

Influence of Cannellini Bean Protein on Human Postprandial Glucose and Insulin Response for Mellitas Health and Foods

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Received: April 18, 2022; **Published:** December 11, 2023

Abstract

This study aims to compare the glycemic response, as well as gut hormonal panel post consumption of a Cannellini Bean Protein beverage and capsules when compared to a control reference food. Area under the curve (AUC) results were analyzed by a one-way ANOVA. The postprandial glucose responses from four panelists were calculated by the incremental AUC. The means of the AUC values for Cannellini Bean Protein beverage resulted in 1635 ± 174 and resulted significantly different (< 0.0001) when compared to its reference (glucose beverage) of 2436 ± 225 . Insulin values as well as the response for capsules treatment did not result in a statistically significant difference. In conclusion, glycemic response to ingestion of the starch from white bread remains as the standard food used as control. Bean protein beverages result in a beneficial blood glucose response. However, additional subjects are needed to verify these preliminary data and assess the glucose-insulin relationship and food intake data.

Keywords: *Cannellini Bean Protein; Human Postprandial Glucose; Insulin; Mellitas Health and Foods; Area Under the Curve (AUC); Enzyme-Linked Immunosorbent Assay (ELISA)*

Introduction

This report presents the results of a two-part study. First, to analyze the human postprandial glucose response calculated by the incremental area under the curve (AUC) and statistical analyses performed by one-way ANOVA, paired t-test and Principal Component Analysis. The collection of the data was done on a two-week period using four subjects. A continuous glucose biosensor monitored the glucose responses at 5- minute interval. The second part of this study includes the results based on the collection of three larger blood samples and the quantitative determination of insulin in serum through a sandwich type enzyme-linked immunosorbent assay (ELISA). The data obtained compared the responses of Mellitas food product: Cannellini Bean Protein beverage with a non-bioactive protein food that contains similar amount of protein and available carbohydrate (Skim or low fat milk). Two individual repeated the glycemic response study with cannellini bean protein capsules.

Materials and Methods

The trial was performed on healthy subjects and the data was collected for two weeks between March 24th and April 17, 2019. Two male and two non-pregnant non-lactating females aged 18 to 67y without diabetic or pre-diabetic condition in the BMI range (in kg/m^2) from 18.5 to 24.9 from Raleigh-Durham area participated in the trial. They were restricted in alcohol consumption and vigorous exercise during the period of the trial. The trial was documented under the NC State University Institutional Review Board (IRB 16892), and each participant signed an approved form and provided informed consent for participation in the study.

The trial was a complete block design with three repeated measurements. One carbohydrate containing food sample was consumed each day to cause a transient increase in blood glucose (glycemic response). A test meal along with a reference meal sample were consumed on alternate days of a 6-day period in the morning after an overnight fast. The reference food consisted of 50g of available carbohydrate from white bread (Pepperidge farm Farmhouse Hearty White) and skim milk-water mixture. The test food was white bread consumed along 3.8g of bean protein powder prepared with water in a beverage form for a total volume of 250 mL. All foods were consumed within 10 minutes after the first blood sample was taken. In a second trial, bread was consumed after taking 2 capsules containing either 700 mg Cannellini Bean Protein or 2 capsules with 700 mg of an isonitrogenous mixture of non-fat dry milk and casein.

Capillary blood samples were taken at fasting time referred to as time 0, time 60 min and time 120 after the consumption of the meals. These glucose responses were obtained using a hand-held glucose monitoring system: ONETOUCH Verio® manufactured by LifeScan (Cilag International GmbH, Zug, Switzerland). Self-retracting safety lancets were used to obtain larger (200 - 600 μ L) of blood samples for later insulin analysis.

A continuous glucose monitor (CGM), iPro2 system, manufactured by Medtronic (Minneapolis, MN) was placed on each participant. CGM contains a biosensor; which is attached to a recorder for 5-minute interval responses. The biosensor requires calibration. For this, each of the participants had to take 3 additional finger pricks each day and record their blood glucose (BG) results before breakfast, lunch, and dinner.

Each participant maintained a 24-h food diary annotating each meal intake and portion size. Additionally, participants were required to wear a pedometer wristband (Willful Direct, China) in order to keep track of the number of steps along with their exertion level (low, medium, high) and any detail of physical activity performed. The responses obtained through the hand-held glucose monitor, food diary and physical activity record were collected in a data sheet.

The insulin concentration was determined using an ALPCO sandwich type enzyme-linked immunosorbent assay (ELISA) (Salem, NH) and the optical density was measured by a Multiskan MCC spectrophotometer at 450 nm (Thermo Electron Corporation).

Calculations and statistical analysis

The incremental area under the curve (AUC) disregarding the area beneath fasting was obtained using the trapezoidal rule. The mean and CV ($CV = 100 \times SD/mean$) of the AUC values for the repeated reference and test foods were calculated to less than 30 for each subject ($CV < 30$).

A preliminary paired t-test was used to compare the means of AUC ($P \leq 0.05$) to ensure that the means of treatment vs reference were statistically significant. A principal component analysis (PCA) is useful as described by Husson., *et al.* (2017) to visualize the data. JMP® Pro 14.1.0 statistical software (SAS Institute, Cary, NC) was used to conduct ANOVAs on the data from the analyses.

The insulin concentration was computed using a 5-parameter logistic regression as explained by Commo and Bot (2016) for calculation of drug concentrations. The MyAssay's Data Analyzing Software was used for ELISA data interpretation. The analyses included a preliminary paired t-test, used to compare the means of the insulin change between time 60 and time 0, to determine whether the pre- and postprandial change was significant. Change was analyzed as mean values for time 60 minus mean values at time 0.

Results and Discussion

Glycemic responses for products consumed

The means and standard deviation of the data collected by 4 subjects are observed on table 1. Figure 1 shows all pair-wise comparisons for placebo beverage (diluted milk) and bean protein beverage mix for the 3 replicates for each of 4 subjects. Data from capsules are only available from 2 subjects at this time.

	AUC reference	AUC Bean Protein	P-value
Beverage	2436 ± 225	1635 ± 174	< 0.0001
Capsules	2796 ± 179	2542 ± 148	0.05

Table 1: Means of AUC of reference food and bean protein beverage (N = 4) and capsules (N = 2).

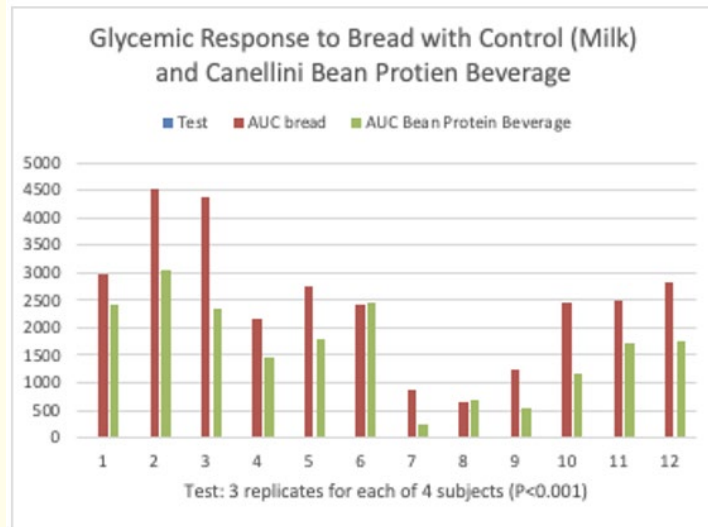


Figure 1: Repeat measures comparison of placebo meal (red) vs bean protein beverage meal (green) for 3 replications in 4 subjects.

For the beverage trial, the glycemic responses for different subjects on all days, replicated pair of days, and the average of the treatment effect (Placebo vs. Bean protein beverage) is shown in figure 2 and the ANOVA table for this analysis is shown in table 2. The subjects had different amounts of glycemic response; there was a slight increase from the first 2 days to the latter 2 replicated tests, and there was a highly significant effect of the bean protein beverage to lower the glycemic response to 50g of carbohydrate from white bread due to intake of the bean protein beverage relative to the placebo when averaged over subjects and replicates, as noted in table 1 and figure 1.

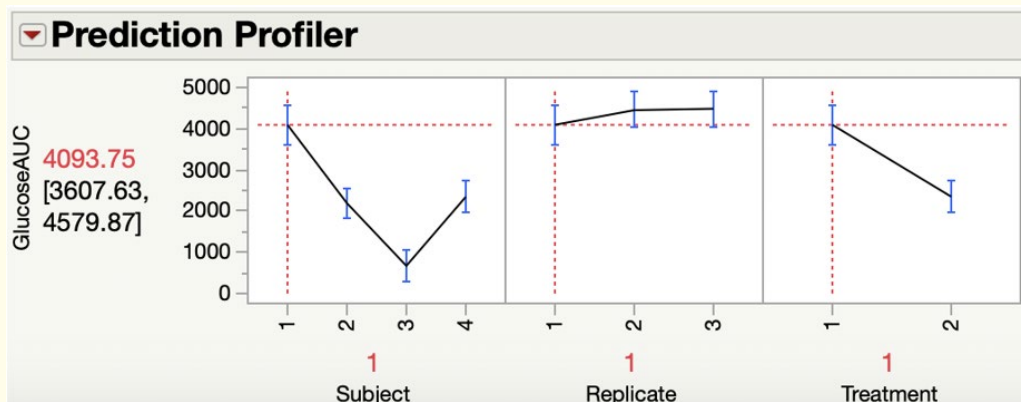


Figure 2: Graph of AUC bean protein trial.

Effect Tests					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Subject	3	3	13889730	65.3145	<.0001*
Replicate	2	2	652517	4.6026	0.0308*
Treatment	1	1	3547441	50.0440	<.0001*
Subject*Treatment	3	3	1386115	6.5180	0.0063*

Table 2: Analysis of variance for bean protein beverage trial.

Insulin responses for products consumed

The placebo reference meal resulted in a larger insulin response than the bean protein meal (Table 3). However, this change was not statistically significant with only 3 subjects tested. The lower average insulin is consistent with a lower rise in blood glucose after intake of the bean protein beverage. Additional analysis of insulin data is in progress.

	Change reference (uIU/mL)	Change in test food (uIU/mL)	P-value
Average	53.308	44.719	0.3007

Table 3: Means of increase in insulin from fasting to 60 minutes after intake of reference meal (bread plus milk) and test meal (Bread plus bean protein beverage) (3 subjects with 3 replicates each).

Conclusion

When tested for ability to alter the glycemic response to ingestion of the starch from white bread, the placebo reference food resulted in a significantly higher AUC blood glucose response than the Bean protein beverage powder food, and bean protein capsules. The insulin response was also numerically but not statistically lower with intake of the bean protein treatments. Additional subjects are needed to verify these preliminary data and assess the glucose-insulin relationship and food intake data [1-4].

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Volume 18 Issue 10 December 2023

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