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Original article

Real-world evaluation of JBA Glucotrojan® supplementation in regulating postprandial glycemic response in healthy adults

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Abstract. *Background and aim:* Our modern diet, abundant in carbohydrates and sugars, often causes surges in blood glucose levels that facilitate the development of diabetes and obesity. This public health crisis has propelled the innovation of GlucoTrojan®, a blend of white mulberry leaf, mango leaf, and banana stem juice extracts. Our study aims to examine the effects of GlucoTrojan® on the postprandial blood glucose response after consuming white rice. *Methods:* We conducted a real-world, prospective interventional study (clinicaltrials.gov registration: NCT06509204) to examine the effects of consuming cooked white rice (82 g of net carbohydrates) with and without GlucoTrojan® on postprandial blood glucose levels among healthy individuals. A continuous glucose monitoring (CGM) device was used to measure blood glucose levels. Tastermonial mobile application was used to collect data on food and drink consumption. The incremental area under the curve (iAUC) measuring glycemic index and postprandial glucose spikes were calculated to assess the effects of GlucoTrojan®. *Results:* Of the 50 individuals who participated, 25 (mean age of 40.3 years; 36% females) provided complete and valid data for analyses. Consuming white rice with GlucoTrojan® significantly reduced the mean postprandial blood glucose levels by 14 ± 6 mg/dl ($P < .0001$) over 2 hours compared to white rice consumption alone, corresponding to a 26.1% reduction. GlucoTrojan® also significantly lowered the glycemic index of white rice by a mean of 1166 ± 610 mg/dL × min, equivalent to a 33% reduction compared to no GlucoTrojan® ($P < .0001$). *Conclusions*: The findings indicate that GlucoTrojan® significantly modulates postprandial glucose responses, lowering blood glucose levels and glycemic index. Overall, our study supports GlucoTrojan® as a promising supplement for managing blood glucose levels.

Key words: GlucoTrojan®, blood glucose, diabetes, postprandial glucose response, glycemic index

Introduction

In most regions, there has never been an easier time to secure food than today, especially calorically dense foods our ancestors would have deemed precious gold. With our survival instincts and physiological mechanisms to store fat being largely intact, it is unsurprising that we have an epidemic of "diabesity," a combination of diabetes and obesity (1). In 2022, about 11% and 42% of the U.S. population have diabetes and obesity, respectively (2, 3). An additional 7.3% of the general population is estimated to have insulin resistance (4). The development of diabetes and obesity results from a complex interplay of genetic, environmental, and lifestyle factors (5). Among these, lifestyle factors, such as physical inactivity and poor nutrition, emerge as the

primary drivers of the diabesity epidemic (5). Regarding nutrition, research indicates that the excess consumption of sugars is a primary contributor to diabetes and obesity, owing to its direct influence on blood glucose and insulin regulation. Excessive sugar intake leads to rapid spikes in blood glucose levels, prompting the pancreas to release large amounts of insulin (6). Over time, repeated exposure to high insulin levels can cause cells to become less responsive to insulin, resulting in insulin resistance. To overcome this resistance, the pancreas heightens insulin production. However, excess insulin facilitates fat storage and obesity, while the constant high blood sugar level, due to ineffective glucose uptake, promotes diabetes (7). By addressing the root cause of insulin resistance, i.e., excess blood sugar, we can pave the way for improved public and individual health. To this end, GlucoTrojan® emerges as a promising powdered supplement that can modulate the postprandial (post-meal) glucose response. The main active ingredient in GlucoTrojan® is white mulberry leaf extract. Mulberry contains iminosugars, which share similar molecular structure to sugars. As such, iminosugars can influence enzymes involved in carbohydrate metabolism. One key iminosugar, 1-deoxynojirimycin (DNJ), is a potent inhibitor of α-glucosidase enzymes that aid carbohydrate digestion in the gut (8). DNJ, thus, mitigates glucose absorption in the intestine and accelerates liver metabolism of sugar to increase energy expenditure (9). Mulberry extract has been demonstrated in multiple randomized clinical trials (RCTs) as safe and efficacious in reducing postprandial blood sugar and insulin levels in healthy individuals and individuals with insulin resistance, obesity, or diabetes (10-17). Although RCTs are the gold-standard method of determining the efficacy of certain interventions, they often recruit participants with similar characteristics to maximize the replicability of results. Hence, real-world studies are needed to extrapolate results derived from the strict laboratory settings of RCTs to the highly diverse general population (18). In this study, we provide further evidence on the health benefits of GlucoTrojan® in real-world settings. Our objective is to compare the effect of consuming white rice with and without Glucotrojan® on the rice's glycemic index and postprandial blood glucose levels over 120 minutes in healthy individuals. We hypothesized that Glucotrojan® supplementation will significantly reduce postprandial blood glucose levels and the glycemic index of white rice, supporting its role as an effective tool for managing blood glucose responses.

Materials and Methods

Sample size

A priori power analysis was conducted to determine the required sample size to detect a clinically significant difference in postprandial glucose levels, measured by the incremental area under the curve (iAUC) in mg/dL × min. Drawing on data from a previous RCT involving 37 healthy participants that evaluated the effects of Reductose® (250 mg) on the 2-hour postprandial glucose response to a sucrose solution (15), we estimated a mean difference of 832 mg/dL × min with a standard deviation of the difference of 1333 mg/dL × min, yielding an effect size (Cohen's d) of 0.62. Using G*Power 3.1 analysis, with a one-tailed test, alpha level of 0.05, and power of 80%, a minimum of 18 participants would be required for paired samples t-test and 19 participants for Wilcoxon matched-pairs signed-rank test.

Participants

To account for potential dropouts or incomplete data, we recruited a conservative sample size of 50 healthy individuals from the Tastermonial network to participate in this prospective interventional study from April 13, 2023, to June 10, 2023, following an open-label, self-controlled (non-randomized) design. This study was registered with clinicaltrials.gov (NCT06509204). The inclusion criteria included a BMI range of 18.5 to 29.9 kg/m^2 and an age range of 21-75 years. The exclusion criteria entailed having type I/II diabetes or underlying health conditions that prevented participation, being pregnant, taking medications that regulate blood glucose or blood pressure, having dietary restrictions preventing the intake of food involved in the study, and being unable to follow remote guidance and instructions to complete the

study. Individuals with pre-diabetes, however, were allowed to participate in the study. Written informed consent was obtained using a digital form compliant with the Health Insurance Portability and Accountability Act (HIPAA) from all participants prior to enrollment. Participants had the right to withdraw from the study at any point. The principal investigator also has the authority to discontinue participation based on medical concerns or non-compliance with the study's inclusion and exclusion criteria. The study was approved by the Institutional Review Board (IRB) of WCG Clinical (Tacking number: 20231417; Princeton, NJ, USA; https://www.wcgclinical.com/) on April 6, 2023.

Procedures

Detailed instructions of the experimental procedures have been published on protocols.io (dx.doi.org/10.17504/protocols.io.kxygxw7yov8j/v1). Specifically, sachets of Glucotrojan® and standardized Test Meals were given to participants. Glucotrojan® was developed by AQP Pharmaceuticals, Inc., formulated with 250 mg of Reducose® and 1000 mg blend of extracts from *Mangifera indica* leaf, banana stem juice, and mint. Reducose® is a patented ingredient consisting of water extract of DNJ-containing White Mulberry Leaf. Test Meals were rice in bone broth from A Dozen Cousins, which contains 82 g of net carbohydrates. Participants were required to wear a CGM device from Abbott Freestyle Libre to track their postprandial glucose responses throughout the study, which records interstitial glucose levels at 15-minute intervals. Participants were also tasked to record their food and drink consumption by scanning the product's barcode in the Tastermonial mobile application. On Day 1, participants were instructed to wear and let the CGM device self-calibrate. Following an overnight fast from 10 pm to 6 am, participants then consumed 1 serving of Test Meal with a glass of water on Day 2 and with Glucotrojan® dissolved in a glass of water on Day 3. The Test Meal must be consumed within 10-15 minutes. From Days 4-8, participants were instructed to consume Glucotrojan® twice daily during their usual breakfast and dinner. From Day 9-13, participants were tasked to resume their normal daily activities while logging their meals during breakfast and dinner without consuming Glucotrojan®. The experiment ended on Day 14. During the study, participants were advised to maintain their usual diet and physical exercise but avoid strenuous physical activities two hours before eating. Participants were also required to maintain consistent conditions for each test. For example, if they consumed black coffee or tea within two hours after the Test Meal, they were instructed to do so consistently across all testing days. To ensure participants consumed meals on empty stomachs and consistent postprandial glucose readings, the resting glucose values were checked to see if they fell within the normal range (70-100 mg/dL). If the test began outside of this normal range, the data is considered invalid and not included for analysis. Participants who completed the experiment according to the instructions were awarded a $20 coupon to use for future purchases at Tastermonial.

Statistical analyses

Participants' eating periods were normalized and overlaid in a data frame that captured glucose values throughout the study. The incremental area under the curve (iAUC), a measure of the total increase in postprandial blood glucose levels (mg/dL) over 2 h after eating, was calculated following the trapezoid rule. This is the gold-standard method of computing glycemic index (19). The time from eating to peak glucose response (mg/dL), i.e., the maximum increase in blood glucose within 2 h of eating, was also recorded. For each participant, reductions in iAUC (glycemic index) and glucose spikes were computed as the differences between values (mean ± standard deviation, SD) obtained when consuming Test Meals (white rice) without and with GlucoTrojan®. These reductions allow us to assess the effectiveness of GlucoTrojan®in modulating postprandial glucose response. Prior to statistical analyses, the data was tested for normality using the Shapiro-Wilk test. Paired t-test was used for data that followed a normal distribution, while Wilcoxon matched-pairs signed rank test was used for data that did not meet this assumption. Statistical significance was set at $p < .05$. All statistical analyses were conducted using GraphPad Prism v8.

Results

Out of the 50 participants initially enrolled, 25 completed the study with valid data, while the remaining 25 were excluded due to non-compliance with study protocols, such as improper logging of food intake or failure to consume the Test Meal (white rice) on an empty stomach (Figure 1). The included participants had a mean age of 40.3 years (range of 24-59), with 36% females' representation, mean body mass index (BMI) of 24.2 ± 3.4, mean fasting glucose levels of 96.8 ± 11.5 mg/dl, and mean haemoglobin A1C (HbA1c) values of 5.2 ± 0.3, indicating normoglycemia.

The Shapiro-Wilk test revealed non-normal distribution in the iAUC data (w = 0.88, $p < 0.01$ for with GlucoTrojan®; $w = 0.94$, $p = 0.14$ for without GlucoTrojan®), while the glucose spike data

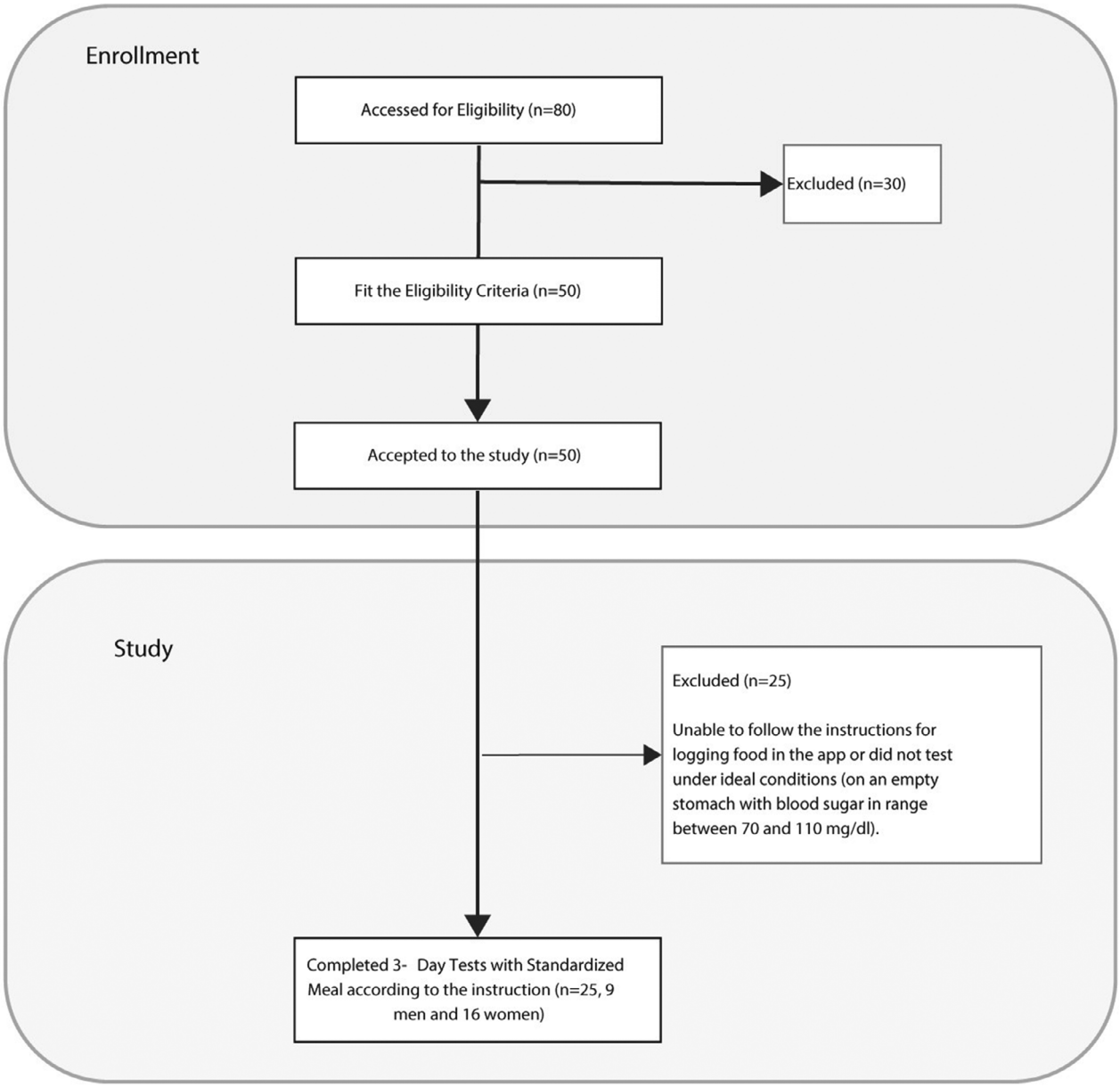

Figure 1. Flow diagram of participant progression through the study.

were normally distributed (w = 0.96, p = 0.37 for with GlucoTrojan®; w = 0.94, p = 0.19 for without GlucoTrojan®). Consequently, iAUC reduction was analyzed using the Wilcoxon matched-pairs signed-rank test, while glucose spike reduction was analyzed using a paired t-test. GlucoTrojan® significantly reduced the iAUC by a mean of 1166 ± 610 mg/dL × min (range of 556-1776), equivalent to a 31% decrease in the white rice's glycemic index (w = −253, p < .0001). Consuming Test Meal (white rice) with GlucoTrojan® also significantly lowered the highest spike in postprandial blood glucose levels by 14 ± 6 mg/dl (range of 8-20 mg/dl), corresponding to a 26.1% reduction (range of 15-37%) ($t(24)$ = 4.9, p < .0001). Importantly, these beneficial reductions in peak glucose and iAUC values (by any amount) were observed in 84 and 88% of participants, respectively (Table 1). These effects were consistent across all age groups (Figures S1 and S2).

In the mean postprandial glucose curve, consuming white rice alone led to a peak increase in blood glucose levels of 47.8 mg/dL at 50 minutes post-meal. In contrast, consuming white rice with GlucoTrojan® reduced the peak by 17.3 mg/dL, allowing faster normalization of glucose levels (Figure 2). No adverse events were recorded in this study.

Table 1. Difference in peak glucose concentration (mg/dl) and difference in iAUC value of healthy individuals after consuming rice with versus rice without GlucoTrojan®.

	Reduction in peak glucose concentration (mg/dl)	**Reduction in iAUC value (mg/dL × min)**
Observed beneficial reduction from consuming rice with GlucoTrojan® compared to rice alone	14 ± 6 (26% ± 11%)	1166 ± 610
Observations with beneficial reduction	21 out of 25	22 out of 25
Percentage of observations with positive reduction relative to total observations	84%	88%
P-value	< .0001	< .0001

Discussion

Per our hypothesis, this study showed that GlucoTrojan® decreased postprandial glucose levels and glycemic index of white rice in healthy individuals, corroborating prior RCTs on Reducose® or mulberry extract (10, 13, 15, 16). Although RCTs can determine cause-and-effect relationships, real-world studies also provide value in generalizing results of RCTs outside of their strict laboratory conditions. Thus, our study showed that the efficacy of GlucoTrojan® remains intact in real-world settings, as well as the feasibility of our Tastermonial application and CGM device in collecting real-world data for analyses (20). However, our study does have limitations. First, half of the invited participants failed to complete the study or provide valid data, which may be due to challenges in comprehending the instructions, navigating the Tastermonial application, or sustaining enthusiasm throughout the study (20). Second, we assessed the effects of Glucotrojan® on postprandial glucose response after white rice consumption. Further research should also be conducted to examine whether such effects of Glucotrojan® extend to other food types or mixed meals. Third, the study's small sample size and short duration may limit the results' generalizability and may not be sufficient to observe the long-term effects of Glucotrojan®. Long-term usage of GlucoTrojan® may have implications beyond immediate glycemic control, such as alterations in glucose metabolism (21, 22). Fourth, while our study minimizes inter-individual variability with a self-control design, it may limit the generalizability across diverse populations. Recognizing this, future studies should explore how various individual variables, such as lifestyle factors and specific dietary habits, affect the efficacy of GlucoTrojan®. Regardless, our study has a few notable strengths, such as the use of self-controlled design (23). Participants were instructed to consume a serving of white rice with a glass of water on Day 2 (control) and a serving of white rice with Glucotrojan® dissolved in a glass of water on Day 3 (intervention). This approach made each participant their own control, which inherently controlled for the countless number of confounding variables that may be present among individuals, such as socioeconomic status or genetic differences (23). The prospective design

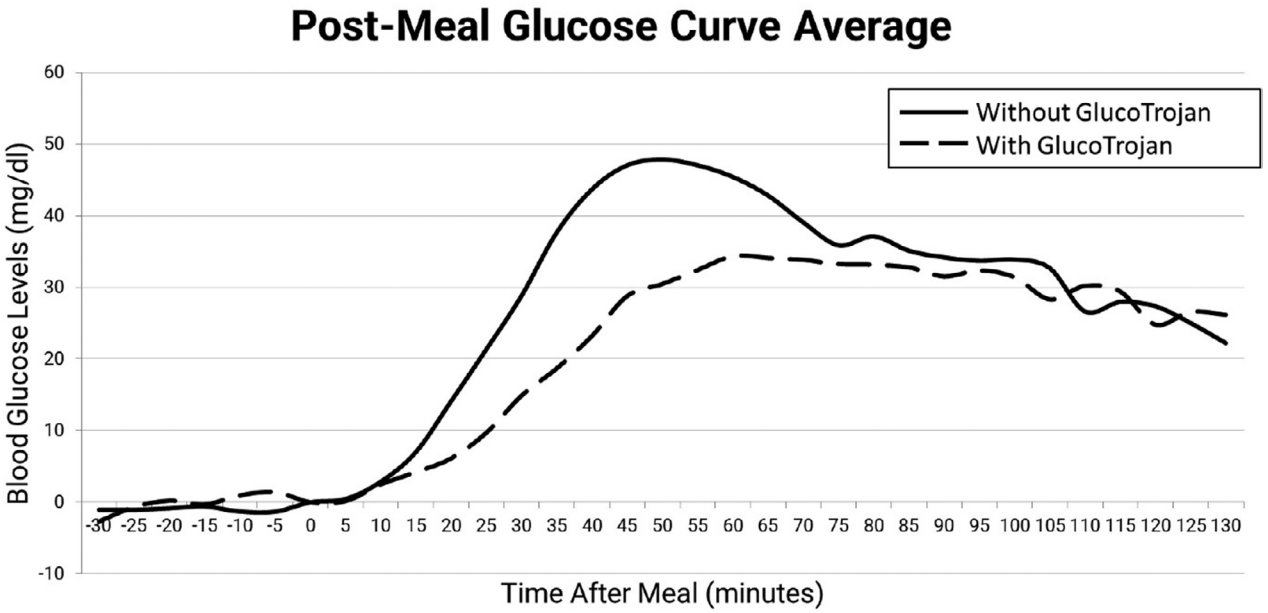

Figure 2. The mean postprandial blood glucose levels (mg/dl) following the consumption of white rice with or without GlucoTrojan®.

of our study further minimizes biases in data collection. Moreover, our choice of white rice as the Test Meal underscores its global relevance as a staple food, highlighting the practical benefits of GlucoTrojan® in everyday diets (24). Research indicates a minimal spike in postprandial blood glucose levels promotes satiety and prevents 'sugar crashes' and subsequent cravings (25). Regulated blood glucose response also prevents insulin resistance and may even allow cells to be re-sensitized to insulin, protecting against the development of diabetes and obesity (26). In addition, the consumption of foods with lower glycemic index is a known protective factor against insulin resistance. This is because such foods undergo slower digestion and absorption processes, allowing better control of postprandial blood glucose and insulin response (27). Unregulated surges in blood glucose also catalyze the formation of advanced glycation end-products (AGEs), which are proteins or lipids that undergo glycation and oxidation in the presence of excess sugar (28). AGEs accumulation accelerates aging and other age-related diseases, such as cardiovascular, neurodegenerative, and cancer diseases, among others (29). Hence, the health benefits of GlucoTrojan® extend beyond diabetes and obesity prevention. Besides mulberry extract, GlucoTrojan® formulation also contains *Mangifera indica* (mango) leaf extract and banana stem juice extract. Mango leaves are rich in phytochemicals (e.g., mangiferin) and have been widely studied for their health benefits, including anti-diabetic effects (30, 31). Similar to DNJ in mulberry extract, mango leaf extract also has the capacity to inhibit α-glucosidase enzymes and decrease glucose absorption (32). Banana stem juice is traditionally used to prevent and treat kidney stones and diabetes, owing to its rich content of antioxidant phytochemicals (33, 34). Banana stem juice extract has also been shown to reduce postprandial glucose and lipid levels, resulting in an improved diabetic condition in rats (35). Therefore, GlucoTrojan® is formulated with three synergistic ingredients capable of improving blood glucose response after meals, contributing to improved metabolic health.

Conclusion

Our prospective interventional study showed that GlucoTrojan® significantly mitigated postprandial glucose spikes in healthy individuals when consumed with white rice, supporting its potential as

a dietary intervention against diabetes and obesity. These findings align with previous randomized clinical trials (RCTs) and demonstrate the effectiveness of GlucoTrojan® in a real-world setting. While our study highlights the immediate benefits of GlucoTrojan®, the long-term implications may require further exploration. Future research should address these aspects, examining the sustained use of GlucoTrojan® across diverse populations to ensure its adaptability and efficacy in managing global dietary challenges and metabolic health.

Conflict of Interest: GDR declares he is a consultant at Golden Gate Biotech Consulting, U.S., who provided consultancy services to Tastermonial Inc., a Contract Research Organization (CRO), which undertakes sponsored research engagement from Affordable Quality Pharmaceuticals (AQP). SJY declares he received financial compensation from Tastermonial Inc. for the preparation of this manuscript.

Authors' Contribution: GDR, LQH, and BS conceptualized and conducted the study. SJY wrote the manuscript and validated the analyses. All authors edited and approved the manuscript for publication.

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ANNEX

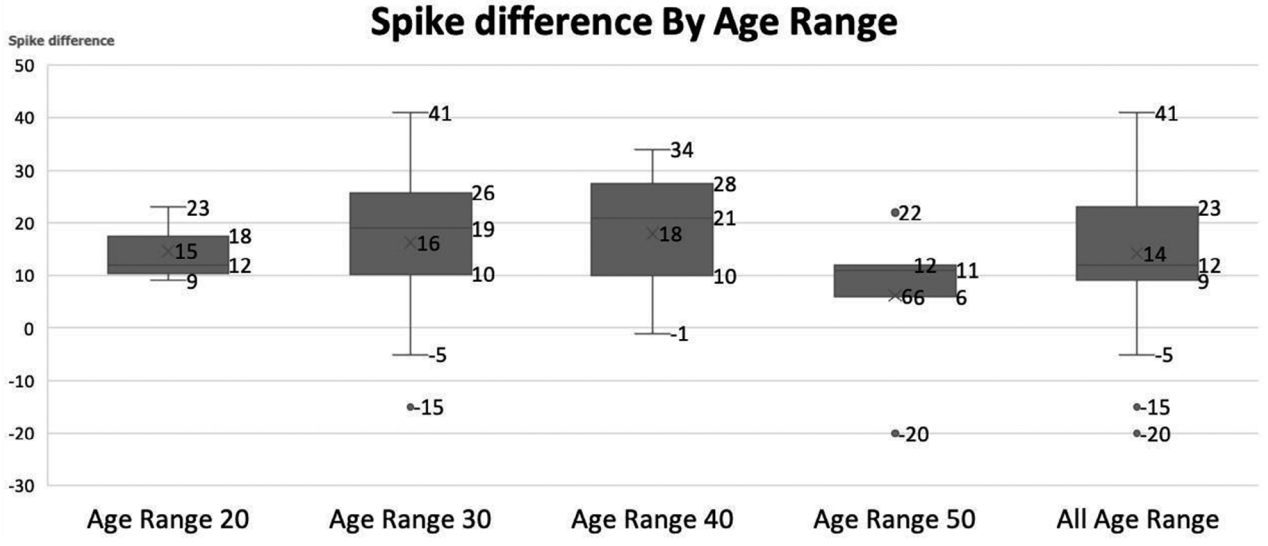

Figure S1. The difference in the spike in the mean blood glucose levels (mg/dl) following white rice consumption with versus without GlucoTrojan® across various age groups.

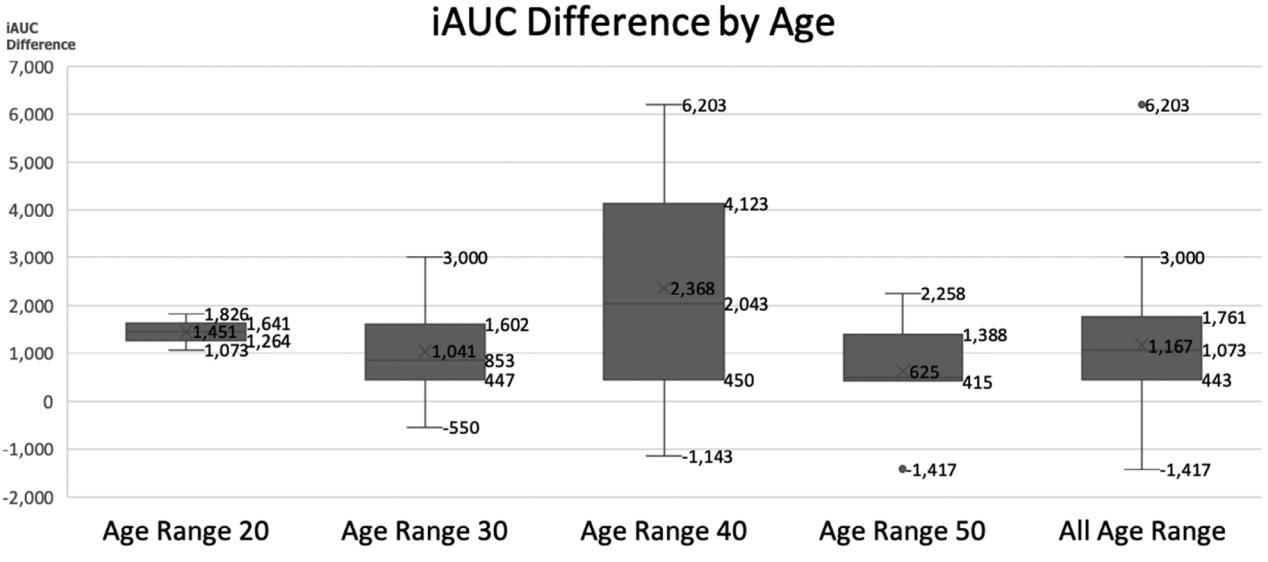

Figure S2. The difference in the incremental area under the curve (iAUC) of the total increase in postprandial blood glucose levels (mg/dL) following white rice consumption with versus without GlucoTrojan® across various age groups.