Study of preB Dietary Supplement: A Randomized, Placebo Controlled, Double Blind Trial

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Abstract

Background: While many whole-food based products have advanced claims about moderating Metabolic Syndrome, few have been tested in controlled trials. **preB** is a Japanese dietary supplement in wide use there and throughout Asia and South America. While it has been extensively tested outside the US, it has not been tested for safety for use in the US. **Methods:** This study was approved by WIRB #20081790, Study #1102926. Subjects (n = 75) were recruited from local clinics and from public advertising. They were randomly assigned to Test and Control groups. Blood, urine, and other measurements were taken of all subjects before and after the administration of the test sample. Using a double-blind procedure for assignment, subjects took either placebo, or the preB (36 mL/day) for six weeks. **Results:** There were no adverse reactions, illness or complications detected in either the control of the preB test group. In addition, several statistically significant results in metabolic variables were noted in the preB group. **Conclusions:** preB is safe to use in the US for the dosage and terms used in the study.

Background

preB has been on the market in Asia and South America for over 7 years. It is used annually by hundreds of thousands of people (sponsor supplied data). To date no adverse reactions have been reported (sponsor supplied data). preB is a thick liquid made of 56 herbs, nuts, seeds, grains, vegetables, and fruits fermented and aged together for a minimum of six months.

preB has previously been tested and shown to be low glycemic (*Final Report*, *Glycemic Index Determination, preB Liquid*, GI 42.3, Protocol GIL-8042, Glycemic Index

Laboratories, Nov. 15, 2008), and high in antioxidants (*Report of Analysis, preB Liquid*, ORAC 79.82 U/g, American Analytical Chemistry Laboratories, Ltd., May 18, 2009). preB also contains other useful ingredients such as lutein, which is a yellow pigment contained in green and yellow vegetables and is known to exert anti-oxidative effects, plus it contains significant carotenoids (pigment group) like β-carotene and lycopene.

A number of studies have been done on the product in Japan, China, and Brazil, that show the product has potential in moderating Metabolic Syndrome along several parameters, including a previous Japanese study (*Safety Study of preB*, Kitasato Institute Medical Center Hospital, Kitasato University, Tokyo, Japan, September 17, 2008, unpublished) which looked specifically at the safety of preB in healthy subjects (n=40) and concluded that it was safe. However, preB has not been studied for its safety in the US.

The purpose of this research was to determine the safety of preB for use in healthy subjects in the US.

Methods

This study was approved by WIRB #20081790, Study #1102926. Subjects were recruited (N=75) from local clinics and public advertising. Volunteers were initially screened by telephone to determine if they had any illness, or metabolic disorders, were on any conflicting medications, or were being treated for depression. Those passing this screen were then interviewed in person by a trained and experienced interviewer to examine more closely their answers to questions concerning their present health and pregnancy status. After signing a disclosure document, they had a number of measurements taken (height, weight, waist circumference, body fat % using the bio-impedance device by Tanita, blood pressure, and resting heart rate). Urine and blood samples were also taken. A total of 43 study variables were tested (see Table 1). Subjects were then assigned to either a Test or Control group, using a double blind procedure, and given either a packet of placebo (36 mL/day of a solution of hydroxypropyl methylcellulose (HPMC), at 0.25 g/100 mL water) in individual serve packets, or preB. Identical looking packets were given to both groups. Subjects were

given written instruction on when to take the samples (any time they wanted during the day), the manner of taking (with or without food or drinks), and not to change their daily eating or exercise habits. Subjects were told to take the samples daily for six weeks. During that period, an interviewer, blinded as to which group the subjects were assigned, contacted each subject weekly, by phone or email, and reminded them to take the samples and to not change their daily exercise or dietary habits. At the end of six weeks the subjects returned to the clinic and had their measurements, blood, and urine samples taken again. Subjects were paid a small amount for their time and travel at the conclusion of the study ($120). Post interviews of the subjects were conducted to determine their self-reported compliance, and reported tolerability of the test samples taken.

Data Analysis and Statistical Power

Data were analyzed using standard inferential statistical approaches. For continuous variables, independent samples t-tests were used when parametric assumptions were met and non-parametric Wilcoxon Rank Sum were used when these assumptions were not met. Dichotomous variables were analyzed with χ^2 (chi-square) analyses or Fisher's exact tests. Due to the exploratory nature of this study, all analyses were conducted using a 2-tailed approach with $\alpha = 0.05$. A two group t-test with a 0.050 two-sided significance level will have 80% power to detect a moderate effect size of 0.661 when the sample sizes in the two groups are 42 and 33, respectively (total sample size of 75).

Results

A total of 90 healthy subjects began the study, and 75 completed it. Two were dropped due to incomplete data sets. A total of 13 subjects dropped out the the study, from both the Control group (n = 2) and Test group (n = 11). There were no significant statistical differences between those who finished the study (n = 75) and those who dropped out with the exception of two items: (1) 4.5% of the Control subjects dropped out versus 25% of the Test subjects (p = 0.016). (2) The mean resting heart rate was 66.84 (sd =7.265) for those who completed the study versus 61.38 (sd = 8.656) for those who dropped out (p = 0.017). This suggests that there was not a systematic pattern of

dropout, other than it appears that those in the Test group were more likely to drop out (probably due to tolerability issues, as suggested by post-test interviews).
Of the 43 variables compared in this study (see Table A), three differed significantly ($p < 0.05$) between the Test and Control groups, with six more having marginally significant differences ($0.05 < p < 0.10$).

The statistically significant differences ($p < 0.05$) occurred among the following variables:

Potassium Serum: While the t1:t2 change in potassium serum went up for the Test group (0.1758), and decreased for the Control group (-0.0857), and while the difference in both groups was significant ($p = 0.040$), both values are in the normal clinical range, and the difference carries no clinical significance.

LDL Cholesterol: While a change occurred in LDL Cholesterol ($p = 0.015$), the change for the Test group (1.3636), and for the Control group (-6.2500), both were within the normal range for healthy individuals, and has no clinical significance.

Erythrocyte Sedimentation Rate (ESR): This is a test that indirectly measures the level of inflammation in the body. The Test group change in the sedimentation rate declined significantly (= -.09394) over the Control group (1.1538), indicating a decline in inflammation ($p = 0.039$).

Discussion

The data supports the null hypothesis on 40 of the 43 variables that there were no significant differences between the Test and Control groups caused by the test substance, preB. Two of the variables (Potassium Serum and LDL Cholesterol) showed statistical changes, but they were not of clinical significance.

The Erythrocyte Sedimentation Rate (ESR) differences between the groups ($p = 0.039$), Test group (= -.09394), versus the Control group (1.1538), indicates a significant decline in inflammation attributable to preB.

Between the groups, the change in C-RP, a more specific measure of inflammation, while showing a similar directional effect for both groups (Test = -0.2212, Control = 0.3000), did not rise to the level of significance (p = 0.507). Even though the previously mentioned Kitasato study did report a more significant impact on C-RP, it was not repeated in this study. The possible effect of preB on C-RP is a good target for a recommended follow-up dosing study.

The Waist/Height Ratio remained unchanged in the Test group (0.0000) during the study period, while it increased in the Control group (0.0120) (p = 0.068). Waist Circumference changes were marginally statistically relevant (p = 0.072), with the Test group change (0.0227) showing a lower rise than the Control group (0.8095). Taken together these two variables could indicate a possible effect of preB on waist circumference, and also waist/height ratio. These relationships need to be tested in further studies to verify it. It is also possible that there is a dose dependent effect (meaning a larger dose could create a larger effect), but that too needs to be confirmed by further clinical study.

Several quintile and inter-variable comparisons were examined. While the results of these examinations showed interesting relationships between variables, and top-quintile versus bottom quintile groups, they did not result in any statistically significant results. They do, however indicate possible directions for further research, especially with larger doses. Unlike drugs that create biologic changes in small doses, it is possible this whole-food product needs to be given in larger doses for its effects to be detected more clearly.

Conclusions

There were no significant statistical differences between the study groups on 40 of the 43 variables. The observed significant statistical differences between groups on the three variables may be inferred by the dietary supplement preB. Even when statistically significant differences occurred between study groups, the overall values are all within normal clinical range.

Therefore, based on the study results, there is no indication of potentially negative impact from the dietary supplement sufficient to cause or lead to physiologic damage or illness to a human. Much higher doses of this product have been consumed in Japan for longer periods of time with no obvious negative effects. Based upon this study, previous clinical studies and clinical observations, and with the absence of reported adverse events among consumers, there is nothing to suggest that such negative effects could be predicted with higher doses.

These results of this study support the conclusion that preB is safe for consumption by healthy humans when used in the dose (36 mL/day) and duration (six weeks) studied.

Now that preB has been shown to be safe, further studies should concentrate on dosing effects at various levels to see if accentuated metabolic relationships develop of clinical significance, as well as to examine the potential relationship between preB and inflammation, and waist circumference.

Competing Interests

The authors have no relationship past or present, with the sponsor of this study, Brazil Products Company, (the owner of preB), other than compensation for their contribution to this study. They therefore declare they have no competing interests.

Authors

John C. Nelson, MD, MPH, was the principal investigator and author of this study, co-investigator was Howard Kadish, MD, MBA. Stephen Alder, PhD, performed the bio-statistical analysis. Study design, coordination of the draft manuscripts, and editing services were performed by Boyd L. Jentzsch, JD. The preB Clinical Study Coordinator was Laura Gontchar, MEd, MBA.

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Table 1: Variables Compared between *Test* and *Control* Groups

1. Waist/Height Ratio
2. Glucose Serum: 65-99 mg/dL
3. BUN: 5-26
4. Creatinine Serum: 0.57-1.00 mg/dL
5. eFGR: >59 mL/min/1.73
6. BUN/Creatinine Ratio: 8-27
7. Sodium Serum: 135-145 mmol/L
8. Potassium Serum: 3.5-5.2/L
9. Chloride Serum: 97-108 mmol/L
10. Carbon Dioxide, total: 20-32 mmol/L
11. Calcium Serum: 8.5-10.6 mg/dL
12. Protein Total Serum: 6.0-8.5 g/dL
13. Albumin Serum: 3.5-5.5 g/dL
14. Globulin, Total: 1.5-4.5 g/dL
15. A/G Ratio: 1.1-2.5
16. Bilirubin, Total: 0.1-1.2 mg/dL
17. Alkaline Phosphatase, Serum: 25-150 IU/L
18. AST (SGOT): 0-40 IU/L
19. ALT (SGPT): 0-40 IU/L
20. Cholesterol, Total: 100-199 mg/dL
21. Triglycerides: -149 mg/dL
22. HDL Cholesterol: >39 mg/dL
23. VLDL Cholesterol Calc: 5-40 mg/dL
24. LDL Cholesterol Calc: 0-99 mg/dL
25. Sedimentation Rate – Westergren: 0-20 mm/hr
26. C-Reactive Protein, Quant: 0.0-4.9 mg/L
27. HbA1C
28. Urine: Glucose
29. Urine: Bilirubin
30. Urine: Ketone, mg/dL
31. Urine: SG (Specific Gravity)
32. Urine: BLD (Blood)
33. Urine: PH
34. Urine: Protein, mg/dL
35. Urine: Urobilinogen, EU/dL
36. Urine: Nitrite
37. Urine: Leukocytes
38. Weight, lbs
39. Waist, inches
40. Body Fat, %
41. Blood Pressure, Systolic
42. Blood Pressure, Diastolic
43. Resting Heart Rate