Investigation of an Amylase Inhibitor on Human Glucose Absorption after Starch Consumption

Joe A.Vinson*, Hassan Al Kharrat and Donna Shuta

Department of Chemistry, Loyola Hall, University of Scranton, Scranton, PA 18510, USA

Abstract: Inhibition of carbohydrate metabolism or absorption can decrease calorie intake to promote weight loss and combat obesity. It is also a mechanism for reducing hyperglycemia in diabetic subjects. A concentrated northern kidney bean extract was found to be an effective *in vitro* amylase inhibitor, thus potentially affecting the absorption of starch carbohydrates. This product was tested in two single dose human studies. Eleven fasting subjects were given 4 slices of white bread and 42 g of margarine with or without 1.5 g of bean extract. Absorption as measured by the area under the plasma glucose-time curve was inhibited 66%. A full meal study with 7 subjects and 0.75 g of extract caused a non-significant 28-41% reduction in absorption. There was a dose-response decrease in glucose absorption by the extract. The bean extract has *in vivo* efficacy for inhibition of starch absorption and may prove beneficial in weight reduction in individuals consuming large amounts of starch. It also may inhibit starch-induced hyperglycemia in normal and diabetic subjects.

Key Words: Glucose, starch, amylase inhibitor, bean extract.

INTRODUCTION

The most common nutritional disorders in the USA are overweight and obesity. The prevalence of obesity has increased more than 75% since 1980 [1]. A 2006 survey by the Centers for Disease Control found that an average of 37% of Americans were overweight [2]. These findings are in agreement with trends seen elsewhere in the world. The number of children and adolescents considered overweight has more than doubled in the US since 1976 [3]. In 1999, over $300 million was spent in the USA for prescription medicines to treat obesity [4]. Being obese increases the risk of arthritis, dyslipidemia, hypertension, diabetes and coronary artery disease. Compounding these health risks, obese individuals have a lower quality of life than those who are not obese [5]. Medications currently approved for weight loss can be divided into two categories based on mechanism: (a) reduction of appetite or increasing satiety, and (b) inhibition of nutrient absorption [6]. There are numerous side effects of these medications, which limit their utility. On the plus side, there is evidence that even modest weight loss (5% of body weight) will decrease the risk of the chronic diseases such as diabetes and cardiovascular disease [7].

The major source of carbohydrates in the US diet is starch and over consumption of carbohydrates is frequently associated with obesity [8]. This idea has been made popular by the low-carbohydrate diet popularized by Atkins and others. White kidney bean (*Phaseolus Vulgaris)* has documented salivary and pancreatic amylase inhibitory effects [9, 10]. The theory behind starch blockers has been explained by Preuss and co-workers [11]. Digestion of starch begins with salivary amylase in the mouth followed by amylase and other pancreatic enzymes in the duodenum. The end result of digestion is the production of mono and disaccharides that are absorbed in the small intestine. The final conversion of disaccharides to monosaccharides occurs during absorption. Slowing of the rapid absorption of carbohydrates would favorably influence the insulin system and in turn lead to lesser fat accumulation [12].

A 2008 search in the National Library of Medicine Database reveals that there are 1552 articles concerning amylase inhibition. A comprehensive experimental investigation found that the major reason commercial amylase inhibitors have failed to influence starch digestion in humans is their low amylase inhibitor activity [13]. We thus investigated a new commercial product for blocking carbohydrates from starch consumption *in vivo*.

MATERIALS AND METHODOLOGY

Phase 2, a concentrated white kidney bean extract (Pharmachem Labs, Kearney, NJ) is in the form of a tan powder. It was found to be a potent *in vitro* amylase inhibitor, 5300 ± 1052 U amylase activity/g using a standard assay done in triplicate at pH 6.8 with potato starch as the substrate and pancreatin as the enzyme source (Lycoming Analytical Laboratories Standard Operating Procedure Revision 5, 2003).

Two random double-blind, crossover human pilot studies were conducted after obtaining approval from the University of Scranton Institutional Review Board and informed consent from the volunteers.

Study 1

There were eleven normoglycemic subjects (6 females and 5 males, aged 21 to 57) who appeared after an overnight fast. After an initial blood draw, they consumed a week apart

*Address correspondence to this author at the Department of Chemistry, Loyola Hall, University of Scranton, Scranton, PA 18510, USA; Tel: (570)-941-7551; Fax: (570)-941-7510; E-mail: vinson@scranton.edu

1876-3960/09

either 4 large slices of white bread with 3 tablespoons of soybean oil margarine (610 calories from 60.5 g carbohydrates, 36.5 g fat, and 10.5 g protein) and 4 g of Sweet N'Low™ with or without 1.5 g of Phase 2 which was mixed with the margarine. Blood was drawn before eating and then every 15 minutes for 2 hours. Plasma glucose measured using an automated clinical procedure.

Study 2

A second pilot study with seven subjects (aged 23 to 43, 4 females and 3 males) included a lower dose of Phase 2 (0.75 g) with a full meal taken after an overnight fast. The subjects consumed a Hungry Man™frozen dinner of country fried steak with gravy, mashed potatoes, green beans and cherry-apple pie containing 630 calories from 64 g carbohydrate (6 g of dietary fiber, 19 g of sugars, and 39 g of starch), 29 g of fat, and 29 g of protein or a control without added Phase 2. The Phase 2 was mixed with the gravy and the subjects were given the dinners blinded. Blood was drawn every 10 minutes for 60 minutes then periodically until 2 hours. For both studies statistical analysis was performed using a paired t test.

RESULTS

Study 1

The bread consumption results are shown in Fig. (**1**). The Phase 2 group experienced a lower change in glucose C_{max} and the glucose returned to baseline earlier than the control (62 vs. 80 min.). The area under the curve (calculated from baseline to when the curve returns to baseline after consumption) for Phase 2 (0 to 62 min) was 66% lower ($p < 0.05$) than for the control (0 to 80 min.). This result indicates only 1/3 of the carbohydrates in the bread were absorbed with Phase 2.

Study 2

The full meal study data are shown in Fig. (**2**). Again Phase 2 caused an earlier return to baseline (58 vs. 70 min). With this dose the average area under the curve was 28% lower, indicating 2/3 of the carbohydrates in the meal were absorbed. This is in fact a conservative estimate, since the label of the frozen dinner meal lists 19 g of the carbohydrates are sugars that are not inhibited from absorption. Assuming all the sugars are glucose and that it is completely absorbed, the maximum inhibition would be 41% of the carbohydrates consumed. The changes in glucose and areas under the curves were not significantly different between the two groups.

DISCUSSION

The product is sold commercially as tablets or in a capsule. We added the product to the meal as a powder to eliminate any effects of an excipient, which could contain starch. Consumption of 0.75g of Phase 2 produced a calculated 28 to 41% inhibition and with 1.5g there was a 66% inhibition of starch absorption as glucose. Thus there was a dose-response inhibition of starch absorption in spite of difference in nutrient consumption between the two meals. The low dose was about 1/2 as effective as the high dose so the effect was linear. However the low dose of Phase 2 did not result in any significant effect perhaps due to small number of subjects. Our results can be compared to a 1987 Mayo Clinic study with a 650-calorie meal in which 2.9 g of an in-house prepared bean extract but not 2.0g reduced postprandial glucose [14]. Also corroborating our single dose results is a study in which fasting subjects consumed 50 g of carbohydrates as white bread plus capsules containing Phase 2 at doses from 1500 to 3000 mg by Udani (personal communication). Reductions in area under the blood glucose-time curve occurred at all doses with a maximal decrease of 34% at the highest dose ($p < 0.05$). The Phase 2 product seems to be much more potent, allowing lower doses to be used. In our single dose study there were no side effects during the day of the study such as flatulence as indicated by questioning the subjects. In the 5 long-term human studies

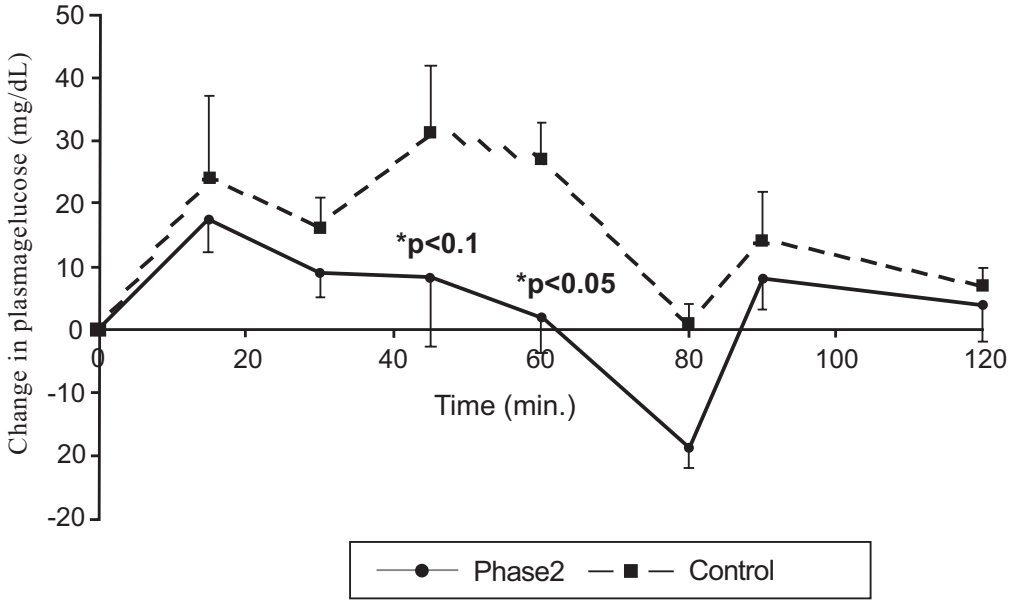

Fig. (1). Comparison of 1.5 g of Phase 2 vs. control on changes in plasma glucose after consumption of 4 slices of white bread and margarine (mean ± standard error of mean). *Significance vs. control.

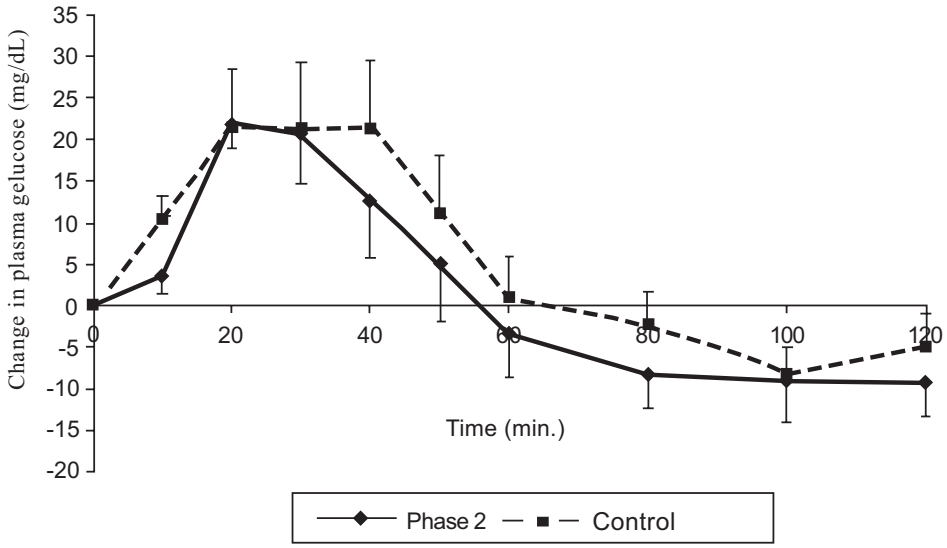

Fig. (2). Comparison of 0.75 g of Phase 2 vs. control on changes in plasma glucose after consumption of a full meal (mean ± standard error of mean). *Significance vs. control.

and an animal study discussed below there were no also no reports of side effects with Phase 2.

A recent animal toxicity study examining acute and sub-chronic dosing of Phase 2 at 0.5 to 5 g/kg body weight showed no toxicity, no adverse reactions, and no effect on renal and hepatic function [15]. A purified white bean extract was shown to decrease plasma glucose in both normal and type 2 diabetic rats [16]. The extract significantly decreased weight gain and food intake in normal animals after 3 weeks and significantly decreased food intake, water intake and body weight gain in the diabetic animals. The extract also normalized the elevated disaccharidases maltase and sucrase in the diabetic groups. The amylase inhibitor might be beneficial to prevent starch-induced hyperglycemia in diabetic subjects which is a contributing factor to the development of cardiovascular disease and diabetic complications [17].

The first paper describing a human study using Phase 2 was in 2000 [18]. The two arm study lasted 12 weeks with 40 obese volunteers produced significant reductions in weight (3.5 kg), body fat and BMI in the Phase 2 group vs. a placebo. Four hundred mg of Phase 2 at each meal were administered in combination with two other extracts so the results are not directly attributable to the Phase 2. An eight-week human placebo-controlled trial with obese subjects given 1500 mg of Phase 2 twice daily with lunch and dinner produced a moderate non-significant weight loss of 0.5 pounds/week compared to the placebo with no adverse effects [19]. The product also produced a reduction of 26 mg/dL in the subjects' plasma triglycerides. A more recent study by the same group included a program of dietary modification, exercise and behavioral intervention [20]. It was a shorter study of 4 weeks with 1000 mg twice per day and showed the highest weight loss of 8.7 pounds in the subjects who consumed the most carbohydrates vs. the placebo group who lost 1.7 pounds ($p < 0.05$) and also a significant reduction in waist size ($p < 0.01$) occurred in the active group. This result best demonstrates the efficacy of the product for inhibiting carbohydrate absorption for those subjects consuming a large amount of carbohydrates. The weight loss from Phase 2 is similar to that found for Orlistat (4 pounds) after 12 weeks [21]. A Japanese group investigated 750 mg of Phase 2 plus several other ingredients given twice per day for 8 weeks [22]. They found a significant reduction in weight, hip, waist, body fat, blood pressure and BMI and a significant increase in basal metabolism for the 10 subjects. The largest and most recent study was a randomized, double-blind, placebo-controlled study conducted on 60 pre-selected, slightly overweight volunteers, whose weight was stable for at least six months. They were divided into two groups, homogenous for age, gender, and body weight. The active product was a table of 445 mg of Phase 2 taken once per day for 30 days before a main meal rich in carbohydrates. There was an average weight loss of 3 kg in the active group and -0.4 kg in the placebo group ($p < 0.001$), a loss of fat mass in active group of 2.4 kg ($p < 0.001$) and a loss of 3 cm in waist circumference and a loss of 1.5 cm in hip circumference ($p < 0.001$). Also adipose tissue thickness taken by a skin echogram decreased 4.2 mm ($p < 0.001$) [23].

The Phase 2 bean extract inhibits starch absorption with normal subjects given either bread or a full meal as a source of carbohydrates. The bean extract also increases the rate of clearance of glucose. Therefore it might also prove to be efficacious for decreasing meal-induced hyperglycemia in diabetic subjects. In November 2006 the FDA in a letter stated that for Phase 2 it was allowed to use the following structure/function claims: "May assist in weight control when used in conjunction with a sensible diet and exercise program" and "May reduce the enzymatic digestion of dietary starches". Long-term weight loss studies (a year in duration) in both overweight and obese normal and diabetic subjects are indicated from these results of this study and published studies.

ACKNOWLEDGMENTS

The authors wish to gratefully acknowledge the volunteers in this study and Pennant Labs for providing the clinical facilities. This research was supported by Pharmachem Labs.

REFERENCES

[1] Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord 1998; 22: 39-47.

[2] Kilmer C, Roberts H, Hughes E, *et al*. Centers for Disease Control and Prevention (CDC). Surveillance of certain health behaviors and conditions among states and selected local areas-Behavioral Risk Factor Surveillance System (BRFSS), United States, 2006. MMWR Surveill Summ 2008; 57: 1-188.

[3] Troiano RP, Flegal KM, Kuczmarski RJ, Campbell SM, Johnson CL. Overweight prevalence and trends for children and adolescents. The National Health and Nutrition Examination Surveys, 1963 to 1991. Arch Pediatr Adolesc Med 1995; 149: 1085-91.

[4] Wilhelm C. Growing the market for anti-obesity drugs. Chem Market Rep 2000; 15: FR23-24.

[5] Hansen B. Emerging strategies for weight management. Post Grad Med Spec Rpt 2001; 3-9.

[6] Yanovski SZ, Yanovski JA. Obesity. N Engl J Med 2002; 346: 591-602.

[7] Hensrud D. Pharmacotherapy for Obesity. Med Clin North Am 2000; 84: 463-76.

[8] Macdonald I. Carbohydrates. In: Olson S, Shike M, Eds. Modern Nutrition in Health and Disease. Pennsylvania: Lea and Febiger 1994.

[9] Meyer BH, Muller FO, Grigoleit HG, Clur BK. Inhibition of starch absorption by tendamistate (an alpha-amylase inactivator). S Afr Med J 1983; 64: 284-85.

[10] Meyer BH, Muller FO, Grigoleit HG, Esterhuysen AJ, Clur BK. Effects of tendamistate on postprandial plasma glucose, free fatty acid and triglyceride levels. S Afr Med J 1984; 66: 224-25.

[11] Celleno L, Tolaini MV, D'Amore A, Perricone NV, Preuss HG. A dietary supplement containing standardized Phaseolus vulgaris extract influences body composition of overweight men and women. Int J Med Sci 2007; 4: 45-52.

[12] Crawford V, Scheckenbach R, Preuss HG. Effects of niacin-bound chromium on body compostion of overweight African-American Women. Diabetes Obes Metab 1999; 1(6): 331-7.

[13] Hollenbeck CB, Coulston AM, Quan R, *et al*. Effects of a commercial starch blocker preparation on carbohydrate digestion and absorption: *in vivo and in vitro* studies. Am J Clin Nutr 1983; 38: 498-503.

[14] Boivin M, Zinsmeister AR, Go VL, DiMagno EP. Effect of a purified amylase inhibitor on carbohydrate metabolism after a mixed meal in healthy humans. Mayo Clin Proc 1987; 62: 249-55.

[15] Kuszhuvelil B, Harikumar B, Jesil AM, Sabu MC, Kuttan R. A preliminary assessment of the acute and subchronic toxicity profile of Phase 12: An α-amylase inhibitor. Int J Toxicol 2005; 24: 1-8.

[16] Tormo MA, Gil-Exojo I, Romero de Tejada A, Campillo JE. White beam amylase inhibitor administered orally reduces glycaemia in type 2 diabetic rats. Br J Nutr 2006; 96: 539-44.

[17] Ceriello M. Postprandial hyperglycemia and diabetes complications. Diabetes 2005; 54: 1-7.

[18] Thom E. A randomized, double-blind, placebo-controlled trial of a new weight-reducing agent of natural origin. J Int Med Res 2000; 28: 229-233.

[19] Udani J, Hardy M, Madsen D. Blocking carbohydrate absorption and weight loss: A Clinical trial using Phase 2™ brand proprietary fractionated white bean extract. Altern Med Rev 2004; 9: 63-9.

[20] Udani J, Singh BB. Blocking carbohydrate absorption and weight loss: a clinical trial using a proprietary fractionated white bean extract. Altern Ther Health Med 2007; 13: 32-7.

[21] Chanoine JP, Hampl S, Jensen C, Boldrin M, Hauptman J. Effect of orlistate on weight and body consumption in obese adolescents: a randomized controlled trial. JAMA 2005; 293: 2873-83.

[22] Koike T, Koizumi Y, Tang L, Takahara K, Saitou Y. The anti-obesity effect and safety of taking Phaseolamin™ 1600 diet. J New Rem Clin 2005; 54: 150-59.

[23] Celleno L, Tolaini MV, D'Amore A, Perricone NV, Preuss HG. A Dietary supplement containing standardized Phaseolus vulgaris extract influences body composition of overweight men and women. Int J Med Sci 2007; 4: 45-52.

Received: November 20, 2008 Revised: January 1, 2009 Accepted: February 3, 2009