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Glycemic Response Assessment of Three Watermelon (*Citrullus lanatus*) Cultivars in Healthy Adults: A Randomized Crossover Trial

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ABSTRACT

The glycemic index (GI) values in nutritional databases for watermelon tend to be generalizable based on sugar content, yet vary among cultivars; however, postprandial glycemia and glucose response vary among individuals and even cultivars. **Objectives:** To compare postprandial glycemia, GI, and glycemic load (GL) after consumption of Charleston Gray, Crimson Sweet, and Sugar Baby cultivars of watermelon by healthy subjects. **Methods:** This study was conducted as a single blind crossover trial in which 20 healthy volunteers (10 male and 10 female subjects; age 22.1 ± 0.8 yr; body mass index 24.4 ± 3.2 kg/m^2) underwent four fasted trials in random order: 25 g anhydrous glucose or a quantity of each watermelon cultivar equaling 25 g of available carbohydrate. Capillary glucose was tested at 0, 30, 60, and 120 min. Calculation of GI was done based on incremental area under the curve (iAUC). **Results:** There was a difference in GI between the cultivars ($F(2,38) = 8.42$, p=0.001, partial eta-squared=0.31). The mean ± SD GI of Charleston Gray, Sugar Baby, and Crimson Sweet were 61.7 ± 41.2, 47.4 ± 35.0, and 31.4 ± 26.8, respectively; and the Friedman sensitivity analysis also confirmed that there was a cultivar effect ($\chi^2(2) = 12.40$, p=0.002). **Conclusions:** Crimson Sweet had the lowest response, while the three cultivars of watermelons had low GL levels in a practical portion of 150 g. Cultivar and size of portion should be taken into account when evaluating the glycemic response of watermelons.

INTRODUCTION

The Glycemic Index (GI) ranks carbohydrate-containing foods based on their postprandial blood-glucose response to a standard portion of digestible carbohydrate, while the Glycemic Load (GL) considers not only the GI but also the amount of carbohydrate in a regular serving [1, 2]. Diet interventions that focus on lower-GI or lower-GL foods can help manage glycemia and reduce cardiometabolic risks; however, the overall dietary pattern must be considered as well [3-5]. Watermelon (*Citrullus lanatus*) is eaten extensively in Pakistan and elsewhere in warmer climates, due to its taste and high-water content, as well as the fact that it provides biologically active components, such as L-citrulline and lycopene, which were studied for their vascular and metabolic properties [6]. Nonetheless, GI values of watermelon reported internationally do not consider different cultivars, ripening degree, geographical origins, or methodology of the study [2, 7]. Published studies have demonstrated moderate GI levels for certain watermelon cultivars. Recent genomic, transcriptomic, and compositional studies show cultivar-specific

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differences in sucrose metabolism, transportation, and soluble solids concentration in the course of fruit development [8-12].

Comparative regional studies on this issue are scarce. Thus, this study aimed to perform and compare the postprandial glucose level, GI, and GL of three watermelon cultivars – Charleston Gray, Crimson Sweet, and Sugar Baby.

METHODS

This randomized single-blind crossover trial was performed at the Rehman Medical Institute from June 2025 until August 2025 and is registered under the trial number NCT07017465. The study has been approved by the Institutional Review Board (IRB/EC-IIT-0028-2025 dated 23 January 2025), and all patients have given their consent to participate. In total, 20 healthy individuals (10 men and 10 women), with a mean age of 22.1 ± 0.8 years and a mean body mass index of 24.4 ± 3.2 kg/m^2, were included. Diabetes mellitus or any other metabolic disease, intake of drugs influencing blood glucose levels, as well as pregnancy or lactation, were exclusion criteria. Sample size was a priori calculated using G*Power 3.1 based on "ANOVA: Repeated measures, within factors" with parameters as follows: one group, three measurements of watermelon, effect size f=0.30 (moderate, partial eta-squared~0.083), α=0.05, power=0.80, correlation among repeated measures=0.50, and nonsphericity correction ε=1.0. This analysis suggested that a minimum total number of subjects of 20 was needed (numerator degrees of freedom=2, denominator degrees of freedom=38, critical F=3.245, non-centrality parameter λ=10.80, power=0.814). A difference of 20 GI units was chosen a priori since it is large enough for a food to change its category (low ≤55, medium 56-69, high ≥70) and is comparable with between-cultivar differences reported before [13, 14]. Each participant attended four sessions after 10-12 h of fasting with a washout of at least 2 days between them. Test meals consisted of 25 g anhydrous glucose in 300 mL of water and portions of Charleston Gray, Crimson Sweet, or Sugar Baby watermelons with 25 g of available carbohydrate. Based on 6 g of available carbohydrate per 100 g of fresh watermelon flesh [15], each watermelon portion was approximately 417 g. The randomization sequence for the four test meals was created before recruiting participants through a computer-generated blocked random procedure. This was done using numbered, opaque envelopes opened right before each test by the research assistant. Participants were not aware of the study hypothesis and code numbers; nevertheless, participant blinding was not completely achieved as the cultivars varied in appearance and taste. Outcome assessment and data analysis were blind to cultivar codes until after the finalization of the database. Of 24 individuals screened, four were excluded: two did not meet the eligibility criteria, and two declined to participate. The remaining 20 participants were randomized, completed all four test sessions, and were included in the final analysis. Participant disposition and completion of the crossover protocol are summarized in Figure 1.

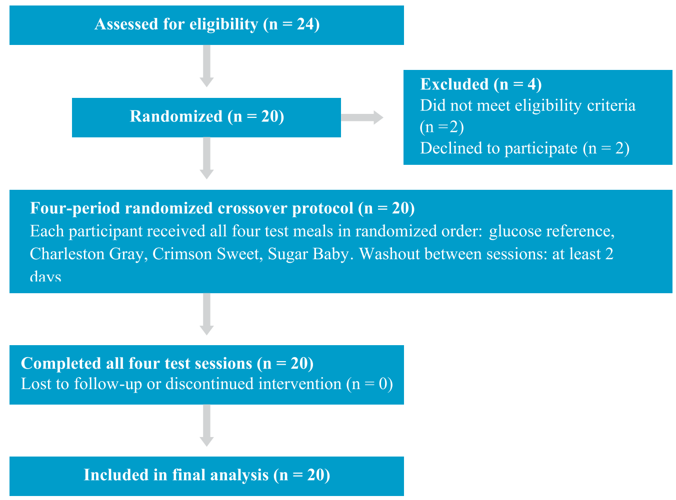

Figure 1: Participant Flow Diagram for the Randomized Crossover Trial

Representative samples of the three watermelon cultivars evaluated in the trial are shown (Figure 2).

Figure 2: Representative Samples of the Three Watermelon (*Citrullus lanatus*) Cultivars Used in the Study: A, Charleston Gray; B, Crimson Sweet; C, Sugar Baby

Capillary blood glucose measurement in the fasting state was done at 0 min using a calibrated glucometer conforming to ISO 15197 criteria, and the test meal was ingested within 10 minutes. Capillary glucose measurements at 30, 60, and 120 minutes thereafter were performed. The four-point testing regime was simplified from the seven-point regime prescribed by ISO 26642 (at 0, 15, 30, 45, 60, 90, and 120 minutes); 15, 45, and 90 minutes were not tested. Incremental area under the curve (iAUC) was determined by the trapezoidal method without considering the areas beneath the fasting baseline. For each subject and cultivar, GI was determined using the formula GI = (iAUC test food / iAUC glucose reference) × 100. Group GI was summarized based on individual GI values. GL was computed as (GI × available carbohydrate per serving)/100. To differentiate between the 25 g available carbohydrates of the test meal and an actual dietary

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portion, GL for a 150 g portion with 9 g of available carbohydrate was considered.

All statistical analyses were carried out in RStudio/R software version 4.5.1. The continuous variables were expressed as means ± SDs and 95% CI. GI values were further analyzed via histograms, Q-Q plots, and the Shapiro-Wilk test, but since the two cultivar groups had a departure from normality, their medians and IQRs were additionally presented. Differences between the GI and iAUC of the three cultivars were investigated using one-way repeated measures ANOVA because all 20 subjects were administered each cultivar. Bonferroni correction was applied for pairwise comparison, and $p < 0.05$ was deemed to be statistically significant. One-way repeated measures ANOVA was maintained as the main analysis tool because it can handle the completely paired data, is tolerant to moderate violation of the normal distribution, and provides an effect size estimator; the Friedman test served as the secondary nonparametric validation tool. Effect size was provided as partial eta-squared for the repeated measures design. The Friedman test was done as the nonparametric sensitivity analysis for the cultivar GI. An exploratory linear mixed-effects sensitivity model used cultivar and sex as the fixed effects and participant as the random effect. There was no power to analyze cultivar by sex interaction, so none was done.

RESULTS

The randomized crossover study involved 20 healthy individuals who were not diabetic (10 males and 10 females). The mean age of the subjects was 22.1 ± 0.8 years, and the mean body mass index was 24.4 ± 3.2 kg/m^2. There was no subject loss to follow-up after randomization, and all randomized subjects were included in analysis. The mean concentration of glucose at each time point is presented in Table 1. The glucose reference caused the highest increase in glucose level post-mealtime, peaking at 30 minutes (115.2 ± 8.4 mg/dL). The responses to all watermelon varieties were smaller, returning to their baselines at 120 minutes (Table 1).

Table 1: Mean ± SD Capillary Glucose Values (mg/dL) at Each Measurement Time Point

Time (min)	Glucose Reference	Charleston Gray	Crimson Sweet	Sugar Baby
0	79.8 ± 7.0	78.3 ± 6.6	75.4 ± 6.3	80.8 ± 6.9
30	115.2 ± 8.4	104.5 ± 9.7	87.4 ± 8.2	103.1 ± 9.5
60	98.3 ± 7.9	80.7 ± 7.1	70.1 ± 6.8	81.3 ± 7.3
120	83.6 ± 7.2	78.8 ± 6.9	77.5 ± 6.7	74.0 ± 6.6

The glucose-time profiles are shown. The mean postprandial glucose curves after the glucose reference and each watermelon cultivar are presented (Figure 3).

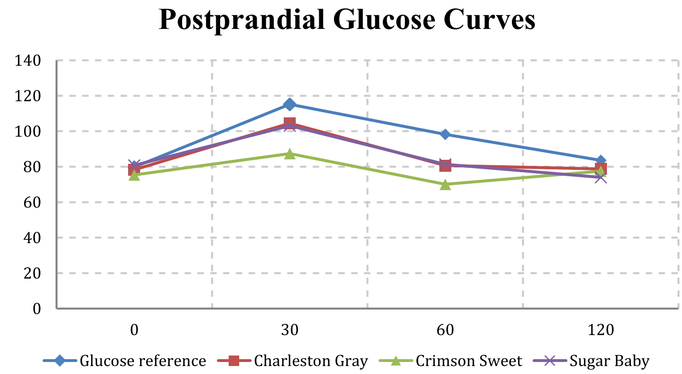

Figure 3: Mean Postprandial Glucose Curves From 0 to 120 Min After Ingestion of the Glucose Reference and Three Watermelon Cultivars in 20 Participants

Repeated measures analysis of variance revealed that there was a significant difference between the GI of the three cultivars ($F(2,38) = 8.42$, p=0.001, partial eta-squared=0.31). Charleston Gray had the highest average GI, followed by Sugar Baby and Crimson Sweet (Table 2). According to the Shapiro-Wilk test, the distribution of GI of Charleston Gray (p=0.019) and Crimson Sweet (p=0.008) was non-normal, while that of Sugar Baby (p=0.149) was normal. As such, the median (IQR) values are reported as well. The nonparametric Friedman test revealed that there was a cultivar effect (χ^2 (2) = 12.40, p=0.002). The study shows the average ± SD GI values. Since the distribution of GI of Charleston Gray was right-skewed, its category depended on the summary measure used; the mean (61.7) was medium, while the median (46.0) fell within the low category (Table 2).

Table 2: Mean, Distributional, and Categorical Summaries of Glycemic Index for the Three Watermelon Cultivars

Cultivar	Mean ± SD	95% CI	Median (IQR)	Shapiro-Wilk p-value	GI Category
Charleston Gray	61.7 ± 41.2	42.4-81.0	46.0 (34.5-85.8)	0.019	Medium
Sugar Baby	47.4 ± 35.0	31.0-63.8	35.5 (17.8-72.3)	0.149	Low
Crimson Sweet	31.4 ± 26.8	18.9-43.9	24.0 (13.5-42.5)	0.008	Low

Post-hoc tests using Bonferroni adjustments revealed a significant difference in GI between Charleston Gray and Crimson Sweet (difference in means=30.3 GI; 95% CI, 8.6 to 52.0; adjusted p=0.004). No significant difference was found between Charleston Gray and Sugar Baby (difference in means=14.3; 95% CI, -7.9 to 36.5; adjusted p=0.240) nor between Sugar Baby and Crimson Sweet (difference in means=16.0 GI units; adjusted p>0.050) (Figure 4).

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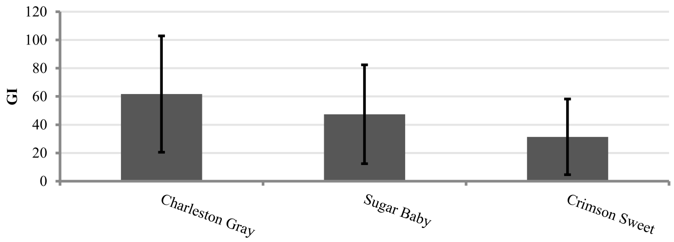

Figure 4: Mean ± SD Glycemic Index Values for Charleston Gray, Sugar Baby, and Crimson Sweet relative to the Glucose Reference (Glucose=100)

Mean iAUC values followed the same pattern as GI, with the largest response after Charleston Gray and the smallest after Crimson Sweet (Table 3).

Table 3: Mean ± SD Incremental Area Under the Curve Values for Glucose Reference and Watermelon Cultivars

Test Food	iAUC mean ± SD (mg.min/dL)	95% CI
Glucose reference	1980 ± 420	1783 to 2177
Charleston Gray	1222 ± 815	840 to 1604
Sugar Baby	939 ± 693	615 to 1263
Crimson Sweet	622 ± 531	373 to 871

The study summarizes the glucose-time, iAUC, and GI results (Figure 5).

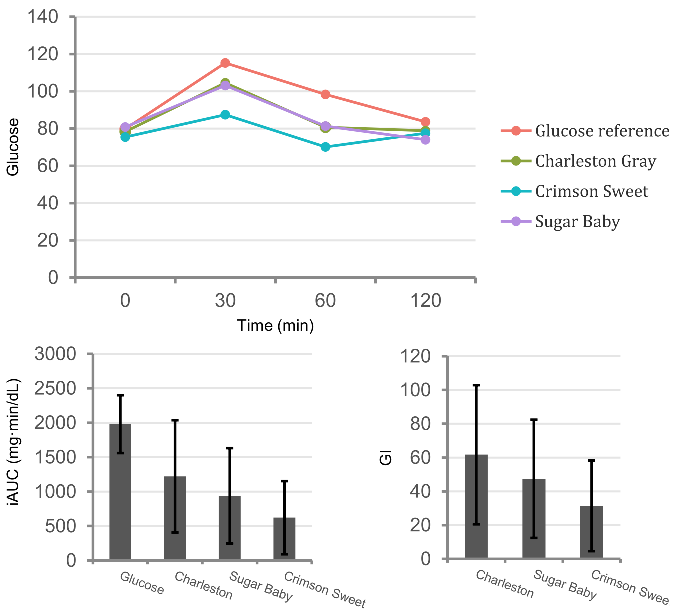

Figure 5: Glucose-Time Curves and Summary iAUC/GI Panels for the Glucose Reference and Three Watermelon Cultivars

Table 4 presents glycemic load values for practical quantities of 150 g. Glycemic load values were less than 10 in all cultivars: 5.6 in Charleston Gray, 4.3 in Sugar Baby, and 2.8 in Crimson Sweet. When considering test portion size with 25 g available carbohydrate, respective GL values were 15.4, 11.9, and 7.9 (Table 4).

Table 4: Glycemic Load Calculations for a Standard 150-g Watermelon Serving

Cultivar	Available Carbohydrate in 150-g Serving (g)	GI	GL Per 150-g Serving
Charleston Gray	9	61.7	5.6
Sugar Baby	9	47.4	4.3
Crimson Sweet	9	31.4	2.8

Variability was captured by the coefficient of variation, which ranged from 66.8% to 85.4%. For that reason, both mean-based and distributional summaries are to be taken into consideration for cultivar GI interpretation. For most subjects, the glucose levels had returned to their baseline values at 120 min after watermelon intake. The results of the exploratory sex-adjusted linear mixed effects model did not show any difference in cultivar effect and ranking ($F(2,38)=8.42$, $p=0.001$): Charleston Gray still retained the largest value, Sugar Baby medium, and Crimson Sweet the smallest. As the comparison between cultivars was done within subjects, sex adjustment did not influence the results substantially. Interaction by cultivar and sex was not considered due to the low number of observations per sex category

DISCUSSION

The results of this randomized cross-over trial suggest a statistically significant overall effect of the cultivar on the postprandial glycemic response to watermelon in the same individuals. On average, GI and iAUC values were highest for Charleston Gray, intermediate for Sugar Baby, and lowest for Crimson Sweet. Only the comparison between Charleston Gray and Crimson Sweet remained statistically significant when adjusted for multiple testing; thus, the intermediate value for Sugar Baby is an estimate but does not suggest a definitive difference from both other cultivars. Repeated measures analysis correctly took into account the paired nature of the design, and the large effect size suggests a meaningful cultivar effect despite large confidence intervals and considerable variability. Biologically plausible mechanisms underlie the cultivar effect. It has been established that there are differences in sugar composition and in the regulation of fructose, glucose, and sucrose accumulation among watermelon genotypes [8-12]. However, carbohydrate composition in the cultivars was not assessed in the current study. These results highlight the reasons why a single universal GI for watermelons might be inaccurate. International tables cite higher combined GI values than what was found in this experiment for Crimson Sweet and Sugar Baby, while GI may differ depending on the cultivar, ripeness, location, standardized carbohydrate intake, and measurement technique [2, 7]. GL depends on serving size, and for all three cultivars, GL values were lower in the case of a 150 g serving size, even though the much larger 25 g carbohydrate test portions had a higher GL value. High between-person variability was noted in this experiment as well. This aligns with precision nutrition studies that prove the existence of heterogeneous glycemic responses to the

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same foods in different individuals [16-18]. Therefore, it is appropriate to present medians and interquartile ranges, and sensitivity analysis for the Friedman test confirmed the main result concerning cultivar despite abnormal distribution in two cases of GI values. Sex-specific sensitivity adjustment did not affect the cultivar hierarchy or statistical significance. However, the equal representation of men and women does not imply equal cultivar effect by sex, as the study lacked power for an interaction. Both cultivar and food serving size could be clinically important factors in the use of watermelon for dietary recommendation, but generalization of results to patients with impaired glucose tolerance and diabetes would require additional studies [19, 20].

There are several limitations to this research. The sample was small and limited to young and healthy adults. The blood sampling was done using capillary glucometers instead of venous plasma glucose or continuous glucose monitors. Moreover, the blood sampling procedure was less frequent compared to the reference method ISO 26642; that is, blood sampling was done at 0, 30, 60, and 120 minutes, while the missing of 15-, 45- and 90-minutes' sampling could lead to underestimation of the early postprandial peak and incorrect depiction of the descending phase of the curve, thereby limiting the accuracy of the iAUC calculations and the GI values calculated based on iAUCs. Furthermore, the glucose reference was conducted only once instead of two or three times, which were necessary for stabilizing the reference denominator in the GI calculation formula, which could be a reason for the high within-cultivar dispersion (66.8% to 85.4%). Carbohydrate content and ripeness of the cultivars were not characterized biochemically. Total blinding of the participants was not possible due to differences in appearance and taste of cultivars. Lastly, the study did not have enough statistical power to analyze the interactions between cultivar and sex.

CONCLUSIONS

When evaluating the effect of various cultivars of watermelon on postprandial glycemic response in healthy young adults, there were differences between cultivars. The lowest mean GI and iAUC values occurred in Crimson Sweet, while the highest were found in Charleston Gray; the GI and iAUC values of Sugar Baby were somewhere in between. After Bonferroni correction, only the comparison between Charleston Gray and Crimson Sweet resulted in a statistically significant difference between cultivars. All cultivars produced low GL values upon calculation for a more realistic 150 g dose of watermelon. These findings suggest that both cultivar and portion size should be taken into account rather than giving a single value of glycemic effect to watermelon.

Authors' Contribution

Conceptualization: FU
Methodology: FU, NA, MT, MAS, HI
Formal analysis: NA, MAS, HI, SS
Writing and Drafting: FU, NA, MAS
Review and Editing: FU, NA, MT, MAS, HI, SS

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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