Title: COMPOSITION AND METHOD FOR REDUCING POST-PRANDIAL BLOOD GLUCOSE

Abstract: A nutritional intervention composition for reducing post-prandial blood glucose levels in humans, including between about 0.1 mg and about 500 mg of a proteinase inhibitor that is administered prior to the meal. The composition is effective for treating or ameliorating the effects of hyperglycemia and Type II diabetes. The composition also is effective in combating obesity. The proteinase inhibitor is preferably isolated from plant material, such as potatoes, soy, and beans. Potato proteinase inhibitor II and soybean Bowman-Birk inhibitor are included in the group of effective proteinase inhibitors.
COMPOSITION AND METHOD FOR REDUCING
POST-PRANDIAL BLOOD GLUCOSE

Background of the Invention

The invention relates to compositions for reducing post-prandial blood glucose in humans and, more specifically, to a proteinase inhibitor that delays gastric emptying and reduces post-prandial glycemia which may be beneficial in combating obesity and Type II diabetes.

Regulation of body weight depends on genetic as well as physiologic and lifestyle factors that are known to influence energy balance, such as diet, appetite control, metabolism, and physical activity (Aronne, L. J. (2001) J Clin Psychiatry 62, 13-22; Fernandez-Lopez, J. A., Remesal, X., Foz, M. & Alemany, M. (2002) Drugs 62, 915-44). Despite measures to combat obesity and an increased awareness of the associated co-morbidities, the condition has become an epidemic, with nearly 60% of Americans classified as overweight or obese (Visscher, T. L. & Seidell, J. C. (2001) Annu Rev Public Health 22, 355-75). Since the gene pool has not changed, researchers believe the culprit is primarily due to a combination of environmental and lifestyle influences. A focus on dietary fat as a leading cause of obesity the last several decades has been successful in reducing overall fat intake by Americans (from 40% to just over 30% of total calories, from the 1960's to present), but has done little to stave the rise in obesity rates (Lichtenstein, A. H., Kennedy, E., Barrier, P., Danford, D., Ernst, N. D., Grundy, S. M., Leveille, G. A., Van Horn, L., Williams, C. L. & Booth, S. L. (1998) Nutr Rev 56, S3-19; discussion S19-28).

Concerns over safety and efficacy of many anti-obesity products have limited their usefulness. Therefore, developments of natural, safe, and effective nutraceutical and/or medications that can help treat or prevent obesity are essential to mitigate this public health crisis.


The development of an efficient proprietary commercial process providing an extract from potatoes containing PI2 has increased the availability of this compound. It was hypothesized that administration of PI2 extract as a nutraceutical ingredient in a low dose, encapsulated form, prior to a meal, might reduce post-prandial glucose levels. This could have important implications for the use of PI2 as part of a diet to help maintain healthy blood sugar levels and reduce the propensity for weight gain.

Summary of the Invention
The invention consists of a method for reducing post-prandial glycogen levels in the blood of humans by the oral administration of a proteinase inhibitor or a combination of proteinase inhibitors. The proteinase inhibitor or combination is administered prior to the ingestion of a meal and reduces not only the initial rise in blood glucose following a meal (Δ Glucose or ΔG) but also the integrated area under the blood glucose curve (AUC) following a meal. The proteinase inhibitor(s) is effective for helping to maintain healthy blood sugar levels and for treating persons, such as those with Type II diabetes, which have adverse health effects due to hyperglycemia. Further, the proteinase inhibitor(s) is expected to reduce the propensity for weight gain by reducing the glycemia experienced by the body.

Proteinase inhibitors which exhibit the property include potato proteinase inhibitor II and soybean Bowman-Birk inhibitor, although other proteinase inhibitors with similar amino acid sequences and with similar proteinase inhibition properties may be used. While single proteinase inhibitors have been shown to be effective, combinations of two or more distinct proteinase inhibitors may also be used.

In a preferred embodiment, a proteinase inhibitor product isolated from potatoes is administered orally prior to a meal. The potato proteinase inhibitor extract contains between about 15% and about 25% by weight PI2 and also contains other proteins, including a protein similar but not identical to soybean Bowman-Birk inhibitor. The potato proteinase inhibitor extract is present in an amount between about 1 mg and about 1000 mg per dose, and preferably between about 5 mg and about 100 mg per dose, and most preferably between about 7.5 mg and about 30 mg per dose. The potato proteinase inhibitor is effective to reduce the blood glucose spike following a meal by between about 5% and about 30% and the AUC glucose by between about 5% and about 40%. Another preferred proteinase inhibitor is Bowman-Birk inhibitor, which is typically isolated from soybeans. The Bowman-Birk inhibitor is present in an amount between about 0.1 mg and about 5.0 mg per dose, and preferably between about 0.5 mg and about 2.0 mg per dose. The Bowman-Birk inhibitor is effective to reduce the blood glucose spike following a meal by between about 10% and about 25% and the AUC glucose by between about 5% and about 30%.

It is an object of the present invention to reduce post-prandial glycemia in humans by the oral administration of one or more proteinase inhibitors prior to a meal.
It is a further object of the invention to reduce the initial blood glucose spike following a meal by the oral administration of one or more proteinase inhibitors prior to the meal.

It is another object of the invention to reduce the total area under the curve blood glucose following a meal by the oral administration of one or more proteinase inhibitors prior to the meal.

It is yet a further object of the invention to treat hyperglycemia by the oral administration of one or more proteinase inhibitors.

It is yet another object of the invention to prevent obesity by the oral administration of one or more proteinase inhibitors.

Yet a further object of the invention is to combat Type II diabetes through the administration of one or more proteinase inhibitors either alone or in combination with other medications that are used in combating diabetes.

These and other objects of the invention will be understood by those skilled in the art upon a review of this specification, the associated figures and the appended claims.

**Brief Description of the Drawings**

Figs. 1a and 1b are HPLC chromatograms of the potato PI2 extract used in the experiments and an authentic PI2 standard, respectively.

Fig. 2 is a photograph of an SDS PAGE of the potato PI2 extract used in the experiments and an authentic PI2 standard.

Fig. 3 is a graph showing the effect of an increasing PI2 dose on post-prandial integrated area under the blood glucose curve (AUC) after a test meal.

Fig. 4 is a graph showing the effect of an increasing PI2 dose on the initial rise in blood glucose above the baseline (Δ Glucose) thirty minutes after a test meal.

Fig. 5 is a schematic diagram of the effects of chronic consumption of a high glycemic load.
Detailed Description of Preferred Embodiments

The composition for reducing post-prandial blood glucose levels in humans is based on a proteinase inhibitor that delays gastric emptying and reduces post-prandial glycemia which may be beneficial in combating obesity and in the therapeutic treatment of patients suffering from hyperglycemia. The proteinase inhibitor is believed to enhance the release of cholecystokinin (CCK), a peptide which regulates gastric emptying. The preferred proteinase inhibitors include potato proteinase inhibitor II and Bowman-Birk inhibitor. In particular, a proteinase inhibitor extracted from potatoes and available commercially from Kemin Consumer Care, L.C., Des Moines, Iowa, under the trademark Biofect™ was used in some of the examples. Biofect™ is also available in tablets formulated to contain 15 mg per dose and sold under the trademark Satise™.

The invention is based on the surprising result that proteinase inhibitors administered orally before a meal have the effect of reducing the initial post-prandial glucose spike and also reduce the total integrated area under the curve blood glucose over more than three hours after a meal. Also surprising is that the proteinase inhibitors are effective when administered in a dose in the less than ten milligram range.

EXAMPLE 1

METHODS

Subjects

Twenty-six men and 13 women, mean age 35 years (range 23-61 years) with a mean body mass index of 27 (range 23-32) participated in the study. Sample size was based on the study by Schwartz et al. who showed significant decreases in mean post-prandial glucose in six type II diabetic subjects following ingestion of a glucose/protein shake in the presence and absence of a high dose of PI2 (1.5 g). All subjects gave informed consent before the study began, and could withdraw at any time.

Study Design

Subjects were randomly allocated to receive placebo and two of the three following doses: 7.5, 15, or 30 mg PI2 extract. On each study day subjects arrived at 8.00 AM after a 10 hour fast. They were given breakfast and 500 ml of water to drink throughout the morning, but ate nothing further until the test meal. Height and weight of all subjects were recorded during their first visit. Three and a half hours after breakfast the first blood glucose measurement was made, and subjects were given the experimental
capsule and 500 ml of water. Thirty minutes later the test lunch was served. As soon as
each subject completed the meal, the timing for post-prandial glucose measurements
began. Subjects recorded any adverse reactions at fifteen minute intervals for 200
minutes after eating the meal.

Test Meal
On each test day subjects were fed a breakfast of granola, skim milk, and orange
juice that contained 330 kilocalories derived from 67 g of carbohydrate, 2.5 g of fat, and
12 g of protein. No other food was permitted until the test meal, which was consumed at
noon on the test day. The test meal was Chicken Teriyaki (Boston Market) and contained
no potato products. The nutritional content of the test meal is set out in Table 1. All
subjects consumed all meals in their entirety.

<table>
<thead>
<tr>
<th></th>
<th>Lunch Test Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>460</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>11</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>53</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>27</td>
</tr>
<tr>
<td>Number of participants taking meal challenge after placebo</td>
<td>39</td>
</tr>
<tr>
<td>Average AUC for placebo participants (SD)</td>
<td>2196.6 ± 1567.2</td>
</tr>
</tbody>
</table>

Glucose Measurements
Finger-prick capillary blood samples were taken 30 minutes before the test meal
(Baseline), and 30, 60, 90, and 120 minutes post-prandially. Glucose measurements were
made with a Dex glucometer, Model # 3952E (Bayer Pharmaceuticals), in accordance
with the manufacturer’s instructions.
Proteinase Inhibitor

PI2 extract was provided by Kemin Consumer Care, L.C. (Des Moines, Iowa), and was supplied in 00 gelatin capsules containing 7.5, 15, or 30 mg, respectively. A mixture of dextrose and whey protein was used to bring all capsules to a uniform weight and volume and also served as a placebo. The doses in the present study were chosen based on previous studies demonstrating efficacy at 30 mg in liquid form (Spiegel, T. A., Hubert, C. & Peikin, S. R. (1999) University of Medicine and Dentistry of New Jersey; Vasselli, J. R., Greenfield, D., Schwartz, L. & Heymsfield, S. B. (1999) Obesity Research Center, St. Luke's-Roosevelt Hospital Center, Columbia University), and 7.5 mg (Gary Green, University of Texas, San Antonio, 1996, 1997, unpublished data). The active material was produced from a single lot of potatoes (Russet Nuggets; Kemin lot 87289C, approximately 244.39 mg PI2 extract/kg).

Measurement of PI2

RP-HPLC: Formulation of the active doses was based on quantitation by high performance liquid chromatography (HPLC). Reversed-phase HPLC (RP-HPLC) analyses were performed on a Hewlett Packard Model 1100 equipped with a diode array detector using a Microsorb C-18, 5 μm particle size, 300 Angstrom pore size, 4.6 X 250mm (Varian Analytical Instruments, Walnut Creek, CA). The chromatographic conditions were as follows: Isocratic elution for five minutes of 80% of 0.1% TFA in H2O (20% of 1% TFA in acetonitrile). Gradient from 80-30% of 0.1% TFA in H2O (20-70 % of 1% TFA in acetonitrile) for 34 minutes. Gradient from 30-0% of 0.1% TFA in H2O (30-100 % of 1% TFA in acetonitrile) for 4 minutes. Flow rate was 1 ml/min for all gradients, and the column temperature was maintained at 30°C. Integration of the HPLC peak area provided the relative concentration of each sample (mg/g solids).

SDS-PAGE: To further characterize the PI2 extract, samples were analyzed by gel electrophoresis. SDS gels were prepared as 4% stacking, 15 % resolving with 1.5 M Tris, 0.5 M Tris, 10% SDS, 30% ammonium persulfate, TEMED, and 40%Acrlyl/Bis. Wells were loaded with pre-stained marker, PI2 standard, and PI2 extract. A current of 80 volts was applied for 1.5 hours. Gels were then stained with Coomasie blue staining. Pure PI2 standard was obtained by sequential RP-HPLC followed by gel filtration chromatography. Western blot using a rabbit polyclonal antibody developed by Kemin
Foods, L.C. against PI2 protein, was used to further determine the identity of the major protein in the potato PI2 extract used in the current study.

Calculations and Statistical Analysis

The difference between the 30-minute post-prandial and baseline blood glucose values was calculated for each subject visit (Δ glucose). The integrated area under the blood glucose-time curve (AUC) after each test meal was calculated using the pre-meal value as the baseline, and integrating the area from 0 to approximately 120 minutes after the meal. Repeated measure analysis of variance was used to test for significant differences between areas. The research design involved repeated measures, so the PROC MIXED function in SAS was used, as this allows a more general specification of the covariance matrix of the dependent variable, and allows random factors of both the model and error terms to be correlated (Hongsen, Z. (2001) Proceedings of the 12th Annual Conference of the Midwest SAS Users Group, 132-140). All subjects received placebo on one visit, but only two of the three possible active treatments during the other visits, so an incomplete block design was used to evaluate the relative effectiveness of the doses. The strategy described by Wolfinger (Wolfinger, R. D. (1993) Communications in Statistics, Simulation, and Computation 22, 1079-1106) was followed to select an appropriate variance-covariance structure for the ANOVA test. The Akaike’s Information Criterion was used to select the appropriate variance-covariance structure for the model. Chi-square analysis was used to evaluate data obtained as discrete variables with p < 0.05 considered to be significant.

Results

Doses of active PI2 extract were quantified by RP-HPLC. The integrated peak representing the PI2 extract co-eluted with a pure authentic PI2 standard, indicating that PI2 is contained in the extract and that it is the major protein (Figs. 1a and 1b). Results of gel electrophoresis further confirm the findings of the analysis by RP-HPLC and show that the PI2 in the extract is likely present as a monomer with a molecular weight of approximately ~12 kDa (Fig. 2). MALDI MS analysis of the purified PI2 protein demonstrated that this protein has a molecular weight of 12 kDa. Western blots of the separated proteins using a rabbit polyclonal antibody for PI2 protein demonstrated that the major protein band separated by SDS-PAGE is PI2. The actual amount of PI2 protein
present in a given extract could vary and ranges from 17 – 20%. The PI2 extract was also characterized for its trypsin and chymotrypsin inhibition activity using an in vitro assay demonstrating both trypsin and chymotrypsin inhibition. PI2 extract product contained a ratio of 0.9 - 1.7:1 units of trypsin: chymotrypsin inhibition activity, respectively.

The volunteers in the present study consumed 120 test meals. Forty placebo doses were administered, along with 27 of each of the 7.5 mg and 15 mg doses, and 26 of the 30 mg dose, respectively (one individual declined to provide blood samples and was included in determination of adverse events monitoring). Table 1 shows the nutrient value of the test meal and the mean glucose AUC following placebo. We first examined the effect of PI2 extract on AUC; the repeated measure ANOVA model used for this analysis showed a statistically significant effect of the experimental treatment (f = 3.3, p < 0.05) but no statistically significant difference between the experimental blocks. Subjects given a dose of 7.5 mg PI2 extract before the test meal experienced no significant reduction in post-prandial glucose compared to placebo. The AUC of subjects receiving both 15 and 30 mg PI2 extract prior to the test meal was significantly reduced compared to placebo, but there was no significant difference in post-prandial AUC between the two higher doses (Fig. 3). The decrease in AUC for 15 and 30 mg was 29.8% and 24.5% respectively, each compared to placebo. There was a significant reduction in Δ glucose at both the 15 mg and 30 mg dose levels compared to placebo, but there was no significant difference in Δ glucose between the two higher doses (Fig. 4). The decrease in Δ glucose for the 15 and 30 mg doses was 25% and 20% respectively, each compared to placebo.

Feeding 120 test meals resulted in 14 reports of an adverse reaction from subjects. These are summarized in Table 2.

<table>
<thead>
<tr>
<th>Table 2 - Subjects recording adverse effects after eating a test meal preceded by PI2 extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI2 Extract Dose (mg)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

Gastrointestinal symptoms included nausea, cramping and diarrhea. Differences in occurrence rates of adverse reactions between the treatments and the placebo were not
significant (p > 0.05, Chi square). Subjects experiencing symptoms rated them as mild, and frequently they were noted at only one of the recording times.

Discussion

The drastic rise in obesity rates over the past 10 years has been accompanied by diets resulting in chronic glycemia and hyper secretion of insulin (Grundy; Wolever, T. M. & Bolognesi, C. (1996) J Nutr 126, 2807-12). This, in turn, initiates a cascade of metabolic and physiologic events resulting in decreased lipolysis, increased de novo lipogenesis, and faster onset of hunger and subsequent food intake (Jenkins; Ludwig).

10 Rapid and drastic excursions in blood sugar may not only contribute to obesity but other chronic diseases, including diabetes and cardiovascular disease (as summarized in Fig. 5 and in Ludwig).

Accordingly, lowering the glycemic load experienced by the body by diet or other means may be an effective way to reduce the post-prandial glycemia that can lead to weight gain and obesity. Findings of the present study suggest that it is possible to lower the glycemic load experienced by the body by ingesting a supplement containing a low dose of P12 extract prior to a meal. Doses of either 15 mg or 30 mg taken 30 minutes before a test meal significantly reduced the subsequent rise in blood glucose (Figs. 3 and 4). A dose of 7.5 mg had no significant effect, indicating that under these test conditions the lowest effective dose lies between 7.5 and 15 mg. This study was limited to acute observations, and the effect of chronic oral administration of P12 extract on blood sugar levels remains to be studied. However, this study is unique because P12 has not previously been administered in solid form in an encapsulated supplement prior to the meal, and because a solid mixed meal was used for the first time. In addition, the dose used was substantially lower and less pure than that previously reported, and a larger cohort of subjects was studied. A dose of 1.5 g P12 (90 - 100% pure) by column chromatography (Clarence Ryan, Washington State University, Pullman, WA), administered in liquid form was used in two previous studies; in one study P12 was added to soup and fed 8 minutes before a test meal, and in the other it was incorporated in a test beverage (Hill et al.; Schwartz et al.). In neither case was it encapsulated. Other differences include the size and glycemic index of the test meals and potential variations in the P12 dose bioactivity. We found a mean reduction in post-prandial blood glucose AUC of 29.8% with a 15 mg dose of P12 extract and 24.5% decrease with a 30 mg active dose (Fig. 3). Schwartz et al. reported a comparable 24.5% reduction in AUC after
feeding 1.5 g PI2 with a liquid glucose and protein beverage administered to diabetics. While the dose of PI2 administered in that study was apparently 100-fold larger, we cannot be sure that it was of the same specific activity as used in our current study. Therefore, it is unclear whether larger doses of PI2 extract would evoke a greater response.

Inspection of the responses of individual subjects to placebo or active dose reveals that 9 subjects experienced no reduction in glycemia with either of the two dose levels of PI2 extract administered (non-responders). There was no significant effect of BMI, age, or fasting blood glucose on responsiveness. Among the 9 non-responders from the initial study, 8 were male and one was female, although this difference was not significant (p = 0.07, Chi-square).

The notion of lowering the glycemic load to reduce or maintain weight is supported by both animal and human studies. Normal rats fed isocaloric diets differing dramatically in terms of glycemic load, experience large differences in post-prandial glycemia and insulin response (Kabir, M., Rizkalla, S. W., Champ, M., Luo, J., Boillot, J., Bruzzo, F. & Slama, G. (1998) J Nutr 128, 35-43; Kabir, M., Rizkalla, S. W., Quignard-Boulange, A., Guerre-Millo, M., Boillot, J., Arduin, B., Luo, J. & Slama, G. (1998) J Nutr 128, 1878-83). Maintaining rats on these diets for weeks at a time results in drastic differences in glucose and lipid metabolism. The levels of fatty acid synthase and de novo lipogenesis, as well as adipocyte size, are higher in rats consuming a high vs. low glycemic load diet (Kabir et al. 35-43; Kabir et al. 1878-83). These data provide evidence at the cellular and metabolic level, that drastic elevations in blood sugar caused by exposing the body to a high glycemic load results in increased fat accumulation over a relatively short window of time. Consistent with this are results from long term research showing that adult rats fed isocaloric diets evoking chronic hyperglycemia gain a significant amount of weight while those fed a diet with moderate glycemia maintain their weight (Pawlak, D. B., Denyer, G. S. & Brand-Miller, J. C. (2000) Proc Nutr Soc Aust 24, 215).

Weight loss studies in humans suggest that reducing the glycemia experienced by the body is an effective means to reduce and maintain weight. Subjects consuming an isocaloric diets consisting of low glycemic index foods lose more weight or maintain their relative to those consuming high glycemic index foods (Slabber, M., Barnard, H. C., Kuyl, J. M., Dammhauser, A. & Schall, R. (1994) Am J Clin Nutr 60, 48-53; Wolever, T. M., Jenkins, D. J., Veksan, V., Jenkins, A. L., Wong, G. S. & Josse, R. G. (1992)
Diabetes Care 15, 562-4; Clapp, J. R. (1997) Arch Gynecol Obstet 261, 101-107). These findings suggest that manipulation of the glycemic load, in these cases by consuming low glycemic load diets, can effectively stimulate weight loss and/or prevent weight gain. Combined with the results from the present study, these support the hypothesis that PI2 extract can serve as an effective nutraceutical to lower the glycemia experienced by the body, and may help promote weight loss and reduce the propensity for weight gain.

PI2 extract is proposed to exert its effect on post-prandial glucose by enhancing the release of a well characterized peptide hormone, CCK, which is naturally secreted into the blood stream by enteroendocrine cells in response to a meal (Crawley, J. N. & Corwin, R. L. (1994) Peptides 15, 731-55). CCK acts on various target tissues throughout the body including the gastrointestinal tract, where it delays gastric emptying leading to feelings of fullness, and the brain leading to feelings of satiety. Although not measured in the present study, previous studies in the late 1980’s and 1990’s demonstrated that large doses of purified PI2 enhance the release of CCK (Peikin et al.; Schwartz et al.) delay gastric emptying time (Schwartz et al.), and decrease energy intake (Hill et al.) in humans. These were followed by studies using a lower dose of less pure PI2 extract which demonstrated reduced hunger and increased fullness ratings (Spiegel et al.; Vasselli et al.) (summarized in Table 3). These findings are consistent with the well established fact that PI’s are potent stimulators of CCK release in rats (Liddle).
Table 3 - Summary of PI2 Clinical Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Institution</th>
<th>Dose</th>
<th>Form</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiegel et al. 1999</td>
<td>Columbia University</td>
<td>30 mg PI2 extract</td>
<td>Liquid (pre-meal shake)</td>
<td>Significant decrease in hunger ratings; increase in fullness ratings; 2 kg weight loss</td>
</tr>
<tr>
<td>Vasselli et al. 1999</td>
<td>Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey</td>
<td>30 mg PI2 extract</td>
<td>Liquid (pre-meal shake)</td>
<td>Significant decrease in hunger ratings; increase in fullness ratings</td>
</tr>
<tr>
<td>Schwartz et al. 1994</td>
<td>University of Texas, San Antonio</td>
<td>1500 mg PI2</td>
<td>Liquid (shake)</td>
<td>Significant increase in plasma CCK; delayed gastric emptying; decreased blood sugar</td>
</tr>
<tr>
<td>Hill et al. 1990</td>
<td>University of Leeds, U.K</td>
<td>1500 mg PI2</td>
<td>Liquid (pre-meal soup)</td>
<td>Significant decrease in food consumption</td>
</tr>
<tr>
<td>Peikin et al. 1987</td>
<td>Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey</td>
<td>1000 mg PI2</td>
<td>Liquid (shake)</td>
<td>Significant increase in plasma CCK levels</td>
</tr>
<tr>
<td>Green, 1996 – 1997*</td>
<td>University of Texas, San Antonio</td>
<td>7.5 – 100 mg PI2</td>
<td>Liquid (shake)</td>
<td>Doses as low as 7.5 mg delayed gastric emptying and reduced blood sugar levels</td>
</tr>
</tbody>
</table>

*Unpublished data

PI2 is a pH, heat, and salt stable protein (Bryant et al.), allowing it to be effective when administered orally, and making it unique among plant PI’s. The extract used in the present study contains PI2 (Figs. 1 and 2), and is derived from white potatoes using a method generally as described in United States Patent Application No. 09/900,555, incorporated herein by this reference. Although normally present in potatoes as a dimer, the PI2 separated from our extract appears to be in the monomeric form. The pure PI2 possess trypsin and chymotrypsin inhibition activities of 1.4 and 3.6 units and the PI2 extract possess trypsin and chymotrypsin inhibition activities of 22 and 13 units. The added beneficial effect of reducing post-prandial glycemia makes PI2 extract a unique and promising nutraceutical.

Some studies involving the direct infusion of CCK have reported minor adverse side effects such as headache, nausea, and diarrhea (Crawley, J. N. & Corwin, R. L.)
(1982) Physiol Behav 29, 627-30). For this reason we questioned participants specifically
about these effects which may ultimately have prompted reporting of events that would
otherwise have gone unnoticed. Although there are a number of reports in the literature
demonstrating morphological changes in the pancreas as a result of long term exposure to
extremely high doses of natural and synthetic PI's in rodents, similar studies in pigs and
primates are not associated with such effects (Struthers, B. J., MacDonald, J. R.,
M., King, N. W., Sehgal, P. K., Nicolosi, R. J., Liener, I. E., Donatucci, D. & Tarcza, J.
Sobotka, T. J., Gaines, D. W., O'Donnell, M. W., Jr., Chi, R., Chirtel, S. J., Barton, C. N.,
Sager, A. O., Sobotka, T. J., O'Dell, R., Thorpe, C. W., Trotter, W. J., Bruce, V. R.,
Dallas, H. L., Poelma, P. L., Solomon, H. M., Bier, J. W., O'Donnell, M. W., Jr., Chi, R.
Chem Toxicol 40, 487-500). Such effects have yet to be observed in humans using PI's
from natural sources. Furthermore, previous studies using PI2 have not demonstrated any
side effects with doses many times that used in this study (Peikin et al.; Schwartz et al.).
Side effects noted by our subjects were mild and inconsistent, and caused no withdrawals
from the study. No increasing dose response was noted for any of these effects and the
rate of occurrence was not different between placebo and treatment. If persistent use of
PI2 extract were contemplated we may see additional mild side effects, although it is
equally possible that tolerance to undesired effects would develop over time.

In conclusion, we have demonstrated in the largest randomized controlled clinical
trial to date that a low dose of PI2 extract prior to a standardized meal reduces
significantly post-prandial glycemia in the majority of healthy subjects. Additional
studies will be required to ascertain long term effects of this supplement on blood
glucose, appetite and body weight. While a mechanism of action has been proposed, it
will be important to confirm this hypothesis in future studies addressing changes in serum
CCK, insulin, and the like. Such studies could be instrumental in applying PI2 to the
clinical problems of obesity and diabetes.
EXAMPLE 2

To better understand if the trypsin/chymotrypsin inhibiting activity of the PI2 protein was related to the glucose response to the potato proteinase inhibitor extract, a preparation that purified the PI2 fraction from the potato proteinase inhibitor extract (abbreviated pPI2) was tested along side a preparation of Bowman-Birk inhibitor after meal challenge. The Bowman-Birk inhibitor used was obtained from Sigma-Aldritch and had a stated purity of greater than 80%. Bowman-Birk inhibitor has similar enzyme inhibiting properties as pPI2. The meal challenge was conducted at a breakfast meal instead of a lunch meal, as in the prior study, and consisted of 390 kcal with 100 kcal from fat and 53 g carbohydrate and was provided to individuals who had been fasting for at least 10 hours. Each participant made two visits to the research center and underwent two meal challenges - one for the placebo and one for an active (15 mg pPI2 or 0.8 mg Bowman-Birk inhibitor) and these treatments were provided in a double-blinded format. The randomization scheme also prevented the participants or the study personnel from knowing at which visit the placebo was given until the code was broken.

In this study we found that among healthy volunteers, both pPI2 and Bowman-Birk inhibitor decreased the post-prandial glucose spike. The data is summarized in Table 4. For example ΔG was decreased by 25.5% compared to placebo among the 10 individuals taking pPI2. This difference (from a mean ΔG of 52.1 mg/dl + 15.9 sd for the placebo dose versus 38.8 + 34.6 sd for those taking the pPI2) was evaluated by one-tailed, paired student's t test and yielded p = 0.065. The Bowman-Birk inhibitor also inhibited the post-prandial glucose spike with a decrease in ΔG of 42.4%. The absolute decrease was from 47.9 mg/dl ± 22.6 sd for the placebo treatments compared to 27.6 mg/dl ± 21.6 sd for the Bowman-Birk inhibitor treatment. This was significant by student's t test with p = 0.04.

Neither pPI2 nor Bowman-Birk inhibitor showed statistically significant differences in AUC, although the trend was toward absolute decreases in this parameter of 17% and 11.5% for both pPI2 and Bowman-Birk inhibitor, respectively.

In the earlier study we did discover that some individuals seemed to be unresponsive to pPI2. Again, we found this to be the case with pPI2. Three individuals were identified as non-responders with pPI2 and two individuals were unresponsive to Bowman-Birk inhibitor. A non-responder was defined as an individual who did not have a lower absolute ΔG after treatment with the active test material than with the placebo.
Thus far we are supporting the hypothesis that the PI2 protein in the potato proteinase inhibitor extract and the trypsin/chymotrypsin inhibiting activity may be related to modulation of post-prandial glucose since the relative concentration of this activity in the pPI2 showed comparable glucose modulating activity as with the original potato proteinase inhibitor extract and the Bowman-Birk inhibitor which also contains an inhibitor or inhibitors of trypsin and chymotrypsin showed glucose modulating activity.

Table 4 - Comparison of purified PI2 and Bowman-Birk Inhibitor on AG and AUC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Purified PI2 Product</th>
<th>Bowman-Birk Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Active</td>
</tr>
<tr>
<td>Mean ΔG ± SD</td>
<td>Study 1</td>
<td>52.1 ±15.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p = 0.065</td>
</tr>
<tr>
<td>Mean ΔG ± SD</td>
<td>Study 2</td>
<td>55.1±21.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p = 0.058</td>
</tr>
<tr>
<td>Mean AUC ± SD</td>
<td>Study 1</td>
<td>2279±1187</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p = 0.15</td>
</tr>
<tr>
<td>Mean AUC ± SD</td>
<td>Study 2</td>
<td>2496.9±1878</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*p = 0.229</td>
</tr>
</tbody>
</table>

* one tailed paired t test (versus placebo)

Although the invention has been described with respect to a preferred embodiment thereof, it is to be also understood that it is not to be so limited since changes and modifications can be made therein which are within the full intended scope of this invention as defined by the appended claims.
We claim:

1. A method for reducing post-prandial blood glucose levels in humans, comprising the step of administering prior to the meal a nutritional intervention composition comprising between about 0.1 mg and about 500 mg of a proteinase inhibitor.

2. A method as defined in Claim 1 wherein said composition is taken 1 to 60 minutes before the meal.

3. A method as defined in Claim 1, wherein the proteinase inhibitor is isolated from plant material.

4. A method as defined in Claim 3, wherein the proteinase inhibitor is derived from the group of plants consisting of potatoes, soy and beans.

5. A method as defined in Claim 1, wherein the proteinase inhibitor comprises proteinase inhibitor II isolated from potatoes.

6. A method as defined in Claim 1, wherein the proteinase inhibitor comprises Bowman-Birk inhibitor isolated from potatoes or soybeans.

7. A method as defined in Claim 1, wherein the proteinase inhibitor reduces the initial blood glucose spike.

8. A method as defined in Claim 1, wherein the proteinase inhibitor reduces the area under the curve blood glucose.

9. A method as defined in Claim 8, wherein the area under the curve blood glucose is measured over a time period of up to 4 hours after the meal.

10. A method as defined in Claim 1, wherein the proteinase inhibitor reduces the initial blood glucose spike by between about 5 percent and about 30 percent.
11. A method as defined in Claim 1, wherein the proteinase inhibitor reduces the area under the curve blood glucose over a period of about 3.5 hours following the meal by between about 5 percent and about 40 percent.

12. A method as defined in Claim 1, wherein the proteinase inhibitor is potato proteinase inhibitor II administered orally in a dose of between about 0.5 and about 20 mg.

13. A method as defined in Claim 1, wherein the proteinase inhibitor is Bowman-Birk inhibitor administered orally in a dose of between about 0.1 and about 2.0 mg.

14. A method of combating obesity, comprising the step of administering orally prior to a meal a proteinase inhibitor other than potato proteinase inhibitor II.

15. A method as defined in claim 15, wherein the proteinase inhibitor is Bowman-Birk inhibitor in a dose between about 0.1 mg and about 2.0 mg.

16. A method of treating hyperglycemia, comprising the step of administering prior to a meal a proteinase inhibitor.

17. A method as defined in Claim 16 wherein the proteinase inhibitor is taken 1 to 60 minutes before the meal.

18. A method as defined in Claim 16, wherein the proteinase inhibitor is isolated from plant material.

19. A method as defined in Claim 18, wherein the proteinase inhibitor is derived from the group of plants consisting of potatoes, soy and beans.

20. A method as defined in Claim 16, wherein the proteinase inhibitor is proteinase inhibitor II isolated from potatoes.

21. A method as defined in Claim 16, wherein the proteinase inhibitor is Bowman-Birk inhibitor isolated from soybeans.
22. A nutritional intervention composition for reducing post-prandial blood glucose in humans, comprising between about 0.1 mg and about 500 mg of a proteinase inhibitor administered prior to the meal.

23. A nutritional intervention composition as defined in Claim 22 wherein said composition is taken 1 to 30 minutes before the meal.

24. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor is isolated from plant material.

25. A nutritional intervention composition as defined in Claim 24, wherein the proteinase inhibitor is derived from the group of plants consisting of potatoes, soy and beans.

26. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor is proteinase inhibitor II isolated from potatoes.

27. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor is Bowman-Birk inhibitor isolated from soybeans.

28. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor reduces the initial blood glucose spike.

29. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor reduces the area under the curve blood glucose.

30. A nutritional intervention composition as defined in Claim 29, wherein the area under the curve blood glucose is measured over a time period of up to 4 hours after the meal.

31. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor reduces the initial blood glucose spike by between about 5 percent and about 30 percent.
32. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor reduces the area under the curve blood glucose over a period of about 3.5 hours following the meal by between about 5 percent and about 40 percent.

33. A nutritional intervention composition as defined in Claim 22, wherein the proteinase inhibitor is potato proteinase inhibitor II administered orally in a dose of between about 0.5 and about 500 mg.

34. A method as defined in Claim 22, wherein the proteinase inhibitor is Bowman-Birk inhibitor administered orally in a dose of between about 0.1 and about 2.0 mg.
FIG. 2

MW/Markers
PI2 Standard
Potato PI2 Extract

21 kDa
14 kDa
PI2 protein
FIG. 5

Chronic consumption of high glycemic load leading to large excursions in blood sugar.

Appetite control/Satiety

Recurrent post-prandial hyperglycemia

Blood sugar regulation PI2

Hypersecretion of insulin

Rapid drop in serum glucose below fasting

Lack of satiation, stimulation of appetite

Increased feeding, increased caloric intake

Increased adipose tissue (increased fat storage)

Pancreatic beta cell "burn out"

Decreased insulin sensitivity, increased insulin resistance

Sedentary lifestyle

Altered circulating lipids: \( \uparrow TG, \downarrow HDL, \uparrow LDL \)

Diabetes

Cardiovascular disease

Obesity