pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 39, No. 6, pp. 602~608 (2024)
https://doi.org/10.13103/JFHS.2024.39.6.602

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Effects of Long-Term Pyridoxal Supplementation on Blood Glucose Levels and Weight Gain in Animal Models

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(Received November 25, 2024/Revised November 30, 2024/Accepted December 6, 2024)

ABSTRACT - We previously reported that pyridoxine and its derivatives exert antidiabetic effects by alleviating postprandial hyperglycemia via inhibition of carbohydrate-hydrolyzing enzymes in normal sprague–dawley (SD) rats. In this study, we aimed to further evaluate whether long-term pyridoxal supplementation decreases the blood glucose levels using SD rats. SD rats were randomly assigned to groups fed a high-carbohydrate diet (66.1% cornstarch) with or without pyridoxal (4%) for 36 days. Changes in body weight, blood glucose levels, and food intake were measured daily for 36 days. Dietary supplementation with pyridoxal significantly decreased the blood glucose levels (P<0.001) and body weight (P<0.001) in mice. Glycated hemoglobin (HbA1c) levels, which are good indicators of plasma glucose concentrations over prolonged periods, were also significantly decreased over five weeks (P<0.001). Similarly, dietary treatment with Acarbose® (0.04%), a positive control, also significantly decreased the blood glucose and HbA1c levels and body weight. Overall, our findings suggest that pyridoxal inhibits weight gain and alleviates postprandial hyperglycemia by decreasing glucose absorption and HbA1c levels.

Key words: Type 2 diabetes, Pre-diabetes, Blood glucose, α-Glucosidase inhibition, Pyridoxal

Type 2 diabetes (or non-insulin dependent diabetes mellitus) is a global problem that provides burden to healthcare systems[1,2]. To prevent the incidence of diabetes, Center for Disease Control (CDC) defined the prediabetes condition, as the state at which individuals have elevated blood glucose levels and if they do not establish dietary and lifestyle changes in their diets, this condition will progress to Type 2 Diabetes[3]. The use of carbohydrate hydrolyzing inhibitors has been extensively studied over the past years to control blood glucose levels in people with hyperglycemia[4,5]. More specifically such inhibitors prevent the enzymatic digestion of dietary carbohydrates (sucrose, lactose, starch, maltose), resulting to controlled glucose uptake in the small intestine and a subsequent dietary caloric load reduction.

Overweight and obesity significantly increase the incidence of noncommunicable diseases, including Type 2 Diabetes[6,7]. We have been observing that globally obesity levels are rising and according to the World Obesity Foundation if we do not develop meaningful solutions, the world will have 4 billion obese individuals[8]. Caloric restriction is a lifestyle intervention that has been suggested to assist towards the management of obesity[9]. Caloric restrictions are classified in four categories[10]. Continuous energy restriction (daily energy intake reduction of 20-30% from daily requirements), short-term fasting (daily energy intake reduction of 25% on either 2-3 consecutive or non-consecutive days of the week), alternate day fasting (consume 20-30% of daily energy needs on fasting days and consume 100% of daily energy needs on non-fasting days), and time-restricted eating (consume within a daily window of less than 12 h). A recent systematic review and network meta-analysis comparing caloric restriction categories for weight management, suggests that all caloric restriction categories offer weight management, with alternate day fasting providing the most significant weight management outcomes[11].

Vitamin B6 vitamers, such as pyridoxine, pyridoxal, and pyridoxime have been shown to have various biological effects, including antioxidant activity, blood lowering, and stress relieving effects[12-15]. Recently we have demonstrated using *in vitro* and *in vivo* models that that pyridoxine and its derivatives can effectively control carbohydrate digestion and uptake in the small intestine by the inhibition of carbohydrate hydrolyzing enzymes[15].

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More specifically, recent findings have demonstrated the inhibitory activity of vitamin B6 and its derivatives against α-glucosidases and pancreatic α-amylase by using *in vitro* and an animal sprague-dawley (SD) rat model[12]. We observed that pyridoxine and pyridoxal supplementation resulted to lower postprandial blood glucose levels SD rat model, after both starch and sucrose loading test[15]. This was the first report for the potential of pyridoxal for type 2 diabetes management. However, there is still limited scientific knowledge about the effect of long-term pyridoxal supplementation on biomarkers relevant to the incidence of hyperglycemia by using an animal *in vivo* model.

Therefore, the aim of this study is to investigate the effect of long-term dietary supplementation of pyridoxal on hyperglycemia-linked biomarkers using a SD rat model and high carbohydrate diet. In this study, pyridoxal was administrated for 36 days in SD rats. The effect of long-term administration of pyridoxal was compared to Acarbose and control for fasting glucose levels, glycated hemoglobin (HbA1c), total cholesterol and triglyceride contents. This study gives information about the possible mode of action of pyridoxal for the management of type 2 diabetes and assist in the design of clinical trials.

Materials and Methods

Materials

Corn starch, casein, vitamin mix, mineral mix, calcium phosphate and sodium chloride were purchased from Raon Bio Co. (Yongin, Korea). Total cholesterol and total triglyceride kits were purchased from Stanbio laboratory Co. Ltd. (LiquiColor® Test series, Boerne, TX, USA). Blood glucose tester was purchased from I-SENS Inc. (Caresens II™, Anyang, Korea) and HbA1c analyzer was purchase from Infopia Inc. (Clover A1c™, Anyang, Korea). Unless noted, all chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Animal and Study Design

Five-week-old male SD rats were purchased from Joongang Experimental Animal Co. (Seoul, Korea) and fed a Pico 5053 diet (Oriental Bio. Co., Seongnam, Korea) for 1 week. The animals were housed in individual cages in a room with a 12 h light/dark cycle (lights on from 06:00) with 50%±7% relative humidity. In this study, ten SD rats were used for each group.

All mice were adapted to a meal-feeding schedule of free access to Pico 5053 diet with or without samples for 36 days (Table 1). The experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Hannam University (Approval number: HNU2024-003). The mice had free access to tap water throughout the experimental period. The mice were anesthetized with pentobarbital and killed, and blood was collected. The cecum weight was determined using analytical balance after biopsy.

Table 1. Composition of diets (g/kg).

High carbohydrate diets	Control	Pyridoxal	Acarbose
Corn Starch	661	621	660.6
Casein	226	226	226
Soybean oil	60	60	60
Vitamin mix[1)]	31	31	31
Mineral mix[2)]	9	9	9
Calcium phosphate	10	10	10
Sodium chloride	3	3	3
Sample (pyridoxal)	-	40	-
Acarbose	-	-	0.4

[1)] Vitamin mixture: AIN-93VX; [2)] Mineral mixture: AIN-93G.

Blood Analysis

The blood glucose level was measured with a glucose analyzer (CaresensII, I-SENS Inc., Anyang, Korea) using the glucose oxidase method, and the plasma total cholesterol and total glyceride concentration was measured using a kit (Liquicolor® test series, Stanbio Laboratory, Boerne, TX, USA). The concentration of HbA1c was measured using Nycocard reader (Clover A1c™, Infopia Inc., Anyang, Korea).

Statistical analysis

All data are described as mean±standard deviation. Statistical analyses were performed by the SPSS 11 program (Statistical Package for Social Science, SPSS Inc., Chicago, IL, USA) and significance in each group was verified with the analysis of one-way ANOVA followed by Duncan's test of $P<0.05$. Additionally, statistical significances in these animal studies were evaluated the Student's *t*-test for comparison of means (*$P<0.05$; **$P<0.01$, and ***$P<0.001$).

Results and Discussion

Postprandial Anti-hyperglycemic Effect of Pyridoxal in SD rat Model

Since the US Food and Nutrition Board has set the maximum daily dose of pyridoxal at 100 mg for adults, in this experiment, it was administered at 17 mg/day, calculated based on the previous rat administration study[15]. Furthermore, based on previous experimental study design[15], we identified 6 evaluation points every 6th day. Statistically significant differences in blood glucose levels were observed since the first evaluation point (6th day) and significant differences in body weight were observed since the thirst evaluation point (18th day). Statistically significant differences were seen in blood sugar from the 6th day and in body weight from the 18th day. Therefore, certain results were obtained by further administration until the 36th day, which is more than twice this period. The effect of pyridoxal administration was evaluated using the SD rat model for 36 days and the effect was compared to that of Acarbose, as described in the

materials and methods[15]. After 36 days we observed that the body weight of pyridoxal treated group was significantly lower compared to control (P<0.001) (Fig. 1). A significant difference between control and pyridoxal can be identified after 24 days and between control and Acarbose after 12 days of administration (Fig. 1). Additionally, it is important to note

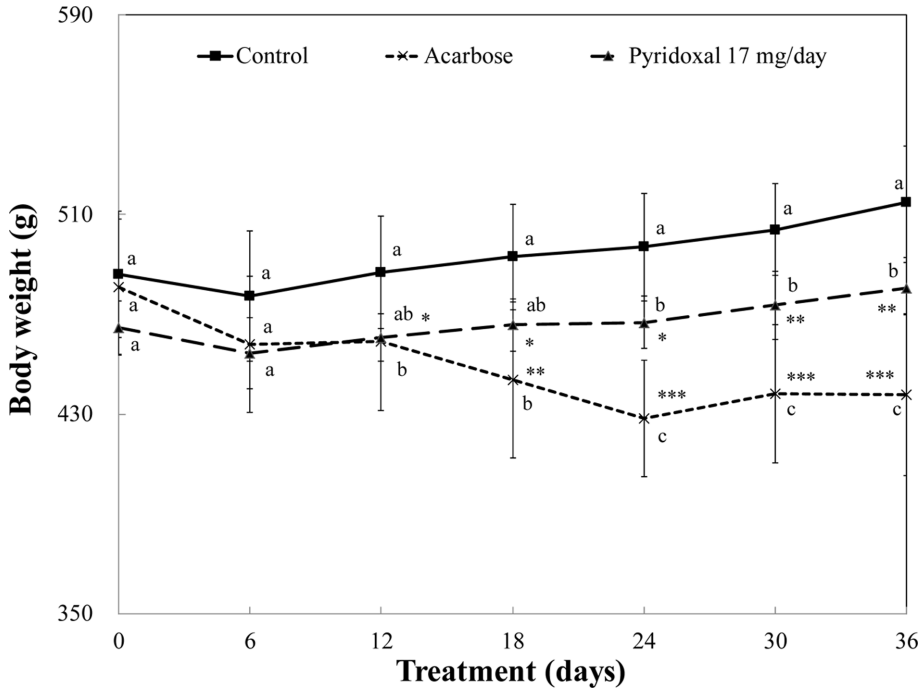

Fig. 1. Changes in body weight gains after administration of pyridoxal. Male SD rats were free access to a high carbohydrate-diet with pyridoxal (4%), Acarbose (0.04%), and vehicle for 36 days. Each point represents mean±standard deviation (SD). (n=10). Body weight levels were compared between control and treatment groups at each time point using unpaired Student's *t*-test (**P*<0.05, ***P*<0.01, and ****P*<0.001). The results are expressed as mean±S.D. [a-c] Different letters indicate statistically significant differences between groups one-way ANOVA followed by Duncan's test of P<0.05.

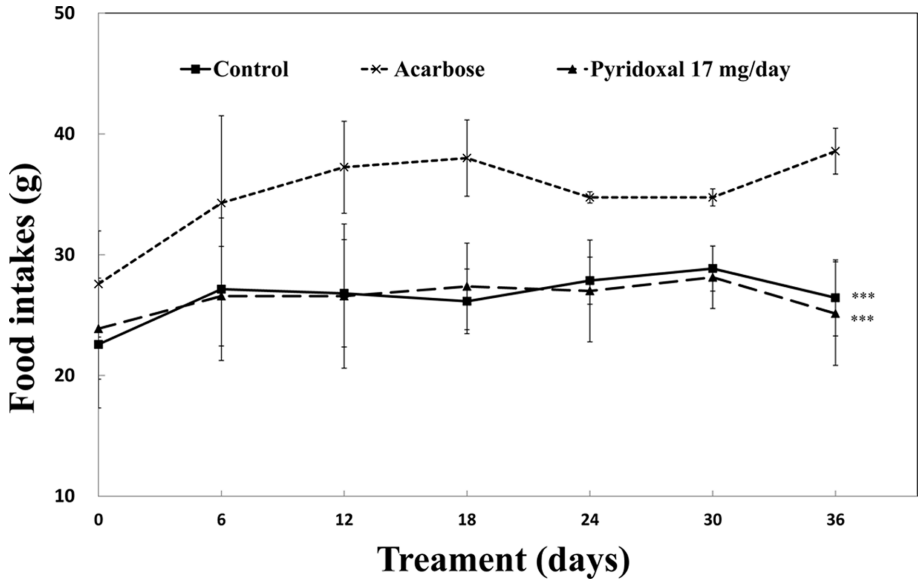

Fig. 2. Changes in food intake after administration of pyridoxal. Male SD rats were free access to a high carbohydrate-diet with pyridoxal (4%), Acarbose (0.04%) or vehicle for 36 days. Each point represents mean±standard deviation (n=10). Food intake levels were compared between control and treatment groups at each time point using unpaired Student's *t*-test (****P*<0.001).

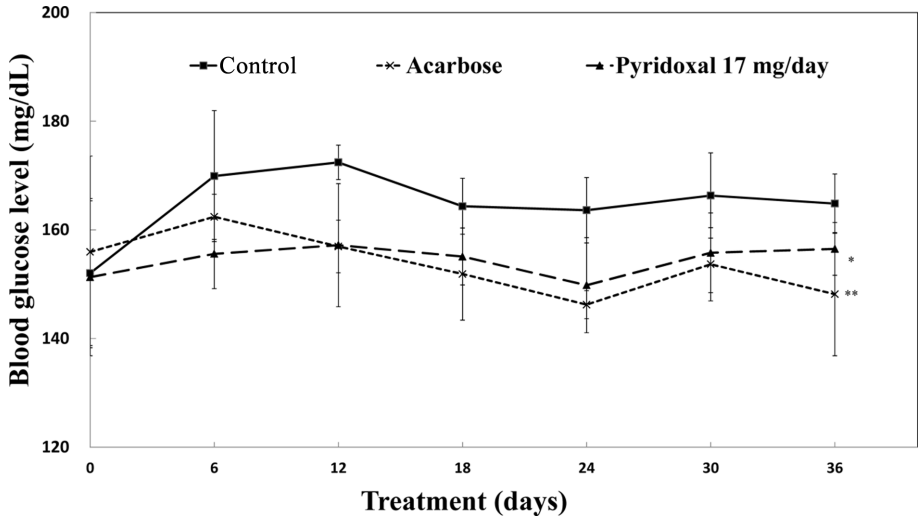

Fig. 3. Changes in blood glucose levels after administration of pyridoxal. Male SD rats were free access to a high carbohydrate-diet with pyridoxal (4%), Acarbose (0.04%) or vehicle for 36 days. Each point represents mean±standard deviation (n=10). Food intake levels were compared between control and treatment groups at each time point using unpaired Student's *t*-test (**P*<0.05 and ***P*<0.01).

that the pyridoxal and control animals appeared to have similar levels of food intake, while in the Acarbose treated group food consumption significantly increased (Fig. 2).

Additionally, the effect of pyridoxal supplementation on fasting blood glucose levels were determined over the period of 36 days. We observed that fasting glucose levels were significantly reduced with pyridoxal supplementation after 36 days to levels like that of Acarbose (Fig. 3). The fasting blood glucose level of control group was around 164.8±5.5 mg/dL, while the levels of pyridoxal and Acarbose groups were 154.9±6.7 mg/dL and 148.2±11.3 mg/dL, respectively, after 36 days (Fig. 3).

Our results suggest that pyridoxal supplementation significantly reduced body weight and fasting glucose levels when compared to control (Fig. 1 and 3). More specifically, we observed that pyridoxal supplementation has a weight management effect by preventing the development of diet-induced obesity, as demonstrated in our SD rat animal model. Another interesting finding is that the observed results were similar to those of Acarbose (Fig. 1 and 3). We expect that the observed pyridoxal effects are due to the inhibitory activity of pyridoxal against carbohydrate hydrolyzing enzymes, which was demonstrated in a previous study[15]. As previously described, starch and sucrose digestion inhibition results to reduced glucose uptake and a subsequent weight-loss, linked to caloric load reduction.

Acarbose treatment group had the largest cecum (1. 60 g, *P*<0.001), followed by control (0.28 g), while pyridoxal treatment had the smallest cecum weight (0.27 g) (Table 2). Acarbose is associated with side-effects due to extensive α-amylase inhibition. As a result, Acarbose supplementation results to increased cecum weight, due to the large load of undigestible starches that end up in the colon. Side effects include flatulence and diarrhea[16,17]. It is important to mention that the cecum weight and volume of the pyridoxal treated group was significantly smaller (5-times smaller) when compared to the Acarbose treatment (Table 2). These findings confirm our previous *in vitro* findings that pyridoxal has minimum inhibitory effect on α-amylase mediated starch digestion[15].

Table 2. Change in cecum weight (g)

	SD rats		
	Control	Acarbose	Pyridoxal
Cecum (g)	0.28±0.09	1.60±0.40***	0.27±0.07

Each point represents mean±standard deviation (n=10). All parameter was compared between control and treatment groups at 36 day using unpaired Student's *t*-test (****P*<0.001).

Another outcome of the excessive α-amylase inhibition resulting from Acarbose supplementation is the increased food intake that we also observed in our study (Fig. 2). It is important to mention that pyridoxal supplementation resulted to similar food intake with the control, which also suggests the reduced α-amylase inhibitory activity[15].

Our previous study demonstrated the reduction of the post-prandial blood glucose levels following starch/sucrose administration along with pyridoxal[15]. In this study we observed reduction of fasting blood glucose levels after 36 days. This is a very interesting finding, suggesting a possible better utilization of glucose by muscle cells, leading to improvement of insulin resistance. To confirm this finding, the HbA1c levels were evaluated at the next part of our study.

Hematological and Serum Biochemical Analysis in SD Rats Trial

Finally, we evaluated the effect of pyridoxal supplementation after 36 days on biomarkers relevant to the incidence of type

Table 3. Effect of pyridoxal and Acarbose treatment on various parameters in SD rats after 36 days

Parameters	Group		
	Control	Acarbose	Pyridoxal
HbA1c (%)	9.51±1.05[a]	5.21±0.82[c]	6.95±0.91[b]
Total cholesterol (mg/dL)	91.03±18.37[a]	68.94±11.93[b]	73.44±14.80[b]
Triglyceride (mg/dL)	76.49±20.35[a]	22.80±10.75[c]	56.68±16.22[b]
HDL-cholesterol (mg/dL)	61.77±11.59[a]	60.40±14.98[a]	57.84±11.54[a]
LDL-cholesterol (mg/dL)	13.96±13.97[a]	3.98±7.91[a]	4.27±22.70[a]
GOT (IU/L)	102.69±21.41[a]	103.60±40.13[a]	111.16±45.43[a]
GPT (IU/L)	42.44±14.09[b]	94.50±65.66[a]	41.43±10.96[b]

Each point represents mean±standard deviation (n=10). All parameter was compared between control and treatment groups at 36 day. The results are expressed as mean±S.D. [a-c] Different letters indicate statistically significant differences between groups one-way ANOVA followed by Duncan's test of $P<0.05$.

2 diabetes and metabolic syndrome, such as HbA1c, highdensity lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride content, and liver function as indicated by glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities (Table 3). Our results suggest that pyridoxal and Acarbose supplementation has significantly lower HbA1c levels when compared to control, suggesting that both treatments prevent the development of type 2 diabetes (Table 3). More specifically, the HbA1c levels of the control group were around 9.51±1.05 while pyridoxal and Acarbose resulted in significantly lower levels (6.95±0.91 and 5.21±0.82%, respectively) (Table 3). Triglyceride levels of pyridoxal treated rats was significantly reduced compared to the control but Acarbose treatment resulted to the lowest triglyceride levels (Table 3). Total cholesterol levels were reduced with both pyridoxal and Acarbose treatments, while HDL-cholesterol levels were similar among all tested groups and control (Table 3). Neither GPT or GOT functions were negatively affected pyridoxal supplementation, but Acarbose treatment resulted to increased GPT levels (Table 3). This is not a surprising finding, since Acarbose treatment has been associated with hepatotoxicity[18].

HbA1c, an accurate biomarker, calculates the average blood glucose level over a period of 12 weeks and helps define how efficient muscle cells utilize blood glucose which is one of the most important factors to define insulin resistance[19]. Additionally, previous reports suggest that HbA1c is an excellent biomarker of overall metabolic wellness and maintain a healthy HbA1c level contributes to better overall health. It was suggested that for every unit that HbA1c is increased, we observe significant increase in cataracts (19%), peripheral blood vessel disease (43%), heart attacks (14%) and death due to diabetic complications (21%)[19]. We observed that pyridoxal administration results to reduced fasting blood glucose levels (Figure 2) and reduce HbA1c (Table 3) when compared to control. These two findings suggest that pyridoxal administration prevents the development of diet-induced insulin resistance, in the SD rat model that we used.

Additionally, pyridoxal supplementation resulted to reduced total cholesterol and triglyceride levels (Table 3). This observation is not surprising, taking into consideration that pyridoxal results in weight management through caloric restriction. Additionally, excess blood glucose can cause diabetic dyslipidemia, which is characterized by elevated fasting and postprandial triglycerides, low HDL-cholesterol, elevated LDL-cholesterol and the predominance of small dense LDL particles[20].

Conclusions

Our findings suggest that pyridoxal has a protective effect against developing diet-induced obesity and type 2 diabetes, without having any negative effects on liver function. A widely employed medicinal strategy to control obesity and type 2 diabetes is the reduction of glucose uptake in the small intestine, resulting to reduced caloric load. One effective strategy to achieve this is the inhibition of the digestion of dietary carbohydrates by small intestinal α-glucosidases[4,5]. a-Glucosidases inhibition reduces the postprandial blood glucose levels following a mixed carbohydrate diet and can contribute to the management of type 2 diabetes[21-23]. Our research demonstrated pyridoxal's efficacy in term of controlling fasting blood glucose, weight gain, HbA1c, triglycerides and total cholesterol in an SD rat model. We believe that the observed health benefits are due to the inhibition of carbohydrate hydrolysis enzymes which were reported in a previous research effort[15]. Our findings provide a strong rational for the further evaluation of pyridoxal for weight and blood glucose control and supports the needs to perform a further clinical trial evaluating this effect.

Acknowledgments

This research was financially supported by research grant from 2024 Hannam University. We would like to thank the Kunpoong Bio Company-affiliated Institute for its research support.

국문요약

본 연구에서는 가장 활성이 우수하였던 pyridoxal의 장기간 투여가 sprague–dawley (SD) 쥐 모델에서 혈당 수치를 효과적으로 감소시키는가를 평가하였다. 이를 위해 pyridoxal (식이 중에 4%)이 함유된 고탄수화물 식이(66.1% 옥수수 전분)와 시료가 함유되어 있지 않은 음성대조군 식이 그리고 식후혈당상승억제제로 시판중인 아카보스(Acarbose®)가 포함된(0.04%) 양성대조군 식이를 제조하고 3군으로 나누어 36일동안 투여하며 체중, 혈당, 당화혈색소, 음식 섭취량, 콜레스테롤등의 각종 혈액생화학적 지표의 변화를 측정하였다. 사전연구를 통해 피리독신과 그 유도체가 소장내 존재하는 탄수화물 가수분해 효소의 억제를 통해 식후 혈당상승을 낮추어 항당뇨 효과를 갖고 있다는 것을 SD 쥐 모델을 이용한 단회 투여 실험결과로 확인했다. 그 결과 pyridoxal을 식이 보충제로 섭취 시킨 시험군의 경우 혈당(P<0.05)과 체중(P<0.01)이 통계적으로 유의하게 감소하였고, 이러한 결과는 양성대조군인 아카보스 투여군에서도 동일하게 나타났다. 또한 장기간에 걸친 혈액내의 포도당 증감을 나타내는 지표인 당화혈색소 수치도 통계적으로 유의하게 감소하였다. 양성대조군인 Acarbose®(식이 중 0.04%)의 식이 투여군도 혈당, 당화혈색소 및 체중 수준을 크게 완화시켰다. 이러한 결과는 pyridoxal 섭취가 식후혈당상승억제제인 Acarbose와 유사한 작용기전으로 소장내에서 전분의 분해와 포도당의 흡수를 억제하고 이를 통해 혈당 및 당화혈색소 증가를 억제시켜 체중 증가를 감소시키고 식후 고혈당 증상을 개선할 수 있다는 것을 시사한다.

Conflict of interests

The authors declare no potential conflict of interest.

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