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Short Report

Effect of Partially Hydrolyzed Guar-gum Treatment on Fecal pH in Mice Fed with a High-fat-diet

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Abstract

To clarify the supplemental effect of partially hydrolyzed guar-gum (PHGG), which is a soluble-dietary fiber, on glucose metabolism and fecal pH in mice fed with a high-fat-diet (HFD), 4-week-old male C57BL/6J mice (n=24) were randomly divided into 4 groups: HFD-0%PHGG (HFD), HFD-1.0%PHGG (G1HFD), HFD-5.0%PHGG (G5HFD) and HFD-7.5%PHGG (G7.5HFD). After the experimental period of 10 weeks, a glucose tolerance test and measurement of fecal pH were carried out. PHGG treatment under HFD feeding did not show improvement in obesity measurements or glucose tolerance. However, both G5HFD and G7.5HFD groups accelerated the decrease of fecal pH ($p<0.01$). These findings suggest that a study using an over 5% PHGG treatment might activate the change in fermentation level of gut microbiota in HFD mice, although PHGG-treatment of less than 10 weeks may not contribute enough to prohibit HFD-induced obesity and diabetes.

1. Introduction

It is known that the ingestion of dietary fiber may lead to numerous health benefits for the host[1]. Guar-gum is one of these dietary fibers, and is classified among soluble fibers. In order to make guar-gum more palatable, hydrolyzed guar-gum (partially hydrolyzed guar-gum: PHGG) was produced resulting in a low viscosity/nonviscous type of fiber. Although PHGG is well fermented by gut microbiota, PHGG intake might not contribute to the improvement of glycemic control[2]. In fact, Aoki et al.[3] showed that there was no difference between the blood glucose area under the curve in HFD and PHGG+HFDC mice when the glucose tolerance test was performed. However, that study used a 5% contained PHGG diet, so a study using a different dose effect of PHGG intake and its reults on glucose metabolism and obesity remains unclear. Furthermore, it is unclear whether the PHGG administered dose-dependently induces the fermentative action via gut microbiota. In this study, we investigated the supplemental effect of PHGG on HFD fed-induced obesity, glucose tolerance and fecal pH via gut microbiota.

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2. Methods

2.1 Animals

In this study, 4-week-old male C57BL/6J mice (n=24) were housed 4 mice to a cage under a controlled environment (22 ± 1 ◦C, 12:12-h light-dark cycle) and were randomly divided into 4 groups: HFD, G1HFD, G5HFD and G7.5HFD. The mice were given access to food and drinking water *ad libitum*. The experiment involving the mice, including the procedures performed, was approved by the Institutional Animal Care and Use Committee of Kawasaki University of Medical Welfare (No.18-017).

2.2 Diet

The mice were fed HFD (D12492Gpx10, Research Diets, New Brunswick, NJ) containing 60 % fat, 20% protein and 20% carbohydrates for 10 weeks. The diet was cellulose free and was administered under three conditions: 1.0% (G1), 5.0% (G5) and 7.5 % (G7.5) PHGG (Sun fiber®, Taiyo Kagaku Co., Ltd, Yokkaichi, Japan) (Table 1).

Table 1 Dietary composition

Contents	HFD	G1HFD	G5HFD	G7.5HFD
	kcal	kcal	kcal	kcal
Protein	20	20	20	19
Carbohydrate	20	20	22	23
Fat	60	60	59	58
Total	100	100	100	100
kcal/g	5.6	5.6	5.4	5.3
	g %	g %	g %	g %
Protein	**28.0**	**27.7**	**26.4**	**25.6**
Casein	27.6	27.3	26.0	25.2
L-Cystine	0.4	0.4	0.4	0.4
Carbohydrate	**32.2**	**33.0**	**36.2**	**38.2**
Corn Starch	0.0	0.0	0.0	0.0
Maltodextrin	17.3	17.1	16.3	15.7
Sucrose	14.9	14.7	14.0	13.6
Cellulose	0.0	0.0	0.0	0.0
PHGG	0.0	1.0	5.0	7.5
Fat	**37.3**	**36.9**	**35.1**	**34.0**
Soybean Oil	3.5	3.4	3.3	3.1
Lard	33.8	33.4	31.9	30.9

2.3 Glucose tolerance test

After the mice fasted for 5 hours blood samples were collected from the tail vein, and then blood glucose levels were measured using the glucose monitoring device Accu-Chek (Roche, Basel, Schweiz) immediately prior to glucose administration, and at 15, 30, 60 and 120 minutes after glucose administration (2 g/kg, i.p.). Each mouse was lightly anesthetized with the inhalant Isoflurane prior to the glucose administration[4].

2.4 Fecal pH measurements

At the 6th and 10th week, fecal materials were collected from mice that were as fresh as possible. The materials were diluted 2-3 folds (w/v) in distilled water and were homogenized by the homogenizer pestle. After calibrating the pH meter (twin pH B-212, Horiba Ltd, Kyoto, Japan), the pH of these diluted samples

was measured[5].

2.5 Measurements of body mass, food intake, cecum contents and epididymal adipose tissue

Body weight and food intake were recorded for the final week. The food intake was expressed as total weekly intake. Two days after the experimental period the mice were euthanized under Isoflurane anesthesia and then the cecum contents and epididymal adipose tissue were collected and weighed.

2.6 Statistical analysis

The statistical analyses were performed using the IBM SPSS Statistics 23.0 for Windows software program. The data were analyzed using the one-way ANOVA, and then a post-hoc test was performed using the Bonferroni's test. P values of <0.05 were considered to be indicators of statistical significance.

3. Results and discussion

3.1 Effect of PHGG on obesity and glucose metabolism

Although body weight was different in the G5HFD and G7.5HFD groups (Figure 1a, $p<0.05$), the G1HFD, G5HFD and G7.5HFD groups did not show any attenuation in body mass gain compared with HFD mice. Since food intake did not differentiate among all groups (Figure 1b), it was suggested that PHGG was ingested in mice in a dose-dependent manner. Nevertheless, we did not observe the inhibition of weight gain by PHGG. Furthermore, adipose tissue mass in HFD mice also was not affected by PHGG intake (Figure 1c). Hence, at the least, our results suggest a small obesity-preventing effect of PHGG. Recently, it was reported that combined with 3% cellulose, 7% guar gum intake did not attenuate body mass gain and adipose tissue

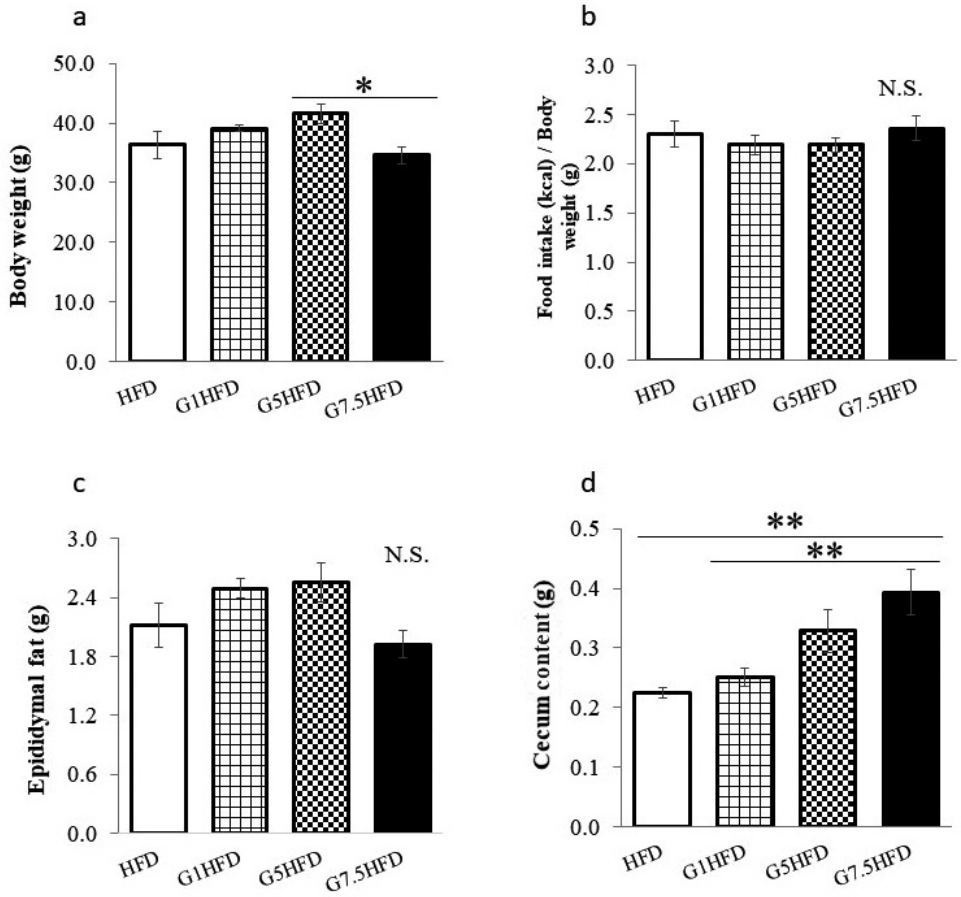

Figure 1 The effect of PHGG treatment on body weight (a), food intake (b), adipose tissue weight (c) and cecum contents (d) in HFD mice. Food intake showed as energy intake (kcal) per body weight at final week. The values were expressed as the mean ± S.E.M. *: $p<0.05$ and **: $p < 0.01$.

mass in HFD mice[6]. It is the considered opinion of this research team that this report contains similar results to our own. On the other hand, cecum contents were increased in a PHGG dose-dependent manner. However, no difference between the cecum content in G5HFD and G7.5HFD groups was observed (Figure 1d). Indigestible carbohydrate supplementation increases cecum content[3,7]. This alteration is caused by indigestibility with increased water-holding capacity. In addition, it was reported that a significant and dose-dependent increase in cecum weight was observed by galactooligosaccharides treatment, and that this was accompanied by an alteration of gut microbiota[7]. Moreover, polyphenol, n-3 polyunsaturated fatty acids and HFD which influences gut microbiota also affects cecum content weight, although they do not have water-holding capacity[8,9]. Therefore, changes in cecum content weight is one of the beneficial indexes of gut microbiota alteration. This result suggests that starting at 5% PHGG treatment might activate the change in fermentation levels of gut microbiota in HFD mice.

Additionally, G1HFD, G5HFD and G7.5HFD groups did not show improvement in glucose tolerance compared with HFD mice (Figures 2a and b). It was already known that any beneficial effect, such as improved glycemic control, is abolished when guar-gum is hydrolyzed to a nonviscous form[2]. In fact, dietary PHGG did not significantly affect plasma glucose levels in a *db/db* mice[10] or in one human study[11]. Therefore, our results suggest that glucose metabolism dose not improve PHGG intake regardless of the intake dose. Interestingly, Aoki et al.[3] recommends that exercise has a complementary effect on insulin resistance in PHGG intake.

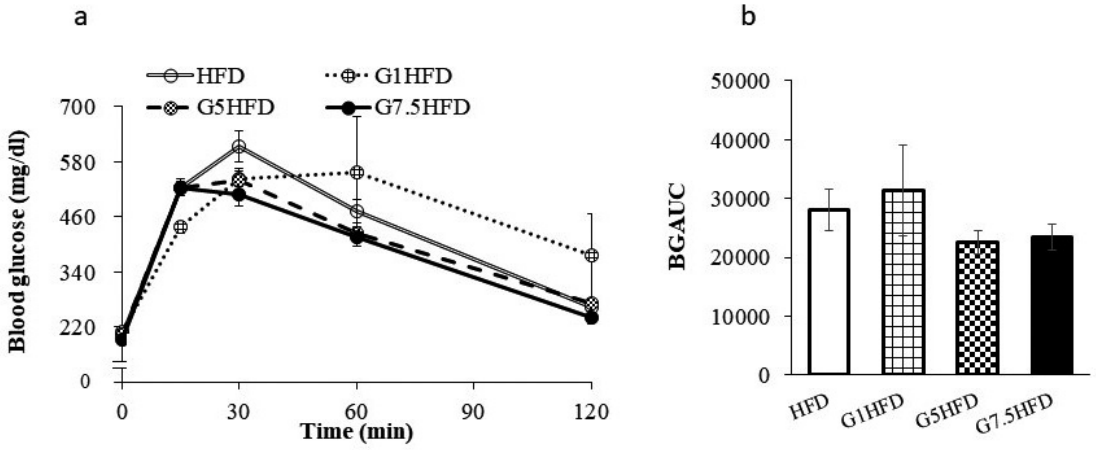

Figure 2 The effect of PHGG treatment on blood glucose concentration and the incremental area under the curve (AUC) of blood glucose during glucose tolerance tests in HFD mice. Time dependent blood glucose levels after glucose load (2 g/kg, i.p.) (a), and the corresponding incremental blood glucose area under the curves (BGAUC) (b). The values were expressed as the mean ± S.E.M.

3.2 Effect of PHGG on fecal pH

In both the 6th and 10th weeks of the experimental period, the reduction of fecal pH depended on the dose of PHGG administration. However, there was no significant difference between the pH levels in G5 and G7.5 groups in both the 6th and 10th weeks (Figure 3). PHGG could elicit constipation relief and modulate gut microbiota, which shows the potential to act as a dietary fiber for constipation treatment[12]. PHGG treatment has been shown to reduce colonic mucosal damage in an animal colitis model[13]. This fermentable fiber is useful as a prebiotic, and it was reported as such in HFD-induced obese mice. At the least, we confirmed that cecum content weights were higher and the fecal pH was lower in mice fed with a HFD diet with PHGG than in those fed diets without PHGG. There was agreement with the results of previous studies, which suggested that the possible mediators of the effects of PHGG were the SCFAs produced by microbial

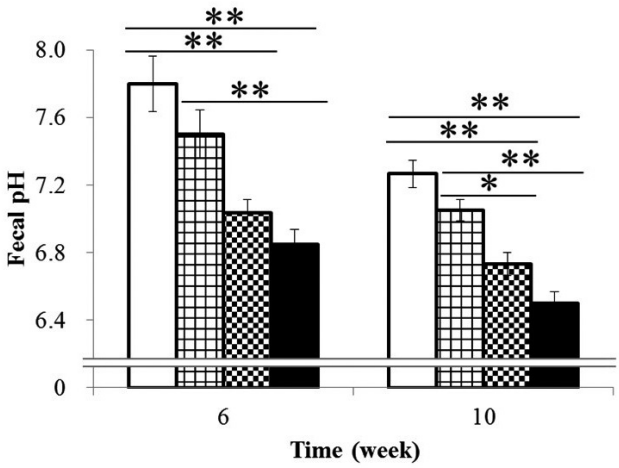

Figure 3 The effect of PHGG treatment on fecal pH in HFD mice. White column: HFD, plaid column: G1HFD, checkered column: G5HFD, and black column: G7.5HFD. The values were expressed as the mean ± S.E.M. *: $p<0.05$ and **: $p < 0.01$.

fermentation of PHGG in the large intestine[3,14]. In addition, those effects were shown to occur at sufficient levels enough with the administration of 5% PHGG. It seems that this is the adequate concentration that should be taken in *in vivo* for PHGG research in the future.

In conclusion, our findings suggest that over 5% PHGG supplementation might activate the fermentation level via gut microbiota in HFD mice, although PHGG alone may not contribute to improvement of HFD-induced diabetes.

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The authors declare no conflicts of interest are associated with the present study.

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