



# Understanding Glycemic Index Variations: A Study on The Effects of Age, BMI, And Gender In A Controlled Setting

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## Abstract

**Purpose:** This study investigates the impact of biological factors, such as age, BMI, and gender, on the variability of glycemic index (GI) responses. It examines how these factors influence postprandial glycemic responses to two common foods, Bread Butter (BB) and Masala Roti (MR), to assess the reliability of GI in predicting health outcomes.

**Design/Methodology/Approach:** The study used an analytical observational cross-sectional design. Participants were divided into experimental groups based on age, BMI, and gender. Blood glucose levels were measured over 180 minutes after consuming BB and MR, and GI values were calculated using the incremental area under the curve (iAUC) method. The control group had broader age, BMI, and gender criteria, allowing for inter- and intra-individual variability assessment.

**Findings:** The results show significant variability in GI responses between and within individuals, with BB producing a higher average GI of 91.99 and 91.91 MR. The range of GI values for both foods demonstrated notable inter-individual differences, with BMI variability being more pronounced for inter-variance BB. Gender differences were also observed, particularly in intra-individual variability, where females showed greater fluctuations in inter and intra-variance.

**Practical Implication:** The findings suggest that relying solely on GI as a dietary tool for managing chronic illnesses may be inadequate. Labeling foods with their GI alone does not account for the variability in glycemic responses due to individual factors, underscoring the need for more personalized dietary strategies and simplified, consumer-friendly food labeling.

**Originality/Value:** This study provides valuable insights into the limitations of GI as a universal dietary tool, emphasizing the role of biological factors in glycemic variability. It offers practical recommendations for improving food labeling and highlights the need for individualized dietary approaches in chronic disease management.

**Keywords:** Glycemic index, variability, inter and intra individuals' variability, Incremental area under the curve (iAUC), Qualitative Research

## Introduction

Diabetes has emerged as a critical global health concern, with its prevalence rising sharply in recent decades. According to the International Diabetes Federation, an estimated 537 million adults worldwide are currently living with diabetes, and this number is projected to increase to 783 million by 2045. Type 2 diabetes, accounting for over 90% of cases, is particularly prevalent and strongly associated with lifestyle factors such as urbanization, aging populations, physical inactivity, and increasing obesity rates. This escalating epidemic has profound health implications, contributing to approximately 6.7 million deaths in 2021 alone.[1]

India is experiencing a severe diabetes crisis, with approximately 77 million adults affected by the condition. Diabetes and its associated complications, including cardiovascular disease and kidney failure, contribute significantly to mortality rates. According to the World Health Organization (WHO), diabetes was responsible for 2 million deaths globally in 2019, with an additional 460,000 deaths linked to diabetic kidney disease. Elevated blood glucose levels have also been implicated in 20% of cardiovascular-related deaths. The rising

burden of diabetes, particularly in low- and middle-income countries, underscores the urgent need for improved prevention, early detection, and comprehensive management strategies.

The glycemic index (GI) is a system that ranks carbohydrates based on their impact on postprandial blood glucose levels. It measures the blood sugar response over two hours after consuming test food compared to reference food, such as glucose or white bread. GI is calculated using the incremental area under the blood glucose response curve (IAUC) for a 50-gram portion of the test food, expressed as a percentage of the response to the reference food. Based on their GI values, foods are classified as low (GI  $\leq$  55), medium (GI 56–69), or high (GI  $\geq$  70). This system is particularly valuable in diabetes management, as it aids in regulating blood sugar levels and mitigating long-term complications.



The application of the glycemic index (GI) at an individual level is challenging due to its inherent variability. Recent research has highlighted significant limitations in the GI, raising concerns about its reliability in accurately predicting metabolic responses and associated health risks. [2]. This variability stems from differences in glycemic responses both within and between individuals, influenced by a range of methodological and biological factors. Individual variations in digestion, insulin sensitivity, gut microbiota composition, and metabolic status contribute to these inconsistencies. Additionally, methodological factors such as food processing, preparation techniques, and testing conditions further impact GI values. As a result, the glycemic index becomes less reliable as a predictive tool for managing health outcomes, limiting its effectiveness in personalized nutrition and disease prevention strategies.[3].

Nevertheless, [4] Contend that this variability does not diminish GI's clinical and public health significance, provided that standardized testing protocols are rigorously followed. Furthermore,[5] Highlight the importance of establishing reliable GI values for region-specific foods to enhance research accuracy and improve clinical applications.

Several factors contribute to the variability in glycemic index (GI) values, including the type and structure of carbohydrates, cooking and processing methods, macronutrient and micronutrient composition, food consistency, and serving temperature. Additionally, methodological differences in GI testing, such as variations in study protocols, reference foods, and participant characteristics, further influence these values. These complexities make it challenging to apply GI as a precise and individualized tool for glycemic management and dietary planning.[6].

This study aims to address these challenges by conducting an analytical observational cross-sectional study to determine the difference in biological variables like Age, BMI, and Gender in determining the glycemic index value of two different types of test food (Bread Butter; BB, and Masala Roti; MR).

### Methodology

Morbidly obese (BMI >35kg/m<sup>2</sup>), diabetic or pre-diabetic patients, Subjects taking medication that affects glucose metabolism, gastric motility, and lipid metabolism, active smokers, alcohol drinkers, pregnant, and lactating females, and participants have a history of major surgery (renal, gastrointestinal, liver, pancreatic and cardiovascular), uncontrolled hypertension. Recent weight gain or loss exceeding 5 kg in the prior 6 months, and those unwilling to participate in the study were excluded.

The dropout (withdrawal) included becoming ill and unable to continue the research, voluntary withdrawal by the participant during the study, and not being compatible with the study. To minimize potential bias, participants for each group were selected randomly, ensuring that no preferential selection influenced the study's outcomes.

Detailed measurements included anthropometric data (Height, Weight, Body mass Index [BMI], Waist Circumference, Hip circumference, and waist-hip Ratio). Height was measured using a non-stretchable measuring tape with an accuracy nearest to 0.1 cm, and weight was measured by Karada Scan (OMRON Body Composition Monitor, HBF-375, China) which calculated the participants' weight with an accuracy nearest to 0.1kg, BMI was calculated by dividing the measurement of weight in kg and height in m<sup>2</sup>, waist, and hip circumference was measured using a non-stretchable measuring tape with an accuracy nearest to 0.1 cm. The hip Ratio was calculated by dividing the measurement of waist in cm and hip in cm. Body composition (Body Fat (%), Fat- Free mass (kg), visceral Fat (%), Resting metabolism Rate (Kcal), Body age(year), Subcutaneous Fat (%), and Skeletal Fat (%)) were done by Karada Scan Body composition analyzer machine. Participants' blood sample was collected in micro-centrifuge tubes containing anticoagulant and held on ice until centrifuged at 3000 rpm at 48C. Blood plasma was pipetted off the packed erythrocytes and stored at 18°C until analysis. (RT-9200 Semi-auto Chemistry Analyzer (Rayto Diagnostics, China)), include the measurements of fasting blood glucose and hemoglobin levels, C-reactive Protein, HbA1C, Lipid Profile, (Low-Density lipoprotein (LDL), High-Density Lipo Protein (HDL), Very Low-Density lipoprotein (VLDL), Triglyceride (TG) and Cholesterol to check whether any inflammation directly or indirectly affects glucose metabolism.

**Biophysical measurements** (DIAMOND Mercurial Blood Pressure Apparatus) were Measured by Sphygmomanometer measured blood pressure according to WHO standard protocol.

### Period and Site of Study

The observational study was conducted from July 2022 to December 2023, at the Food Science and Nutrition Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

### Sample Population

36 participants are voluntarily selected from Ahmedabad, Gujarat, India. Consent forms are given to each participant who joins in this study. The study was approved by the local institutional ethics committee GU-ICE(NIV)/02/PhD/028; 23/06/2022 at Gujarat University.



### Standardization of Test Food

Two test foods, Bread Butter (BB) and Masala Roti (MR) were standardized for equal carbohydrate content. (50 g)

**Table 1 Proximate Composition of the Test Foods**

Proximate	Method	Wheat Masala Roti	White bread
Ash	Gravimetric Analysis (using Muffle furnace)	1.97gm $\pm$ 0.19	2.8 $\pm$ 0.023
Moisture	Gravimetric Analysis (using Hot air oven)	19.34 % $\pm$ 0.35	25.84 $\pm$ 2.05
Fat	Soxhlet Analysis (solvent Extraction)	12 gm $\pm$ 0.12	11.2 $\pm$ 0.29
Protein	kjeldal method	10.25 gm $\pm$ 0.37	8.2 $\pm$ 0.08
Crude Fiber	Treatment of Acid and alkali	2.97 gm $\pm$ 0.29	2.5 $\pm$ 0.17
Carbohydrate	Calculated	53.25 gm $\pm$ 0.32	51.34 $\pm$ 1.85
Energy	Computed	362 kcal $\pm$ 1.6	333.72 $\pm$ 28.29

Glucose was used as a reference food for the GI study.

Initial screening is obtained based on demographic and clinical factors in 36 volunteers.

**Estimation of GI and Test the Variability:** 36 participants were recruited and divided into two groups: the Experimental Group and the Control Group. The Experimental Group comprised participants aged 18-22 years, with a BMI range of 18.5-22.9 kg/m<sup>2</sup>, while the Control Group included participants aged 18-55 years, with a BMI range of 18.5-29.9 kg/m<sup>2</sup>. In terms of gender distribution, the Experimental Group consisted of 9 males and 12 females, while the Control Group included 12 participants of both genders.

Each participant in the study fasted for 12 hours on nine separate occasions, avoiding alcohol, smoking, and vigorous physical activity to ensure accurate blood glucose measurements. Blood glucose levels were recorded at fasting (baseline) and after consuming the test foods. After the fasting period, participants ingested either Bread Butter, glucose, or Masala Roti within a 10-minute window, followed by blood glucose measurements at intervals of 30, 60, 90, 120, 150, and 180 minutes post-consumption.

A Dr. Morepen Gluco One (BG 03) Digital machine was used for blood glucose measurements, calibrated daily for precision. The fasting sample time served as the starting point (0 minutes), and participants were instructed to remain seated to minimize physical activity effects during the test. The glycemic index (GI) was calculated using the incremental area under the curve (iAUC) method, as described by [7], facilitating a detailed assessment of postprandial glycemic responses and highlighting inter- and intra-individual variability in GI values.

### Statistical Analysis:

Statistical analyses were performed on data from the 36 volunteers who completed the study by using Descriptive data expressed as means  $\pm$  SE. SD (Standard Deviation) was used to derive the interindividual (square root of subject variance divided by the mean GI value) and intra-individual (square root of error term divided by the mean GI value). The F-test was used to assess the significance of variability. Baseline characteristics, Anthropometric Data, Biophysical and Biochemical Data were done by using Mean  $\pm$  SE, and percentages by Microsoft Excel 365. Minimum Maximum value and Mean GI values were done By GraphPad Prism 8. Calculation of the GI values at 30, 60, 90, 120, 150, 180. min by using the iAUC method.

### Results

This study evaluated the impact of controlled biological variables—specifically age, body mass index (BMI), and gender—on the inter and intra-individual variation of glycemic index (GI) measurements among 36 healthy volunteers. (Table 2), Participants had a mean age of 22.13  $\pm$  0.26 years with all being unmarried, 77.77 %

postgraduates, and 80.86 % vegetarian. **Anthropometric and Body Composition Data**, (Table: 3) The mean BMI was 23.28  $\pm$  0.62 kg/m<sup>2</sup>. The mean weight and Height of study participants were 61.81  $\pm$  1.71 and 162.5  $\pm$  1.49 respectively. leading to a waist-hip ratio of 0.82  $\pm$  0.012. Body fat and fat-free were 23.74  $\pm$  1.70 and 27.51  $\pm$  2.10 respectively. The Visceral fat of study participants was 5.27 $\pm$ 0.67 and Resting metabolism



**Table 2 Demographic Characteristics of Study Participants**

Demographics Characteristics		Subjects (n=36)
Age(year)	Mean $\pm$ SE	22.13 $\pm$ 0.26
Education	Graduate	08 (22.22)
	Postgraduate	28 (77.77)
Marital Status	Unmarried	36 (100)
Food Habits	Vegetarian	29(80.55)
	Non-vegetarian	07(19.14)

\* Values in parenthesis show the percentage

**Table 3 Mean Value of Anthropometric and Body Composition Measurement of Study Participants. (n=36)**

Anthropometric Data	
Measurements	Mean $\pm$ SE of Subjects(n=36)
Weight (kg)	61.87 $\pm$ 1.71
Height (cm)	162.55 $\pm$ 1.49
BMI (kg/m <sup>2</sup> )	23.28 $\pm$ 0.62
Waist Circumference (cm)	81.44 $\pm$ 1.95
Hip Circumference (cm)	98.19 $\pm$ 1.61
Waist-Hip Ratio	0.82 $\pm$ 0.01
Body Composition	
Body Fat (%)	23.74 $\pm$ 1.70
Fat-Free mass (kg)	27.51 $\pm$ 2.10
Visceral fat (%)	5.27 $\pm$ 0.67
Resting Metabolism Rate (kcal)	1357.94 $\pm$ 33.95
Body Age (year)	29.83 $\pm$ 1.50
Subcutaneous Fat (%) (Whole Body)	23.60 $\pm$ 1.32
Skeletal Muscle mass (%) (Whole Body)	30.48 $\pm$ 0.98

#### Biochemical Parameters:

Table 4 presents the biochemical parameters of the subjects (n=36) indicating a generally healthy lipid profile. The mean HDL (good cholesterol) is 48.54 mg/dl, which is in the optimal range, while LDL (bad cholesterol) is 82.88 mg/dl, indicating low cardiovascular risk. Total cholesterol is 124.68 mg/dl, reflecting a favorable profile. The CRP level, at 1.22 mg/L, suggests low inflammation. Glycated hemoglobin (HbA1C) is 5.85%, indicating normal blood sugar levels over time. Triglycerides are at 72.29 mg/dl, and VLDL is 14.45 mg/dl, both within healthy limits.

**Table 4 Mean Value of Biochemical Parameters of Study Participants**

Bio-Chemical Parameters	Mean $\pm$ SE of Subjects(n=36)
High-density lipoprotein (HDL) mg/dl	48.54 $\pm$ 1.10
Low-density lipoprotein (LDL) mg/dl	82.88 $\pm$ 4.39
Cholesterol (mg/dl)	124.68 $\pm$ 5.75
C- Reactive Protein (CRP) mg/l	1.22 $\pm$ 0.18
Glycated Hemoglobin (HbA1C) %	5.85 $\pm$ 0.090
Triglyceride mg/dl	72.29 $\pm$ 4.94
Very Low-density lipoprotein (VLDL) mg/dl	14.45 $\pm$ 0.98

**Table 5: Mean Value of Glycemic index when Biological Variable was controlled For Both Test Food (Bread Butter & Masala Roti): (n=24)**

Age Control	Experimental Group(n=12) (Age- 18 to 22 year)		Control Group (n=12) (Age- 18 to 30 Year)	
	Bread Butter	Masala Roti	Bread Butter	Masala Roti
GI	80.69	91.91	76.09	89.85
Min of GI	35.59	43.10	20.14	33.04
Max of GI	144.04	172.26	144.97	231.22
Inter variance	34.09	34.46	35.94	50.88
Intra variance	12.68	18.96	15.05*	15.60*

**Table 6 Mean Value of Glycemic Index when Biological Variable was controlled For Both Test Food (Bread Butter & Masala Roti): (n=24)**

BMI Control	Experimental Group (n=12) BMI- 18.5 to 22.9 Kg/m <sup>2</sup>		Control Group (n=12) BMI- 18.5 to 29.9 Kg/m <sup>2</sup>	
	Bread Butter	Masala Roti	Bread Butter	Masala Roti
GI	74.85	90.61	88.98	84.88
Min of GI	35.59	38.08	20.14	18.72
Max of GI	134.15	162.21	156.72	231.22
Inter variance	29.33	33.63	39.87	54.67
Intra variance	8.04**	17.51**	18.88	13.21

**Table 7: Mean Value of Glycemic Index when Biological Variable was controlled For Both Test Food (Bread Butter & Masala Roti): (n=33)**

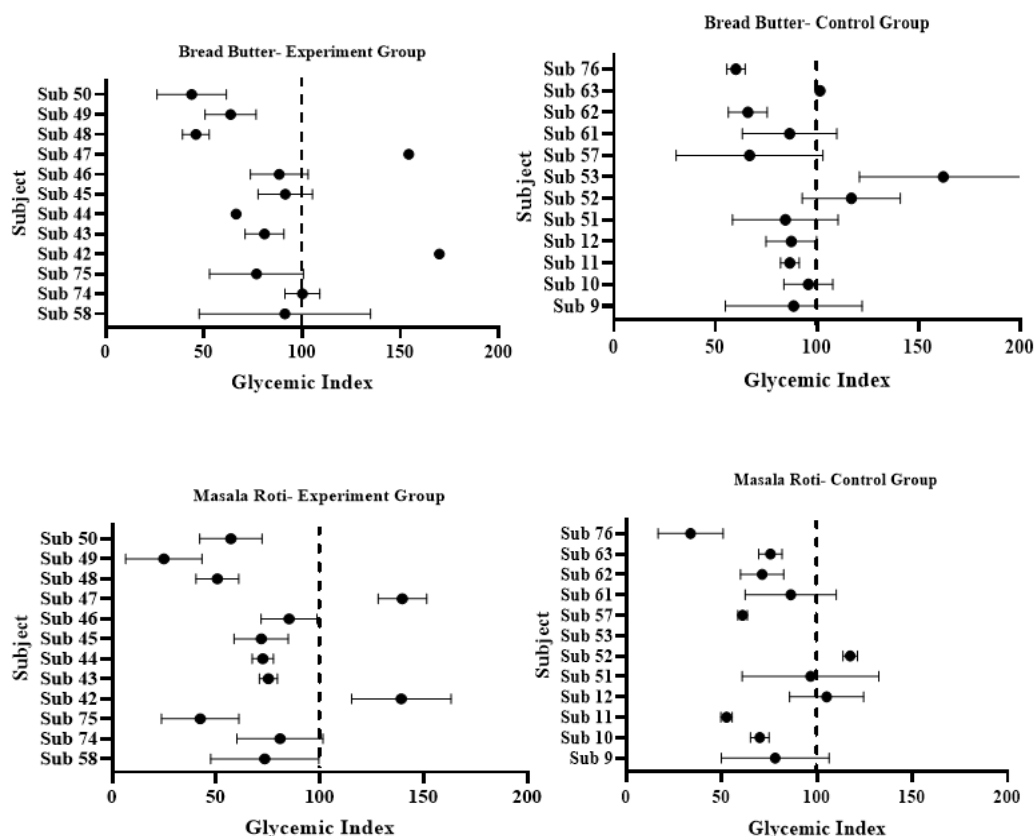
Gender Control	Experimental Group(n=21)				Control Group (n=12)	
	Bread Butter		Masala Roti		Bread Butter	Masala Roti
	Male (n=9)	Female (n=12)	Male (n=9)	Female (n=12)	Mixed Gender	Mixed Gender
GI	91.99	84.15	68.41	79.20	110.97	106.95
Min of GI	43.04	44.11	34.87	20.14	32.41	18.72
Max of GI	145.93	140.53	122.40	143.57	191.57	231.22
Inter variance	31.69	28.14	27.67	32.63	45.36	57.73
Intra variance	29.11	16.82	18.03	13.25*	15.63	18.31*

**Glycemic Index estimation:**

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In this study, 36 participants were recruited and assigned to either the Experimental or Control Group to evaluate glycemic index (GI) variability for two test foods: Bread Butter (BB) and Masala Roti (MR). Key biological factors—age, BMI, and gender—were controlled to minimize confounding effects.

**Age:** The Experimental Group consisted of 12 participants aged 18–22 years, whereas the Control Group included 12 participants aged 18–55 years.

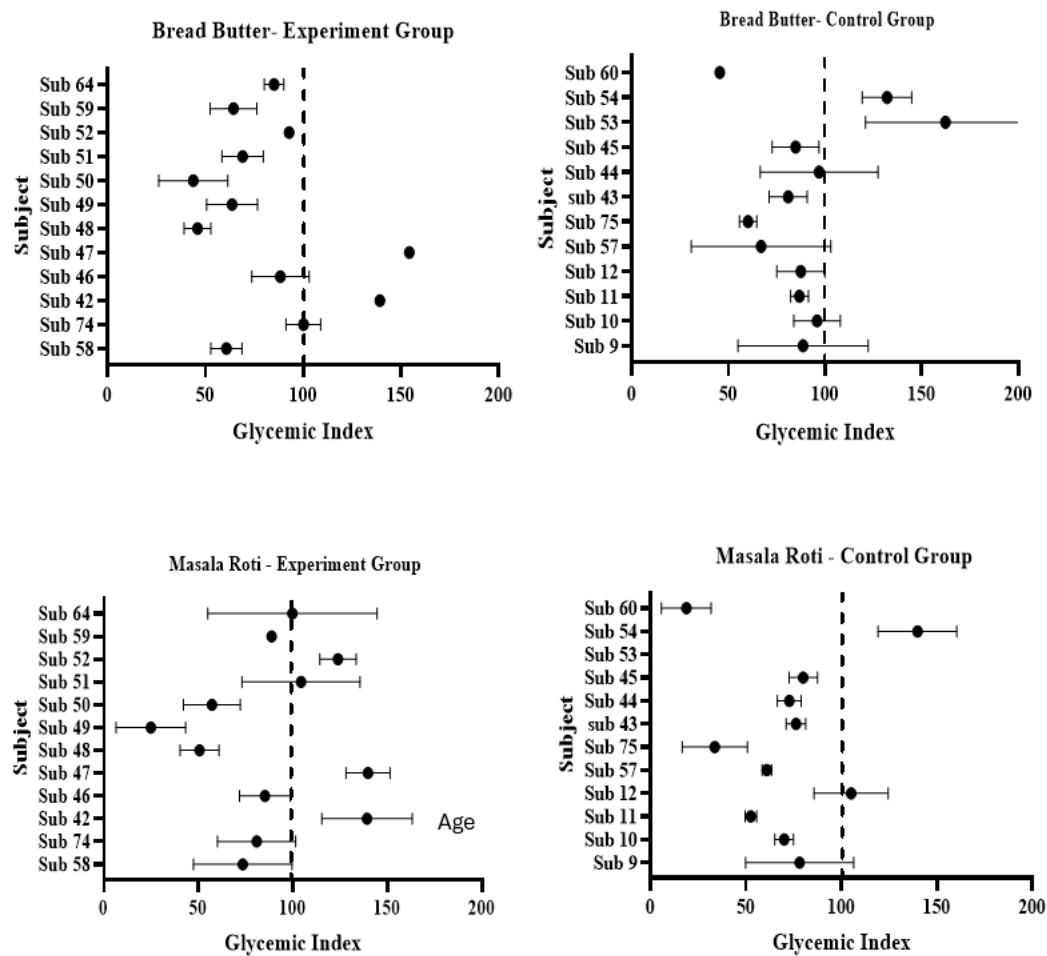


**Figure A- Individual glycemic index values of participants of Experimental (age Range 18-22 years) and Control Group (age Range 18-55 years) GI was calculated for the participants who were tested three times test food and reference food (n =12). Represent the mean of three glycemic index determinations; horizontal lines represent  $\pm$  SD.**





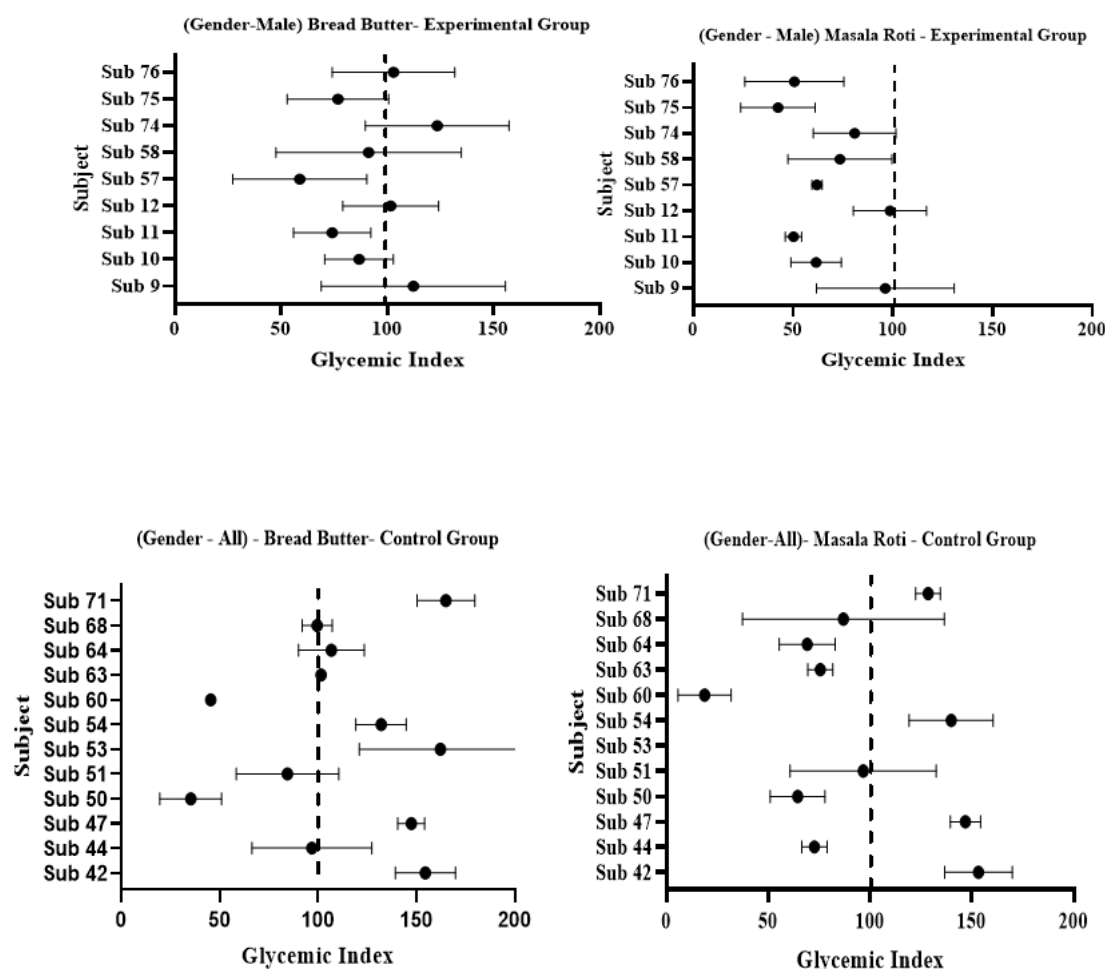
**BMI:** Participants in the Experimental Group had a BMI range of 18.5–22.9 kg/m<sup>2</sup>, while those in the Control Group had a broader BMI range of 18.5–29.9 kg/m<sup>2</sup>.



**Figure B-Individual glycemic index values of participants of Experimental (BMI 18-22.9 Kg/m<sup>2</sup>) and Control Group (BMI 18.5-29.9 Kg/m<sup>2</sup>) GI was calculated for the participants who were tested three times test food and reference food (n =12). Represent the mean of three glycemic index determinations; horizontal lines represent  $\pm$  SD.**



**Gender:** The Experimental Group analyzed males ( $n = 9$ ) and females ( $n = 12$ ) separately, whereas the Control Group included 12 participants of both genders. This experimental group ensured a systematic assessment of GI variability under differing physiological conditions.



**Figure C - Individual glycemic index values of participants of experiment Group ( $n=9$  male,  $n=12$ , female) and Control Group ( $n=12$ , mixed gender) GI was calculated for the participants who were tested three times test food and reference food ( $n=12$ ). Represent the mean of three glycemic index determinations; horizontal lines represent  $\pm$  SD.**

In the Age-controlled group, Figure A, bread butter (BB) exhibited a Higher mean GI of 80.69 in the Experimental Group compared to 91.91 in the Control Group, Similarly, Masala Roti (MR) had a lower mean GI of 76.09 in the Experimental Group but a higher GI of 89.85 in the Control Group. Minimum and maximum GI values in the Experimental Group for bread butter ranged from 35.59 to 144.04, while in the control group, the range was 43.10 to 172.26, for Masala Roti the GI Range was 20.14 to 144.97 in the Experimental and 33.04 to 231.22 in Control Group.

Variance analysis revealed that there were significant differences in the inter- and intra-variability between the experimental and control groups. In the Experimental Group for Bread Butter, an inter-individual variance was 34.09, an intra-individual variance was 12.68, and 34.46 and 18.96 for the control group respectively.





For Masala Roti in the Experimental Group, an inter-individual variance was 35.94 and control group was 50.88, while the intra-individual variance for masala Roti was 15.05 for the Experimental group and 15.60 for the Control Group, suggesting a significant difference among subject variability, In the BMI-controlled groups, Figure B, Breadbutter (BB) exhibited a Lower mean GI of 74.85 in the Experimental Group compared to 90.61 in the Control Group, Similarly, Masala Roti (MR) had a Higher mean GI of 88.98 in the Experimental Group but a Lower mean GI of 84.88 in the Control Group. Minimum and maximum GI values in the Experimental Group for breadbutter ranged from 35.59 to 134.15, while in the control group, the range was 38.08 to 162.21, for Masala Roti the GI Range was 20.14 to 156.72 in the Experimental and 18.72 to 231.22 in Control Group.

Variance analysis revealed that there were significant differences in the inter- and intra-variability between the experimental and control groups. In the Experimental Group for Bread Butter, an inter-individual variance was 29.33 for the Experimental Group and 33.63 for the Control Group, and intra-individual variance was 8.04 for the Experimental Group and 17.51 for the Control Group which is highly Significant. For Masala Roti Inter-Individual Variance was 39.87 for the Experimental Group and 54.67 for the Control Group and Intra Individual Variance for the Experimental Group was 18.88 and 13.21 for the Control Group, indicating more variability in responses among individuals with higher BMI. This aligns with findings from [8], which linked higher BMI to increased glycemic variability and related metabolic disorders, including type 2 diabetes.

In the gender-controlled groups, males in the Experimental Group showed a Lower mean GI for Bread Butter 91.99, and females 84.15 Compared to the Control Group 110.97, suggesting more pronounced glycemic responses. For Masala Roti, males and Females in the Experimental Group had a lower mean GI of 68.41 and 79.20 respectively, and mixed genders in the Control Group displayed higher GI values of 106.95. This supports research by [9], indicating that gender differences significantly impact glycemic responses and the risk of type 2 diabetes, with men generally exhibiting higher glycemic indices and being diagnosed at younger ages than women due to biological factors such as hormonal differences and body composition. These findings highlight the importance of considering both BMI and gender when assessing glycemic responses and their implications for dietary management and diabetes risk. Minimum and maximum GI values in the Experimental Group for breadbutter for males and females ranged from 43.04 to 145.93, and 44.11 to 140.53 respectively, while for masala Roti range was for males and Females 34.87 to 122.40 and 20.14 to 143.57 Respectively, In the control group the GI Range was 32.41 to 191.57 for Bread Butter and 18.72 to 231.22 for Masala Roti.

The Inter-individual variance for Bread Butter shows that males have an inter-variance of 31.69 and females 28.14 and for the control group higher inter-variance of 45.36 compared to the experimental group. Masala Roti Females in the experimental group show an inter-variance of 32.63 and males 27.67 and for the control group, a higher inter-variance of 57.73 compared to the experimental group. While Experimental groups have lower intra-variance overall for Masala Roti, with males 18.03 and females 13.25, and for Bread Butter with males 29.11 and females 16.82, and Control groups have intra variance for Mixed genders for bread butter 15.63 and For Masala Roti 18.31, indicating significance variability in glycemic responses over time.

Overall, Bread Butter elicited higher glycemic responses than Masala Roti in both Male and Female groups, with significant variability observed, especially for Masala Roti, Females in the experimental group. Gender and BMI were influential in glycemic variability, with females showing higher variability, particularly in the Masala Roti group. These findings highlight the complexity of glycemic responses and underscore the importance of individualized approaches when assessing the glycemic index of foods.

## Discussion

The findings of this study underscore the significant variability in glycemic index (GI) responses to Bread Butter and Masala Roti across different experimental conditions. By controlling key biological factors such as age, body mass index (BMI), and gender, the study provides valuable insights into inter and intra-individual differences in glycemic responses, shedding light on how these factors modulate postprandial glucose levels. In the age-controlled analysis, the results demonstrate that dietary interventions were more effective in reducing the glycemic index (GI) and variability for Masala Roti (MR) compared to Bread Butter (BB). The experimental group for MR showed lower mean GI, minimum and maximum GI, and significantly reduced variability in Masala Roti, with variability in intra-variance in the Masala Roti, [10] suggesting it may lead to rapid postprandial glucose spikes. These findings suggest that while age is a factor in glycemic response variability, it is not the sole determinant, with other physiological factors also playing significant roles.

The BMI-controlled analysis further reinforced these trends. Individuals in the experimental group, with lower BMI, exhibited a more uniform and pronounced glycemic response to Bread Butter. In contrast, the control group, with a wider BMI range, showed greater variability in their glycemic responses. This variability may be

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attributed to differences in insulin sensitivity, adiposity, and metabolic regulation. Notably, Masala Roti elicited a lower glycemic response in both the experimental group and control group, suggesting that BMI may impact the metabolism of complex carbohydrates differently than refined carbohydrates like Bread Butter. Bread butter shows high significance Intra Individual Variability The broader glycemic response range in the control group likely reflects greater heterogeneity in metabolic health, further complicating predictions of postprandial glucose levels.

Gender-controlled analyses revealed additional distinctions in glycemic responses. Males generally exhibited higher glycemic responses to breadbutter compared to females, which can be attributed to physiological differences such as greater muscle mass. [11], differential insulin sensitivity [12], and variations in sex hormones [13] That influences carbohydrate metabolism. Interestingly, males showed a lower glycemic response to Masala Roti compared to females, suggesting gender-specific differences in the digestion and absorption of complex carbohydrates. Another study suggested that women may show different glycemic

responses in adulthood due to hormonal variations across the menstrual cycle, affecting substrate utilization and insulin sensitivity. These factors highlight the complexity of gender differences in glycemic control and the importance of tailoring nutritional interventions to optimize health outcomes in both sexes.[14]

The significant intra-individual variability in females' responses to Masala Roti suggests that factors like energy expenditure and hormonal fluctuations contribute to more variable glycemic outcomes. Males, on the other hand, showed more consistent responses to Bread Butter but greater intra-individual variability to Masala Roti, likely linked to hormonal cycles. Both foods displayed distinct patterns of variability, but Masala Roti showed higher intra-individual variability, possibly due to fiber content, gut microbiota, and enzyme activity. Bread Butter showed greater inter-individual variability in younger individuals, highlighting the complexity of predicting glycemic responses to both refined and complex carbohydrates.

Overall, the results suggest that more uniform and elevated glycemic response across different biological parameters shows greater variability in glycemic responses both between and within individuals. The broader variability in the Experimental group indicates that factors like lifestyle habits, physical activity, and metabolic conditions may contribute to extreme glycemic fluctuations. The greater intra-individual variability, particularly with Masala Roti, emphasizes the need to account for fluctuating glycemic responses in dietary recommendations.

A study [15] Found that greater intra-individual variability 42.8% contributed more to the overall variability of glycemic index (GI) values for white bread than inter-individual variability 17.8%. This indicates that an individual's response to the same food can vary significantly over time. In our study, we observed significant inter-individual variability across different biological variables. For Masala Roti Showed significance variability for age, 15.05 and 15.60, suggesting that age influences glycemic responses more in controlled conditions. Similarly, BMI variability was highly significant for Bread Butter which was 8.04 and 17.51 indicating that body composition plays a significant role in GI variation. Regarding gender, females displayed significance variability of 13.25 and 18.31 compared to males These findings highlight the importance of considering individual biological characteristics when assessing glycemic variability in dietary interventions.

In a study by [16], the reliability of glycemic index (GI) measurements was evaluated, revealing moderate consistency. The intra-class coefficients (ICCs) were 0.50 for glucose and 0.49 for white bread, indicating a significant level of variability in glycemic responses among participants. While some individuals demonstrated stable responses, others experienced considerable day-to-day fluctuations in an incremental area under the curve (iAUC) values. This inconsistency complicates the ranking of foods by GI and raises concerns about its reliability as a dietary tool, suggesting that GI may not provide a stable metric for all individuals. These findings highlight the challenges of using GI in clinical and dietary settings for effective glycemic control. T.M.S. Wolever addressed criticisms regarding the glycemic index (GI) as a marker of carbohydrate quality, defending its methodology, accuracy, and relevance. He argued for the integration of GI into dietary guidelines, citing substantial evidence supporting its health benefits. Wolever suggested that including GI alongside other nutritional factors, such as whole grains and dietary fiber, would provide more comprehensive dietary advice. He also highlighted the importance of education and regulation surrounding GI to reduce misunderstandings and enhance its perceived health benefits among healthcare professionals and the public [17].

Similarly, [18] Emphasized the importance of glycemic load (GL), which considers both the quality (GI) and quantity of carbohydrates consumed. The authors argued that both GI and GL are critical metrics for assessing carbohydrate quality and its impact on health outcomes, thereby broadening the scope of how carbohydrate-rich foods should be evaluated in dietary recommendations.

Addressing biological factors like age, BMI, and gender did not significantly enhance the consistency of glycemic index (GI) values. While the controlled setting allowed for a more focused examination of these variations, additional research is required to extend these findings to broader populations with diverse dietary habits. A notable aspect of our study is that glycemic status, even among healthy individuals, plays a critical role in the variability of GI estimates, which may limit its clinical and public health significance.

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Previous studies have demonstrated that individual differences in GI responses can also be attributed to factors such as gut microbiota composition, enzyme activity, and even the timing and composition of meals. [19]. Our experimental data reinforces this notion, showing a considerable degree of fluctuation in GI responses, even when controlling such variables. This high level of intra-individual variability suggests that metabolic responses to carbohydrate intake are highly personalized and influenced by a complex array of physiological factors.

Furthermore, studies by [3] Have identified additional biological determinants, such as the insulin index and HbA1c levels, which help explain some of the variability in GI responses. However, these factors alone are not wholly predictive, suggesting a complex interplay of determinants that influence how an individual's blood glucose levels fluctuate post-consumption.

The significant differences in GI responses, especially the higher intra-individual variability in the experimental group, suggest that even in a controlled environment, individual metabolic responses to glucose can vary widely. These findings align with previous research indicating that GI responses are highly individualized and influenced by factors beyond the food's macronutrient composition. [20].

## Conclusion

This study highlights the individualized nature of glycemic responses to carbohydrate-rich foods, emphasizing the significant variability in glycemic index (GI) values. Despite controlling for age, BMI, and gender, inter- and intra-individual differences persisted, with Masala Roti showing more consistent responses than Bread Butter. Notably, Age, BMI, and Gender emerged as a significant factor influencing GI variability, with the control group exhibiting Significantly high intra-variance for both foods, suggesting that Age, body composition, and Gender play a critical role in modulating glycemic responses. This reinforces the need for personalized dietary interventions, as glycemic responses can vary widely even under controlled conditions, highlighting the importance of considering individual metabolic factors such as Age, Gender, and BMI in dietary recommendations.

## Limitations

Food's glycemic index (GI) can be influenced by various factors, such as preparation methods, processing, and the combination of foods consumed. As a result, GI values can vary even for the same food, leading some to argue that it may not be a consistently reliable guide for making food choices.

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## Author Contribution

NC wrote the manuscript; NC performed experiments, and prepared tables; SB performed experiments; PP performed experiments; HP assisted in the manuscript; DS collected data; MNA collected data; RS conceptualized the work and supervised

## Identification of the corresponding author

\* Indicates the corresponding Author

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## Conflict of interest

The authors declare no conflict of interest.

## Other Information

Ethical permission for the Clinical trial was obtained from an ethical committee of Gujarat University (Number: GU-ICE(NIV)/02/PhD/028; 23/06/2022)

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