose level of subjects who ate control noodles containing no Ca-Alg showed the highest value at 41 ± 12 min after ingestion. The $\Delta C_{max}$ was 50 ± 14 mg/dL, and $\Delta AUC$ was 3365 ± 1302 mg·min/dL. A significant decrease in $\Delta C_{max}$ was observed in subjects who ate noodles containing 5 or 8% Ca-Alg, compared to the control. The $\Delta C_{max}$ values were

**Table 2. Changes of Biochemical Parameters in Blood**

<table>
<thead>
<tr>
<th>(mg/dL)</th>
<th>30min</th>
<th>120min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ca-Alg 5%</td>
</tr>
<tr>
<td>$\Delta$IRI</td>
<td>34.23±19.41</td>
<td>37.97±17.53</td>
</tr>
<tr>
<td>$\Delta$T-Cho</td>
<td>-6.93±5.12</td>
<td>-7.13±4.34</td>
</tr>
<tr>
<td>$\Delta$HDL-Cho</td>
<td>-1.73±1.58</td>
<td>-1.67±1.54</td>
</tr>
<tr>
<td>$\Delta$LDL-Cho</td>
<td>-3.40±3.38</td>
<td>-3.73±3.13</td>
</tr>
<tr>
<td>$\Delta$UA</td>
<td>0.007±0.059</td>
<td>-0.007±0.080</td>
</tr>
<tr>
<td>$\Delta$Ca</td>
<td>-0.173±0.162</td>
<td>-0.127±0.158</td>
</tr>
</tbody>
</table>

Mean±S.D.: n=15, **p<0.01.

Fig. 1. Changes in Blood Glucose Level (ΔC) after Eating Test Noodles
The data are mean±standard deviation (S.D.). n=15.

Fig. 2. Effect of Ca-Alg on Blood Glucose Level
(A) $\Delta C_{max}$: The difference between the maximum blood glucose level $C_{max}$ and the pre-feeding blood glucose level $C_0$; (B) $\Delta AUC$: Area under the blood glucose level–time curve at 0 to 120 min ($\Delta AUC$, with $C_0$ as the baseline), calculated from $\Delta C_n$. The data are mean±S.D., n=15. *p<0.05, compared with control.
similar to findings with (ΔCa in the amount of Ca change at 30 min after noodle feeding of noodles (Figs. 1, 2).

24) and is by Ca-Alg. This is consistent with previous findings was no tendency for the absorption of glucose to be delayed.

C max was observed, suggesting that there no significant difference in the time of maximum blood glucose level (T max) was observed among the three kinds of noodles (Figs. 1, 2).

Blood Biochemical Parameters No significant difference in the amount of Ca change at 30 min after noodle feeding (ΔCa30min) was found between the 5 and 8% Ca-Alg groups compared to the control, but ΔCa at 120 min (ΔCa120min) showed a significant increase in both groups. In addition, ΔT-Chol30min showed a slight tendency to decrease in both groups, and ΔT-Chol120min was slightly decreased in the 8% Ca-Alg group. There were no significant changes in other blood test values (Table 2).

Questionnaire Subjects evaluated chewiness, thickness and favorability of the noodles using a questionnaire after eating. There was no significant difference in the evaluation of the three kinds of noodles (Fig. 3).

DISCUSSION

Noodles containing 5 or 8% Ca-Alg caused a significant decrease in ΔC max compared to control noodles. Moreover, ΔAUC also showed a significant decrease in both groups. In addition, no delay in T max was observed, suggesting that there was no tendency for the absorption of glucose to be delayed by Ca-Alg. This is consistent with previous findings and is similar to findings with α-glucosidase inhibitors, except miglitol.27 These results indicate that Ca-Alg suppresses the postprandial increase in blood glucose and reduces the total absorption amount of glucose. Thus, our previous finding that 5% Ca-Alg had a blood glucose-suppressing effect in rats was reproduced in humans.

We found that the blood Ca concentration at 120 min after eating 5 or 8% Ca-Alg-containing noodles remained within the normal range, 8.5 to 10.4 mg/dL, but was increased significantly compared with the control noodles, suggesting that Ca derived from Ca-Alg was absorbed into the body. The recommended amount of Ca intake in adults to help prevent diseases such as osteoporosis is 600–900 mg/d.28 Since the amounts of Ca in noodles containing 8% Ca-Alg and 5% Ca-Alg would be 500 and 320 mg, respectively, it is suggested that about half of the recommended daily intake might be provided by these noodles. Moreover, the upper limit of tolerable daily Ca intake for Japanese adults is 2500 mg.29 Even if these noodles were eaten three times a day, the upper limit would not be reached, and therefore the likelihood of excessive Ca intake appears to be low. In addition, the Ca-Alg-containing foodstuffs might promote excretion of sodium into feces ion exchange, and therefore may have potential to ameliorate hypertension.

Various substances are known to moderate glucose absorption, including indigestible dextrin, a dietary fiber made from corn starch hydrolyzate,31,32 salacinol derived from plant salacia,33 proanthocyanidin derived from acacia,34,35 and degraded guar gum.36 In particular, indigestible dextrin acts by inhibiting α-glucosidase,23 and is already widely used in many foods for specified health use (FOSHU) and functional display foods.38 Our previous work showed that Ca-Alg also inhibits α-glucosidase,23 and its effect on blood glucose level was similar to or more potent than that of indigestible dextrin.31 On the other hand, the side effects of α-glucosidase inhibitors are abdominal distention and flatus, because some undigested sugars are transferred to the large intestine, where gases accumulate due to fermentation by intestinal bacteria. According to our previous in vitro study,23 ingestion of noodles containing 8% Ca-Alg is expected to show an α-glucosidase-inhibitory effect equal to about 1/40 of that of a single dose of acarbose. Therefore, it is considered that the likelihood of side effects arising from α-glucosidase inhibition due to ingestion of noodles containing Ca-Alg is extremely low.

In this connection, it is noteworthy that the questionnaire results indicated that adding Ca-Alg to noodles did not significantly affect their acceptability, supporting the idea that the use of Ca-Alg as a food supplement to suppress hyperglycemia after meals might not adversely affect individual eating habits.

Taken together, our results raise the interesting possibility that the introduction of food ingredients containing Ca-Alg into the regular diet may be helpful in preventing lifestyle-related diseases, particularly diabetes and osteoporosis.

Conflict of Interest Kazuyo Shiragami, Mariko Koike, Fumiya Nishibori, Miho Tomokane, Takahiro Seki, Keiko Itabashi and Nobuyuki Obara are employees of Shimadaya Corporation. The other authors have no potential conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

REFERENCES


