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A Novel Method of Lowering the Glycemic Index of White Bread Using a White Bean Extract¹

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42 **Abstract**

43

44 Phase2® is a dietary supplement derived from the common white kidney bean (*Phaseolus*
45 *vulgaris*). The Phase2® product has been shown to inhibit the complex carbohydrate
46 digesting enzyme alpha-amylase. This process may result in the lowering of the effective
47 Glycemic Index (GI) of certain foods. The objective of this study was to determine whether
48 the addition of Phase2® would lower the GI of a commercially available high glycemic food
49 (white bread). An open-label 6-arm crossover study was conducted with 13 randomized
50 subjects. Standardized GI testing was performed on Wonder Brand white bread with and
51 without the addition of Phase2® capsules and powder each in dosages of 1500mg, 2000mg,
52 and 3000mg. Reductions in the GI of Wonder Brand white bread were seen at all dosages and
53 formulations except the 1500mg capsule dose. These reductions reached statistical
54 significance with 3000mg of Phase2® in powder form (-20.23 or 34.11%, $p=0.0228$). The GI
55 of Wonder Brand white bread was significantly reduced by the addition of 3000mg of the
56 Phase2® brand white bean extract in powder form with other dosages and formulations
57 showing clinically meaningful reductions without reaching statistical significance. In the
58 appropriate dose and formulation, the Phase2® white bean extract appears to be a novel and
59 potentially effective method for reducing the GI of existing foods without modifying their
60 ingredient profile. Given the potential health benefits of a low GI diet, further study of
61 Phase2® with other high GI foods should be considered.

62 **Key Words:** Glycemic Index; White Bean Extract; Alpha-Amylase; *Phaseolus vulgaris*

63 Introduction

64

65 Phase2® is a dietary supplement derived from the common white kidney bean (*Phaseolus*
66 *vulgaris*) and has been shown to inhibit the digestive enzyme alpha-amylase (1). This
67 proprietary extract alpha-amylase is secreted in saliva and by the pancreas and is responsible
68 for breaking down complex carbohydrates for absorption. Alpha-amylase activity determines
69 the rate of breakdown of complex carbohydrates in glucose and since the GI is a function of
70 the rate of absorption of glucose in the gut, inhibition of this enzyme may result in a lowering
71 of the GI. The GI is defined as “the incremental area under the blood glucose response curve
72 of a 50g carbohydrate portion of a test food expressed as a percent of the response to the
73 same amount of carbohydrate from a standard food taken by the same subject”(2). The GI
74 standardizes the glycemic response and accounts for between subject variability by averaging
75 the results of testing at least 10 persons. Therefore the GI is more reliable than standard
76 glucose response testing as it can predict the response in any individual. The GI has also been
77 shown to be reliable in mixed meal testing environments demonstrating that the inclusion of
78 fat or protein in a meal does not preclude the measurement of the GI of the carbohydrate
79 content of that meal (2-5).

80

81 The benefits of low GI diets have been well studied. Epidemiologic data have demonstrated
82 that low GI diets decrease the risk of progressing to Diabetes (6;7) and decrease the risk of
83 Coronary Heart Disease (8). Controlled clinical trials have shown that low GI diets can lower
84 cholesterol (9), reduce HbA1c and improve insulin sensitivity in diabetics (10), delay the
85 return of hunger (11), and may decrease weight and BMI in adolescents (12;13).

86

87 Foods may have inherent GI values, and there are several methods for effectively lowering
88 the GI of a particular food. The addition of resistant starches or fiber products (psyllium,
89 blackgram fiber, barley, oat beta-glucan) to the food may lower the GI and the concomitant
90 use of prescription alpha-glucosidase inhibitors may also lower the effective GI of a food (14-
91 20). Each of these methods has its limitations. Changing the recipe of a commercial food
92 product is difficult and may alter the taste or texture of the food, and the use of prescription
93 products for such a purpose can only be accomplished under the supervision of a physician.
94 Given the overall benefit of a low glycemic diet coupled with the reluctance of most people
95 to change their diet, the objective of this study was to determine whether the addition of the
96 Phase2® product could lower the effective GI of a common high glycemic food product. We
97 hypothesized that addition of the Phase2® would affect the GI of the high glycemic food
98 product, white bread.

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105 Materials and Methods

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107 The Phase2® product is a water extract of the white kidney bean (*Phaseolus vulgaris*). Non-
108 GMO whole white kidney beans are ground and then extracted for 4 hours. The liquid is
109 filtered and concentrated under vacuum. The extract is filtered again, and then
110 pasteurized before being spray dried. The product was dosed as powder (mixed in butter)
111 or in capsule form. Further characterization of the extraction process of this proprietary
112 product is considered confidential intellectual property of the manufacturer (Pharmachem
113 Laboratories, Kearny, NJ). Wonder brand white bread (Interstate Bakeries, Kansas City,
114 MO) was utilized in this study.

115

116 **Subjects and Study Design**

117

118 Sixteen healthy volunteer subjects between the ages of 24 and 44 and a BMI between 18 and
119 25 (kg/m²) were screened at the Medicus Research facility in Northridge, CA. Thirteen
120 subjects (38% men and 62% women) were eligible and entered into this 6-arm open-label
121 controlled crossover trial.

122

123 IRB approval was obtained from the Copernicus Group IRB (Cary, NC) prior to any study
124 related procedures. Good Clinical Practices (GCP) were followed throughout the study. All
125 subjects gave informed consent according to GCP guidelines prior to initiating any study
126 procedures. Screening fasting glucose levels were ≤ 100 mg/dL. Subjects with any active
127 eating disorders, gastrointestinal illness or history of gastrointestinal surgery, Diabetes or
128 other Endocrinologic disorders were excluded. Subjects underwent a history and physical

129 examination by a board certified physician and all women of child bearing potential were
130 given a urine pregnancy test. Patients were required to use appropriate methods of
131 contraception during the active study. In order to standardize the glycemic response on the
132 each study test day, subjects were required to consume only a diet of standardized prepared
133 low-fiber frozen foods (21) containing a minimum of 100g of carbohydrates. The purpose of
134 the low-fiber diet is to minimize the potential residual blood sugar effects of slowly digested
135 and absorbed complex carbohydrates which may be present up to 1 day after consuming
136 them. Subjects were also required to fast for 10 hours prior to their study visit.

137

138 GI testing was performed according to the FAO/World Health Organization (WHO)
139 standard methodology using glucose as the standard food (22). During the standardization
140 phase of the study, subjects reported to the study center 3 times during which they
141 received 50g net carbohydrates in the form of glucose. At each visit subjects had their
142 capillary blood glucose measured 9 times over 2 hours. Capillary blood collections and
143 multiple GI measurements were performed during the two hour interval as the
144 recommended technique to reduce the measurement errors(23).

145

146 During the active phase of the study, subjects reported to the study center 7 times during
147 which they received 50g net carbohydrates in the form of Wonder brand white bread with
148 butter either by itself or with one of the test products. The serving of bread used to obtain 50g
149 of net carbohydrates was determined from the package label information. Butter was
150 obtained in standardized plastic “pats” and each serving was 5g, 36kcal and contained 0
151 carbohydrates. The amount of butter was standardized for each test dose so that each subject
152 received the same amount of butter at each visit regardless of how much test product they

153 received. It has been documented that fat does not affect the GI of foods (24). The test
154 product was given at dosages of 1500mg, 2000mg, and 3000mg in capsule form and
155 1500mg, 2000mg, and 3000mg in powder form. The powder form of the test product was
156 mixed into the butter which was spread on the bread. During each visit subjects again had
157 their capillary blood glucose measured 9 times over 2 hours.

158

159 The white bread was consumed within 5 minutes after which subjects remained in a semi-
160 recumbent position throughout the duration of the study visit (unless they need to use the
161 restroom) to variability in oro-cecal transit time (25). Subjects were only allowed to drink ice
162 water only until each testing session was complete.

163

164 **Analyses**

165

166 Capillary blood was analyzed for blood glucose using the Bayer brand Ascensia Contour
167 glucometer (Bayer Healthcare, Mishawaka, IN). Blood was drawn twice at baseline and
168 then at times 0 (start of meal), 15 min, 30 min, 45 min, 60 min, 90 min, and 120 min.

169

170 **Questionnaires**

171

172 10 point Likert scales for Diarrhea, Flatulence, Abdominal Bloating, Abdominal
173 Cramping, Nausea, Boborygmi (bowel sounds), and Soft Stools were filled out hourly
174 and at the end of each test period.

175

176 **Statistical Analysis**

177

178 Statistical analysis was performed by one-way ANOVA of all seven treatment groups
179 using unadjusted multiple comparisons (t tests) to the white bread control.

180

181 **GI Calculation**

182

183 The GI was calculated according to the FAO/WHO standard (22), which utilizes capillary
184 blood glucose measurements. This method obtains the mean representative response to the
185 Wonder brand white bread by averaging the 3 responses to the standard food (glucose). The
186 Incremental Area Under the Curve (iAUC) is calculated geometrically by applying the
187 trapezoid rule. The iAUC equals the sum of the area of triangle A, trapezoid B, trapezoid C,
188 triangle D, triangle E, and trapezoid F and the GI is calculated by the following formula:

189

190 $GI = 100 \times (iAUC \text{ of test food}) / (\text{mean } iAUC \text{ of standard food})$

191

192 As per this protocol, when a blood glucose value fell below the baseline, only the area above
193 the fasting level was included. Statistical analysis was performed using a one-way ANOVA of
194 all treatment groups with unadjusted multiple comparisons (t-tests) to the white bread control.

195

196 **Results**

197

198 Of the 15 subjects who began the study, 2 subjects were withdrawn because their blood
199 glucose went above 200mg/dL during the glucose tolerance testing during the
200 standardization phase.

201

202 **Impact on GI**

203

204 There was a dose dependent response observed in the reduction of the GI of the Wonder
205 brand white bread with both the powder and capsule formulations of Phase2®. These
206 reductions reached statistical significance with 3000mg of Phase2® in the powder form.
207 The reductions were trending statistical significance for the 2000mg Capsule dose
208 (P=0.076). The remainder of the dose formulations showed non-statistical reductions
209 (except for the 1500mg Capsule dose).

210

211 TABLE 1 – The GI of white bread with different doses and formulations of Phase2® and
 212 % change from white bread control.

213

Formulation	GI	% Change from white bread control	P Value
White Bread (Control)	59.3±24.7		
1500mg Capsule	61.9±2.6	-4.39	0.7659
2000mg Capsule	45.1±14.2	24.01	0.0762
3000mg Capsule	46.8±12.5	21.05	0.1064
1500mg Powder	43.6±15.7	26.41	0.1101
2000mg Powder	45.2±14.1	23.76	0.1561
3000mg Powder	39.1±20.2	34.11	0.0228

214 Values are presented as mean ± SD, n=13. All the doses and formulations were consumed
 215 with white bread. P value represents the mean changes between control and treatment.

216

217 Safety

218

219 All of the dosages and formulations appeared to be well tolerated as no differences were
 220 seen on any of the questionnaires (Diarrhea, Flatulence, Bloating, Cramping, Nausea,
 221 Abdominal Pain, Bowel Sounds, and Stool Softness). No adverse events were observed
 222 or reported during the study.

223

224 **Discussion**

225

226 The data from this study demonstrates clinically meaningful decreases in the GI of
227 Wonder brand white bread with Phase2® in both capsule and powder form. These
228 decreases were statistically significant in the powder form at 3000mg and showed non-
229 significant but clinically meaningful reductions for all dosages and formulations except
230 for the 1500mg capsule dosage, which showed no change at all. The data suggests a
231 possible dose dependency. There may be several reasons why GI lowering effects were
232 not significant for the other formulations and dosages. First, the mechanism of action of
233 the Phase2® test product may require complete saturation of gut alpha-amylase. This
234 may not occur at lower dosages and may occur more slowly with a capsule than a powder
235 due to capsule dissolution time. Second, this study had a relatively small sample size
236 which was based solely upon the FAO/WHO guidelines for GI testing. This data may
237 provide a baseline to perform effect size calculations for future studies.

238

239 Several methodologies were employed to diminish the inter and intra-subject variability
240 inherent in GI testing including the use of glucose rather than white bread during the
241 standardization phase, standardization of meals the day prior to each visit, restricting the
242 inclusion criteria to certain age and BMI criterion, and the semi-recumbent position
243 during the study to standardize oro-cecal transit time. There is inherent person-to-person
244 variability in these results which is to be expected and GI calculation as an average does
245 take these factors into account (26).

246

247 These results certainly merit further study. Future study designs should utilize the glucose
248 standardization and should incorporate a larger sample pool to further decrease

249 variability. In addition, it would be worthwhile to test the 2000mg and 3000mg powder
250 and capsule formulations on other high GI foods (such as pasta or rice) to identify
251 whether or not their GI can be lowered as well.

252

253

254 **Conclusions**

255

256 The GI of Wonder Brand white bread was significantly decreased by the addition of
257 3000mg of the Phase2® brand white bean extract in powder form. All other
258 dosages/formulations (except the 1500mg capsule form) showed clinically meaningful
259 reductions in the without reaching GI statistical significance. With the appropriate dose
260 and formulation, the Phase2® white bean extract appears to be a novel and potentially
261 effective method for reducing the GI of existing foods without modifying their ingredient
262 profile. Given the potential health benefits of a low GI diet, further study of Phase2® at
263 adequate dosage / formulation combinations with other high GI foods should be
264 considered.

265

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267

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273 Reference List

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275 (1) Meyer BH, Muller FO, Kruger JB, Grigoleit HG. Inhibition of starch absorption
276 by alpha-amylase inactivator given with food. *Lancet* 1983; 1(8330):934.

277 (2) Wolever TM, Nuttall FQ, Lee R, Wong GS, Josse RG, Csima A et al. Prediction
278 of the relative blood glucose response of mixed meals using the white bread
279 glycemic index. *Diabetes Care* 1985; 8(5):418-428.

280 (3) Collier GR, Wolever TM, Wong GS, Josse RG. Prediction of glycemic response
281 to mixed meals in noninsulin-dependent diabetic subjects. *Am J Clin Nutr* 1986;
282 44(3):349-352.

283 (4) Wolever TM, Bolognesi C. Prediction of glucose and insulin responses of normal
284 subjects after consuming mixed meals varying in energy, protein, fat,
285 carbohydrate and glycemic index. *J Nutr* 1996; 126(11):2807-2812.

286 (5) Wolever TM. Glycemic index and mixed meals. *Am J Clin Nutr* 1990;
287 51(6):1113-1114.

288 (6) Salmeron J, Ascherio A, Rimm EB, Colditz GA, Spiegelman D, Jenkins DJ et al.
289 Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* 1997;
290 20(4):545-550.

291 (7) Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC.
292 Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus
293 in women. *JAMA* 1997; 277(6):472-477.

294 (8) Liu S, Manson JE, Buring JE, Stampfer MJ, Willett WC, Ridker PM. Relationship
295 between a diet with high glycemic load and plasma concentrations of high-
296 sensitivity C-Reactive Protein in middle aged women. *Am J Clin Nutr* 2002;
297 75(3):492-498.

298 (9) Jenkins DJ, Wolever TM, Vidgen E, Kendall CW, Ransom TP, Mehling CC et al.
299 Effect of psyllium in hypercholesterolemia at two monounsaturated fatty acid
300 intakes. *Am J Clin Nutr* 1997; 65(5):1524-1533.

301 (10) Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the
302 management of diabetes: a meta-analysis of randomized controlled trials.
303 *Diabetes Care* 2003; 26(8):2261-2267.

304 (11) Roberts SB. Glycemic index and satiety. *Nutr Clin Care* 2003; 6(1):20-26.

305 (12) Spieth LE, Harnish JD, Lenders CM, Raezer LB, Pereira MA, Hangen SJ et al. A
306 low-glycemic index diet in the treatment of pediatric obesity. *Arch Pediatr*
307 *Adolesc Med* 2000; 154(9):947-951.

308 (13) Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High
309 glycemic index foods, overeating, and obesity. *Pediatrics* 1999; 103(3):E26.

- 310 (14) Boby RG, Leelamma S. Blackgram fiber (*Phaseolus mungo*): mechanism of
311 hypoglycemic action. *Plant Foods Hum Nutr* 2003; 58(1):7-13.
- 312 (15) Anderson JW, Randles KM, Kendall CW, Jenkins DJ. Carbohydrate and fiber
313 recommendations for individuals with diabetes: a quantitative assessment and
314 meta-analysis of the evidence. *J Am Coll Nutr* 2004; 23(1):5-17.
- 315 (16) Wolever TM, Vuksan V, Eshuis H, Spadafora P, Peterson RD, Chao ES et al.
316 Effect of method of administration of psyllium on glycemic response and
317 carbohydrate digestibility. *J Am Coll Nutr* 1991; 10(4):364-371.
- 318 (17) Frati Munari AC, Benitez PW, Raul Ariza AC, Casarrubias M. Lowering
319 glycemic index of food by acarbose and *Plantago psyllium* mucilage. *Arch Med*
320 *Res* 1998; 29(2):137-141.
- 321 (18) Jenkins DJ, Vuksan V, Kendall CW, Wursch P, Jeffcoat R, Waring S et al.
322 Physiological effects of resistant starches on fecal bulk, short chain fatty acids,
323 blood lipids and glycemic index. *J Am Coll Nutr* 1998; 17(6):609-616.
- 324 (19) Liljeberg HG, Granfeldt YE, Bjorck IM. Products based on a high fiber barley
325 genotype, but not on common barley or oats, lower postprandial glucose and
326 insulin responses in healthy humans. *J Nutr* 1996; 126(2):458-466.
- 327 (20) Jenkins AL, Jenkins DJ, Zdravkovic U, Wursch P, Vuksan V. Depression of the
328 glycemic index by high levels of beta-glucan fiber in two functional foods tested
329 in type 2 diabetes. *Eur J Clin Nutr* 2002; 56(7):622-628.
- 330 (21) Hallfrisch J, Behall KM. Breath hydrogen and methane responses of men and
331 women to breads made with white flour or whole wheat flours of different particle
332 sizes. *J Am Coll Nutr* 1999; 18(4):296-302.
- 333 (22) Anonymous. The role of the glycemic index in food choice. Carbohydrates in
334 human nutrition. (FAO Food and Nutrition Paper - 66). 1997.
- 335 (23) Hatonen KA, Simila ME, Virtamo JR, Eriksson JG, Hannila ML, Sinkko HK et
336 al. Methodologic considerations in the measurement of glycemic index: glycemic
337 response to rye bread, oatmeal porridge, and mashed potato. *Am J Clin Nutr* 2006;
338 84(5):1055-1061.
- 339 (24) MacIntosh CG, Holt SH, Brand-Miller JC. The degree of fat saturation does not
340 alter glycemic, insulinemic or satiety responses to a starchy staple in healthy men.
341 *J Nutr* 2003; 133(8):2577-2580.
- 342 (25) Staniforth DH, Rose D. Statistical analysis of the lactulose/breath hydrogen test in
343 the measurement of oro-caecal transit: its variability and predictive value in
344 assessing drug action. *Gut* 1989; 30(2):171-175.
- 345 (26) Wolever TM, Csima A, Jenkins DJ, Wong GS, Josse RG. The glycemic index:
346 variation between subjects and predictive difference. *J Am Coll Nutr* 1989;
347 8(3):235-247.

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