Food Sci. Biotechnol. 25(3): 911-914 (2016)
DOI 10.1007/s10068-016-0149-9

Food Science
and
Biotechnology

Research Note

# Effect of Supplementation of Low-Molecular-Weight Chitosan Oligosaccharide, GO2KA1, on Postprandial Blood Glucose Levels in Healthy Individuals Following Bread Consumption

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*Received November 13, 2015*
*Revised February 10, 2016*
*Accepted March 21, 2016*
Published online June 30, 2016

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pISSN 1226-7708
eISSN 2092-6456



**Abstract** The effect of chitosan oligosaccharide (GO2KA1) administration on postprandial blood glucose levels of subjects with normal blood glucose levels was evaluated following bread consumption. Postprandial blood glucose levels were determined for 2 h after bread ingestion with or without 500 mg of GO2KA1. GO2KA1 significantly lowered the mean, maximum, and minimum levels of postprandial blood glucose at 30 min after the meal. Postprandial blood glucose levels were decreased by about 25% (from 155.11±13.06 to 138.50±13.59, $p<0.01$) at 30 min when compared to control. Furthermore, we observed that the area under the concentration-time curve (AUC*t*) was decreased by about 6% (from 255.46±15.43 to 240.15±14.22, $p<0.05$) and the peak concentration of blood glucose ($C_{max}$) was decreased by about 11% (from 157.94±10.90 to 140.61±12.52, $p<0.01$) when compared to control. However, postprandial the time to reach $C_{max}$ ($T_{max}$) levels were the same as those found in control. Our findings suggest that GO2KA1 limits the increase in postprandial blood glucose levels following bread consumption.

**Keywords:** chitosan oligosaccharide, GO2KA1, anti-hyperglycemic effects, healthy individuals, postprandial hyperglycemia

## Introduction

Type 2 diabetes is a disease linked to high blood glucose levels due to inadequate insulin secretion, resistance to insulin or both. Chronic hyperglycemia due to diabetes is associated with many long-term adverse effects on various organs (1). According to CDC, more than 29 million people are estimated to have either diagnosed or undiagnosed diabetes in the US and it is expected that by 2050 one in three US adults will have diabetes (2-3).

Postprandial blood glucose management is a strategy used to treat diabetes. α-Glucosidase inhibitors in particular (acarbose) are considered to have good efficacy when compared to other oral blood glucose-lowering interventions. α-Glucosidase inhibitors act on reducing the digestion of starch and disaccharides in the proximal small intestine, resulting in lower postprandial blood glucose levels. Finally, research findings suggest that such inhibitors are effective and safe not only in monotherapy but also when used synergistically with other drugs (4-6).

Chitosan is obtained from the deacetylation of chitin using basic conditions. This is the most important chitin derivative in applications. Chitosan and some chitosan derivatives are known for their anti-bacterial activity (7), anti-diabetic activity (8,9), lowering of liver cholesterol (10), anti-tumor activity (11,12) and antioxidant activity (13,14). Also, it was reported to have different biological properties depending on the molecular weight (8,14,15). Recently, the chitosan oligosaccharide molecular-weight-dependent effect on the inhibition of carbohydrate-hydrolyzing enzymes was evaluated and it was suggested that MW < 1,000 Da (GO2KA1) has a more positive effect towards postprandial glucose management in both animal and in vitro models (15).

The objective of this study is to test the hypothesis that 500-mg GO2KA1 administration prior to bread consumption will lead to reduced postprandial blood glucose levels due to the inhibition of carbohydrate-hydrolyzing enzymes.

## Materials and Methods

**Preparation of GO2KA1** GO2KA1 was prepared from low-molecular-weight chitosan, water soluble in neutral and alkali pH. Low-molecular chitosan was obtained from KPB (Kunpoong Bio, Co., Seoul, Korea) and produced by enzymatic hydrolysis. Low-molecular chitosan was fully lysed by soaking in 18% (v/v) ascorbic acid and succinic acid solution as 5% (w/v) for 2 h and then was titrated to pH 4.5-5.5. Three to five grams of chitosanase (from *Bacillus circulans*) was added to a prepared 50 g sample of low-molecular chitosan. The mixture was incubated at 50-60°C with shaking (120 rpm) for 15 h to prepare GO2KA1. The obtained product was filtered by a 0.5-μm filter and was sterilized at 121°C for 15 min to deactivate any remaining enzymatic activities. The sterilized product was used for clinical testing. The GO2KA1 used in this study was composed of 45.1% dimers, 24.8% trimers, 12.8% tetramers, 7.0% pentamers, 3.7% hexamers, and 6.6% of more than heptamers based on Matrix-assisted laser desorption/ionization Time-of-flight MS (Microflex series; Bruker, Billerica, MA, USA) and HPLC (1260 Infinity LC system; Agilent Technologies Inc., Santa Clara, CA, USA) analysis. The GO2KA1 composition therefore comprised 82.7% monomers to tetramers and 17.3% of more than pentamers.

**Experimental protocol for postprandial blood glucose control** The study was a randomized crossover trial performed at Hannam University, Korea, from October to December 2013. The Hannam University Institutional Review Board (IRB) approved the trial (HNU 2013-10K), and written informed consent was obtained from all participants before screening.

For this clinical study, 9 healthy volunteers (all are students of Hannam University, male=6; female=3) were recruited after explanation about experimental protocols and models. Mean age, mean weight, and mean height of volunteers were 22±2.5 years, 65.9±15.6 kg, and 166.3±8.3 cm, respectively. On the first day, a control experiment was conducted without oral take of GO2KA1. All participants consumed only bread as per the 58 g carbohydrate requirement, along with 200 mL of water. On the second day, the same procedure was repeated as the comparative experiment (GO2KA1 experiment) with an oral take of 500 mg of GO2KA1 in 200 mL of water while eating bread. The blood samples were collected after ingestion, and blood glucose levels were measured at 0, 0.5, 1, and 2 h. The blood glucose level was determined using a blood glucose monitoring kit (Caresens II, ICENS Co., Ltd., Wonjoo, Korea) and compared with that of the control group. The blood glucose parameters were calculated using PKSolver, a freely available menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications. The maximum observed peak blood glucose level ($C_{max}$), the time at which it was observed ($T_{max}$), and the area under the concentration-time curve (AUC*t*) were estimated based on the observed data. AUC*t* was calculated by the linear trapezoidal rule.

**Statistical analysis** All data are presented as mean±SD. Statistical analyses were carried out using the statistical package SPSS 12.0 (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) program and significance was verified using Student's *t*-test for comparison of means.

## Results and Discussion

**Regulation of postprandial blood glucose levels using GO2KA1** The changes in postprandial blood glucose in healthy volunteers with normal blood glucose levels were measured (Table 1). Blood glucose levels were measured as described in the Materials and Methods section. We observed that GO2KA1 significantly lowered postprandial blood glucose concentration at 30 min after ingestion (Table 1 and

**Table 1.** Postprandial blood glucose levels (BGL; mg/dL) and extent of blood glucose increase (BGI; mg/dL) in control and after administration of GO2KA1

| | Groups | 0 h | 0.5 h | 1 h | 2 h |
|---|---|---|---|---|---|
| BGL (mg/dL) | Control | 98.06±5.81[1)] | 155.11±13.06 | 132.00±16.70 | 108.78±14.38 |
| | GO2KA1 | 95.61±6.24 | 138.50±13.59** | 125.78±8.22 | 105.33±13.92 |
| BGI (mg/dL) | Control | NA | 57.06±15.39 | 33.94±16.86 | 11.61±13.62 |
| | GO2KA1 | NA | 42.89±14.39** | 30.17±7.72 | 11.00±10.92 |

[1)]Each bar represents mean±SD (*n*=9). Control vs. GO2KA1 treatment; *$p$<0.05, **$p$<0.01, ***$p$<0.001.

**Table 2.** Pharmacodynamic (PD) parameters in control and after administration of GO2KA1

| | Groups | PD parameters | | |
|---|---|---|---|---|
| | | $C_{max}$ (mg/dL) | $T_{max}$ (h) | AUC*t* (h·mg/dL) |
| BGL (mg/dL) | Control | 157.94±10.90[1)] | 0.61±0.22 | 255.46±15.43 |
| | GO2KA1 | 140.61±12.52** | 0.61±0.22 | 240.15±14.22* |
| BGI (mg/dL) | Control | 59.89±13.98 | 0.61±0.22 | 59.71±16.98 |
| | GO2KA1 | 45.00±12.59* | 0.61±0.22 | 49.57±11.53 |

[1)]Each bar represents mean±SD (*n*=9). Control vs. GO2KA1 treatment; *$p$<0.05, **$p$<0.01, ***$p$<0.001

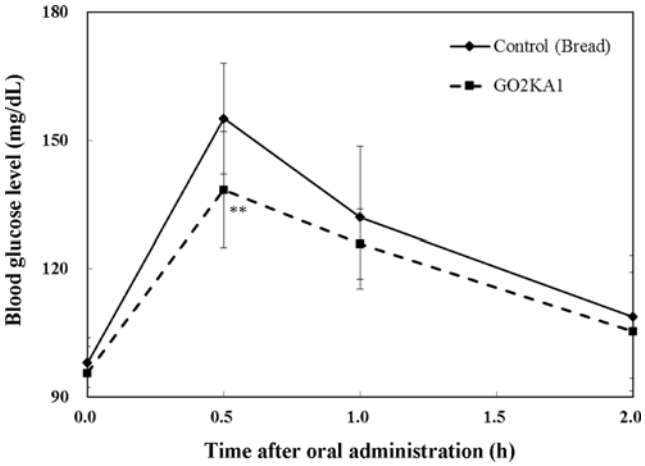


**Fig. 1.** Changes in postprandial blood glucose levels in control and GO2KA1 groups. Each bar represents mean±SD (*n*=9).

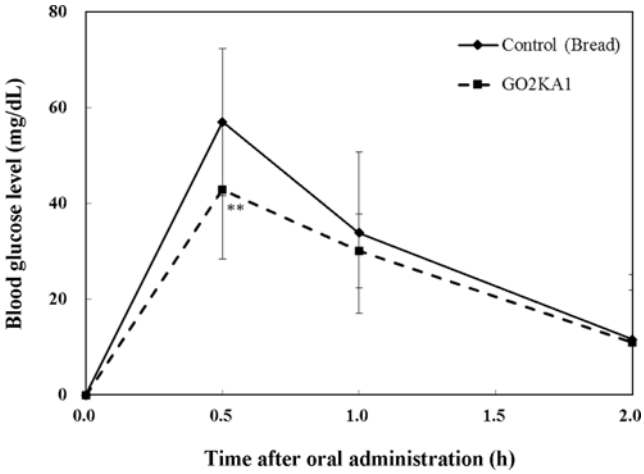


**Fig. 2.** Extent of blood glucose increase in control and GO2KA1 groups. Each bar represents mean±SD (*n*=9).

Fig. 1). More specifically, postprandial blood glucose level was reduced from 155.11±13.06 to 138.50±13.59 at 30 min. That is, we observed blood glucose reduction of around 11% at 30 min, when compared to the control group. In addition, after pharmacodynamic assessment, we observed that GO2KA1 significantly lowered $C_{max}$ (maximum blood glucose concentrations) and AUC*t* (area under the curve), but did not affect $T_{max}$ (time when glucose peak is observed), when compared to control (Table 2). More specifically, $C_{max}$ was decreased by 8.9% and AUC*t* was decreased by 6.8% when compared to control. In our previous clinical trial using sucrose loading (16), we observed increased $T_{max}$ and reduced $C_{max}$. Since bread's major carbohydrate component is starch, we believe that the lack of effect on $T_{max}$ is due to a different inhibition mechanism of GO2KA1 on maltase and $\alpha$-amylase compared to sucrase.

When these results were expressed in terms of increase in blood glucose concentrations, we observed similar trends (Table 1 and 2), but the reduction rate was much higher compared to control. This method of data analysis gives a better understanding of the effect since we normalize all samples to the same initial blood glucose levels. More specifically, we observed that GO2KA1 significantly inhibited the increase in postprandial blood glucose levels at 30 min by about 25%. In addition, $C_{max}$ was decreased by 11% and AUC*t* was decreased by 6% when compared to control.

As a result of this clinical study, we confirmed that GO2KA1 supplementation leads to reduction of postprandial blood glucose levels as indicated by the reduced $C_{max}$, AUC*t*, and glucose concentration at 30 min after ingestion (Table 1, Table 2, Fig. 1, and Fig. 2). At the same time, these results suggest that GO2KA1 reduced post-prandial blood glucose levels following bread administration. This observation is possibly due to the inhibition of carbohydrate-hydrolyzing enzymes in the small intestine (15) resulting in reduced glucose release from starch.

Finally, our current findings indicate a trend similar to the results of our previous observation in animal trials and human clinical trials using sucrose (15,16), indicating that GO2KA1 administration results in reduced postprandial blood glucose levels via inhibition of carbohydrate hydrolysis enzymes (15).

**Acknowledgments** This research was financially supported by the Korea Institute for Advancement of Technology (KIAT) through the Industry-Academy Cooperation Campus grant for Industry-Academy Convergence Laboratory in 2015.

**Disclosure** The authors declare no conflict of interest.

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