RESEARCH ARTICLE

ESTABLISHING PROTOCOLS FOR GLYCAEMIC INDEX CLINICAL TRIALS IN AN ACADEMIC SETTING IN SRI LANKA

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ABSTRACT

The University of Peradeniya in Sri Lanka has initiated a Healthy Human Clinical Trial Unit (CTU) dedicated to post-prandial glycaemic response (PPGR) analysis. The present study, involving 42 participants aged 22-25 years, assessed the glycaemic index (GI) of four rice varieties: Super Kernel, Rathu Suduru, Ceylon Purple, and Red Fragrant. The results identified GI values ranging from 40 to 61, categorizing these varieties into low and medium GI groups, with Super Kernel and Rathu Suduru representing the lowest (40 ± 5.5) and the highest (61 ± 5.5) GI values, respectively. Glycaemic load (GL) values ranged from 8 to 12, falling into low and medium categories. This pioneering initiative validates the effectiveness of CTU in precise GI and GL evaluation for food labelling, which are essential for informing consumers about how different types of food impact the sugar levels in the bloodstream. Also, it is a precedent for future clinical nutrition research, emphasizing the need for consistent and harmonized methodologies in academic and regulatory contexts to support broader public health goals by providing accurate nutritional information to consumers. The processes and standards are detailed in a comprehensive guidebook (ISBN 978-624-94637-0-7), supporting the continuation and expansion of further PPGR studies.

Keywords: Clinical trial unit; Glycaemic index; Glycaemic load; Nutritional labelling; Post-prandial glycaemic response

INTRODUCTION

The assessment of dietary components and their impact on human health has garnered considerable attention in recent years, particularly concerning carbohydrate-rich diets (Jung and Choi, 2017). Among the pivotal indicators used to assist individuals in managing their blood glucose levels and enhancing overall health, the glycaemic index (GI) and glycaemic load (GL) stand out as crucial metrics (Pustozerov *et al*., 2020; Chekima *et al*., 2022). These indices have become increasingly valuable in light of the escalating frequency of diabetes mellitus and other non-transmissible diseases (NCDs) (Nusrianto *et al*., 2019; Cione *et al*., 2021), which demand effective preventative

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strategies and management approaches (Hawkes *et al*., 2013). In this context, researchers are placing a significant emphasis on dietary interventions that can mitigate the progression of such health challenges (Budreviciute *et al.*, 2020).

Low GI foods, characterized by their relatively modest glycaemic response (GR) following consumption, have emerged as a promising avenue for supporting these efforts, offering a range of health benefits compared to high GI counterparts (Durganaudu *et al*, 2020). As a consequence, the interest of the food and beverage industry in studying, comprehending, and quantifying the GI and GL of their products has surged, driven by a dual commitment to health-conscious consumers and sound nutrition (Barclay *et al.*, 2021; Lim *et al.*, 2021).

Notably, the inception of GI testing laboratories worldwide has revolutionized the assessment of these nutritional parameters. The Sydney University GI Research Service (SUGiRS) sets a pioneering precedent by establishing the world's first GI testing laboratory in 1995. SUGiRS provided not only a reliable commercial GI testing facility but also a GI determination protocol in strict accordance with internationally recognized guidelines. This endeavour, later emulated by various countries globally, enables food manufacturers to certify their products with a GI-tested label, signifying rigorous clinical assessment in research participants (Barclay *et al.*, 2021).

However, despite the global proliferation of GI testing laboratories, the need for such facilities remains unmet in certain regions. Sri Lanka, for instance, lacks an established GI testing research laboratory, hindering its ability to offer GI research services to the local food industry. This absence poses a critical limitation, as it restricts the assessment of the GI score of available food products and the application of GI labelling, which, in turn, could promote healthier food choices among consumers.

The Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, recognized this imperative need of the local food industry. The department sought to initiate a well-developed GI testing laboratory to address the dearth of a GI testing laboratory in Sri Lanka and foster healthier dietary options. This laboratory would provide essential GI testing services to the local food industry, enabling the measurement of GI values for various food items. The primary goal of this endeavour was to establish and manage a healthy human panel for clinical trials, specializing in the analysis of the post-prandial glycaemic response of food. Such a facility promises to significantly contribute to advancing nutritional science in Sri Lanka and facilitating healthier dietary choices for the benefit of its population.

MATERIALS AND METHODS

Establishment of a Healthy Human Clinical Trial Unit (CTU) for Advancing Research on Post-prandial Glycaemic Response

Recruitment of participants for clinical trials

The process of selecting potential subjects involved choosing first-year undergraduates from the Faculty of Agriculture, totalling 308 individuals.

An online questionnaire was distributed to all participants to collect information, including age, gender, medical history, dietary habits, and socio-demographic characteristics at the baseline visit. The study commenced with the measurement of subjects' height, weight, hip circumference (HC), and waist circumference (WC) using techniques used in standard methodologies and calibrated scales in the Nutrition Laboratory, Department of Food Science and Technology Faculty of Agriculture, University of Peradeniya, Sri Lanka. All anthropometric measurements were obtained with subjects dressed in lightweight clothing and without footwear. Height measurements (in cm) were taken using a wall-mounted stadiometer (seca 213, Munich, Germany), and weight (kg) using a

body weight scale (seca 813, Munich, Germany), WC (cm) and HC (cm) utilizing a measuring tape. Body Mass Index (BMI) was determined as the weight (kg) divided by the square of the height (m): BMI = Weight (kg)/ Height^2 (m^2). The mathematical formula, which division of WC (cm) by the HC (cm), was used to determine the waist-to-hip ratio (WHR). Simultaneously, consent was given with full understanding and awareness after being provided with information from eligible, healthy participants. This comprehensive dataset was crucial for thoroughly understanding the participants' health and background for the clinical trials.

Screening of enrolled participants

The screening of enrolled participants was carried out meticulously, following the ISO 26642:2010 (E) standard and adhering to established inclusion and exclusion criteria for GI studies (ISO 26642:2010). The initial screening process considered various factors, including anthropometric measurements, place of residence, long-term medical treatments, supplement usage, the presence of chronic medical conditions, food allergies, alcohol consumption, and smoking habits.

Participants having a BMI falling below 18.5 kg/m² or above 24.9 kg/m² were excluded (WHO, 2006). Furthermore, female participants with a WHR greater than 0.85 and male participants with a WHR above 0.90 were also not eligible to participate (WHO, 2008).

Participants with chronic health conditions under medical treatments and those with alcohol consumption and smoking habits were considered ineligible. Additionally, individuals with documented food allergies or those taking dietary supplements were excluded during the initial screening process.

Training of screened participants

Training sessions were conducted for the screened participants to provide them with an understanding of the GI test procedure. These sessions covered various aspects, including the details of the GI tests, the requirements for participating in a GI test, the responsibilities of the participants, and the role they would play in the GI study. Before obtaining consent, participants were received with a comprehensive information sheet outlining the study procedure. All participants were provided informed written consent following the training sessions.

Development of healthy human clinical trial unit

The healthy human CTU was organized into three clusters based on participants' degree programs, with each cluster comprising 14-15 individuals who provided their consent and were available after screening. Glucose tests were administered to these three clusters to identify the most appropriate panel for GI studies. The participants were instructed to consume a glucose solution, which consisted of 50 g of 100% pure glucose mixed with 250 mL of water, in a comfortable setting within 10 min. Blood samples were collected through finger prick at each 15 min in the first hour and each 30 min in the second hour following the ingestion of the glucose solution. Capillary blood was collected by finger-prick using sterile lancets; the blood sample was placed on a test strip inserted into a calibrated glucometer, and the direct reading was within 45 seconds by a registered nurse. Additionally, a bio-clinical evaluation was carried out on randomly selected screened participants using venous blood samples to obtain data about their blood glucose levels. This evaluation encompassed fasting blood sugar (FBS) and 2-hour post-prandial blood glucose (2h-BG) measurements.

Determination of *in vivo* Glycaemic Response in the Developed Human Clinical Trial Unit

Chemical analyses of selected rice varieties

The four rice varieties used in this study were named as 'Ceylon Purple,' 'Red Fragrant,' 'Super Kernel,' and 'Rathu Suduru.' The rice samples were ground to fine flour using a domestic mixer grinder (Panasonic, MX-AC400, Mumbai, India) and sieved through a 250 µm - mesh stainless steel sieve before analysis, and samples were stored in a refrigerator (LG, GR-282MF, Seoul, South Korea) for further analysis.

The total carbohydrate content of the rice samples was calculated by difference rather than by direct analysis. Under this approach, rice sample components (protein, fat, moisture, and ash) were determined individually per 100 g, then added and subtracted from the total weight of the rice sample (100 g). Here, the protein, fat, moisture, and ash content of the rice samples were determined using the methods of the Association of Analytical Chemists (AOAC) (AOAC, 2000).

All tests were conducted three times, and analytical findings were reported based on dry matter. The amount of the total dietary fiber of all rice samples was determined according to the AOAC 991.43 standard method (AOAC, 1991) using a buffer composed of 2-(N-morpholino) ethanesulfonic acid and Tris (hydroxymethyl)aminomethane (MES-TRIS). The samples were first defatted and dispersed in the buffer. Starch was gelatinized and partially broken down using alpha-amylase, followed by additional digestion with protease and amyloglucosidase enzymes. The total dietary fiber content was determined by subtracting the residue weight from the protein and ash weight, which is reported as a percentage of the original sample weight. The available carbohydrate content of each rice sample was determined as a subtraction of the weight of total dietary fiber from the weight of the total carbohydrates.

Preparation of rice samples

Preliminary studies were undertaken to determine the appropriate cooking time and water-to-uncooked rice ratio for each rice variety. All rice samples were cooked 30 minutes before the experiment using a domestic-scale rice cooker (Singer, SRC-18W -SL08701295, Zhanjiang, China).

Procedures for determination of glycaemic index and glycaemic load

The human CTU was initially used to assess the postprandial glycaemic response (PPGR) to rice, to provide practical experience to selected individuals and validate the results within the laboratory, considering the available GI of rice.

The procedures recommended by the FAO/ WHO (FAO/WHO, 1998) were followed to measure GR and calculate the GI value. Subjects from three clusters within the developed human CTU participated separately in the GI testing protocol.

Initially, all participants were given the reference food, prepared as 50 g of 100% pure pharmaceutical-grade glucose dissolved in 250 mL of water. The participants were required to report to the nutrition laboratory at 6:30 AM after fasting for 10 hours on the designated days. A glucometer (CLEVER CHEK TD-4279, MedNet EC-REP GmbH, Münster, Germany) was standardized by comparing finger-prick and capillary blood glucose levels of the selected human clinical panel. Fasting blood glucose levels were measured using the standardized glucometer, and information regarding their meals from the previous day was collected through a questionnaire.

On another day, participants were asked to consume the glucose solution for 10 min. Blood samples were taken every 15 minutes in the first hour and every 30 minutes in the second hour after consuming the glucose solution, using finger-prick blood samples collected by sterile lancets. The blood samples were placed on test strips inserted into a calibrated glucometer, providing straightforward readings within 45 seconds by a registered nurse. The blood glucose response curves of individuals in the three CTU clusters were analysed to select the most suitable cluster based on their GR to the standard glucose solution.

The most suitable healthy individuals were chosen for GI testing of four newly developed rice varieties by CIC Seed Farm, Palwehera, Dambulla, Sri Lanka named ‘Ceylon Purple’, ‘Red Fragrant’, ‘Super Kernel’, and ‘Rathu Suduru’. All rice samples were cooked 30 min before the experiment using a rice cooker. Participants were asked to consume the test food containing 50 g of available carbohydrate with 250 mL of water, and the same procedure was followed to obtain post-prandial blood glucose concentrations. GI and

GL scores were analysed geometrically using the GI and GL calculation software 'Glycaemic SPY' (Version 1.0.0).

Validation of the healthy human clinical trial unit

Inter-laboratory validation was conducted using GI data from one local and international GI testing laboratories. The GI data obtained from the CIC Food and Nutrition Research Laboratory in Palwehara, Dambulla, Sri Lanka, was used as the data from the local laboratory. The GI data obtained from the Glycaemic Index Research Unit (GIRU) at the Temasek Analytical Services Facility in Singapore was used as data from the international GI testing laboratory.

Establishing and maintaining a healthy human panel for long-term GI analysis

Ensuring the continuity of GI analysis for the specified foods in the Department of Food Science at the Faculty of Agriculture, University of Peradeniya, requires maintaining a healthy human panel for the long term. Therefore, comprehensive documentation of the entire process, including recruitment, screening, training, and the methodology for GI analysis, has been completed through a guidebook. This documentation is essential for the smooth operation of the laboratory and for reducing variations in results between different laboratories.

Ethics review statement

Institutional ethical approval (ECC/2022/ E/034) was taken from the Ethical Clearance Committee of the Faculty of Agriculture, University of Peradeniya. All methods adhered to relevant guidelines and regulations, as outlined in the International Organization for Standardization (ISO) concerning GI analysis. Assessors were required to report any indispositions or allergies, and if applicable, it was required to ensure that the affected subject did not participate in the tests. Furthermore, participants had the option to withdraw their consent without any justification.

Experimental Design

The present study was open, randomized, non-blinded, controlled study conducted at the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka. The participants were recruited from first-year university students at the Faculty of Agriculture as voluntary participation in the study. Various methods, including email circulation, social media messages, and word of mouth, were employed for recruitment. All participants received a written consent form to voluntarily join the study, along with a detailed explanation of the study procedures and the chance to inquire any doubts in relation to the experiment.

Statistical Analysis

Data collected from each type of rice were organized into a Completely Randomized Design (CRD) and analysed using analysis of variance (ANOVA). Tukey's post-hoc HSD test was used to find out the statistical significance among the *in vivo* GR of the three developed clusters and to identify the statistical significance between rice varieties ($P<0.05$). Anthropometric results and chemical composition of the rice varieties were presented as mean ± standard deviation (SD). GI values of rice varieties were presented as mean ± standard error (SE).

RESULTS

Establishment of a Healthy Human Clinical Trial Unit (CTU) for Advancing Research on Post-prandial Glycaemic Response

Recruitment of participants for clinical trials

The study conducted at the Faculty of Agriculture, University of Peradeniya, involved a meticulous participant recruitment process. From an initial group of 308 potential participants, 301 participants were enrolled in the investigation. This cohort comprised 202 female and 99 male participants. Exclusions were made for seven individuals based on criteria such as exceeding the age limit, lack of consent, or ongoing medical treatments that could influence study outcomes. Upon their first visit, each participant was provided with informed consent, ensuring ethical compliance

and understanding of the study's objectives and procedures.

To provide a comprehensive understanding of the participant profile, Table 1 details the demographic data of the enrolled individuals.

Table 1: Comprehensive overview of demographic characteristics of participants

Characteristics (unit)	(Mean ± SD) (N= 301)
Age (years)	23 ± 0.9
Body weight (kg)	54.6 ± 10.4
Height (m)	1.6 ± 0.1
BMI (kg/m^2)	21.1 ± 3.4
WC (cm)	71.3 ± 8.2
HC (cm)	89.9 ± 7.4
WHR	0.8 ± 0.1
Characteristics	**N (%)**
BMI category	
Underweight	71 (24)
Normal	194 (64)
Overweight	30 (10)
Obese	6 (2)
WHR category	
Male	
Low risk for metabolic implications	96 (97)
Moderate risk for metabolic implications	1 (1)
High risk for metabolic implications	2 (2)
Female	
Low risk for metabolic implications	157 (78)
Moderate risk for metabolic implications	37 (18)
High risk for metabolic implications	8 (4)

BMI: Body mass index (kg/m2), WC: Waist circumference (cm), HC: Hip circumference (cm), WHR: Waist to hip ratio, SD: Standard deviation

Table 2, on the other hand, offers insights into the demographic distribution and clinical backgrounds of the participants, highlighting the diverse nature of the study group.

The physical attributes of the recruited participants were as here: mean body weight 54.6 ± 10.4 kg, mean height 1.6 ± 0.1 m, BMI 21.1 ± 3.4 kg/m^2, WC 71.3 ± 8.2 cm, HC 89.9 ± 7.4 cm and WHR 0.8 ± 0.1. Most of the participators were in normal BMI range as 64% and highest percentage of both male and female were low risk for the metabolic

Table 2: Summary of demographic and clinical information in recruited participants

Characteristics	N (%)
Sex	
Female	202 (67)
Male	99 (33)
Ethnicity	
Sinhala	259 (86)
Tamil	35 (12)
Muslims	6 (2)
Other	1 (0)
Alcohol	
Never	270 (90)
Rarely	18 (6)
Occasionally	12 (4)
Usually	1 (0)
Smoking	
Never	259 (86)
Rarely	36 (12)
Occasionally	4 (1)
Usually	2 (1)
History of food allergies/ intolerances	
Yes	24 (8)
No	277 (92)
On supplement	
Yes	13 (4)
No	288 (96)
On medication	
Yes	7 (2)
No	294 (98)
Residence	
Hostels	258 (86)
Boarding place	12 (4)
Home	30 (10)
Other	1 (0)

implications as 97% and 78% respectively. The reason for using the body weight and height measurements were convenient and inexpensive, making them useful tools for gathering information from a large number of people in order to determine physical health conditions.

The demographic characteristics and medical conditions were necessary in recruiting participants because analysing these factors contributed to identifying subgroups that may make the findings more applicable to clinical practices. The highest percentages of participants were females, 67%, whereas males, 33%, based on the gender distribution. Ethnicity distribution showed the highest

proportion of Sinhalese, 86% of participants. Data on the alcoholic and smoking behaviour of recruited participants showed the highest percentage of non-alcoholic and non-smoking behaviours at 90% and 86%, respectively. Considering the data on food allergenic conditions, supplement intake and oral drug treatment, most participants were non-occurrence of all the above three conditions.

Selective screening and exclusion criteria: Shaping a cohort for future studies among enrolled participants

The screening process for enrolled participants is outlined in Table 3, offering a comprehensive view of their age and anthropometric details. Of the initial participants, 107 were excluded based on BMI values, leaving 194 within the normal BMI range of 18.5 - 24.9 kg/m². Notably, 48 participants at moderate and high risk of metabolic implications were excluded, while 253 were retained with low risk based on WHR values. Additionally, 73 participants were excluded due to alcoholic and smoking behaviours, and 258 participants progressed to the following screening phase as they resided in university hostels. A further 24 respondents with food allergens and intolerances were excluded. The screening process also identified 20 participants using oral drug treatments and supplements who were subsequently excluded. Following these meticulous screening procedures, 99 participants were screened as candidates for future studies.

Table 3: Age and anthropometric measurements of screened participants

Characteristics (unit)	(Mean ± SD) (N=99)
Age (years)	23.0 ± 0.9
Body weight (kg)	54.0 ± 6.7
Height (m)	1.6 ± 0.1
BMI (kg/m^2)	21.1 ± 1.8
WC (cm)	70.3 ± 5.3
HC (cm)	90.4 ± 5.0
WHR	0.8 ± 0.1

BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, SD: Standard deviation

The age and physical attributes of the screened participants were as follows: age 23.0 ± 0.9 years, mean weight 54.0 ± 6.7 kg, height 1.6 ± 0.1 m, BMI 21.1 ± 1.8 kg/m2, WC 70.3 ± 5.3 cm, HC 90.4 ± 5.0 cm, and WHR 0.8 ± 0.1. Data on BMI and WHR were obtained in the normal range and low risk for metabolic implications, respectively.

Training of healthy human clinical trial unit

Ninety-nine screened participants qualified for training sessions, with 83 actively participating based on their availability. Following the training, 63 participants provided informed consent for future GI studies. Additionally, 42 participants were identified and proposed as healthy human CTUs for prospective GI studies, considering both consent and availability.

Development of healthy human clinical trial unit

A healthy human CTU was established, comprising 42 participants selected from those who were both screened and trained, ensuring a well-rounded representation. The age and anthropometric details of this developed healthy human CTU are detailed in Table 4.

Table 4: Selected age and anthropometry for development of CTU.

Characteristics (unit)	(Mean ± SD) (N=42)
Age (years)	23.0 ± 0.8
Body weight (kg)	54.4 ± 6.1
Height (m)	1.6 ± 0.1
BMI (kg/m^2)	20.9 ± 1.7
WC (cm)	71.5 ± 5.1
HC (cm)	89.8 ± 5.0
WHR	0.8 ± 0.1

BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, SD: Standard deviation

According to the data, the age and physical attributes of the healthy human CTU were as follows: age 23.0 ± 0.8 years, mean body weight 54.4 ± 6.1 kg, height 1.6 ± 0.1 m, BMI 20.9 ± 1.7 kg/m^2, WC 71.5 ± 5.1 cm, HC 89.8 ± 5.0 cm and WHR 0.8 ± 0.1. The healthy human CTU was made into three clusters based on participants' degree

programs, with 14 individuals for each cluster who provided their consent and were available after screening. According to the procedures recommended by the ISO 26642:2010 (E) standard, the minimum number of subjects involved in a GI study is 10. However, outliers must be considered and not included those data in GI calculation. Therefore, 14 subjects were chosen for each cluster based on consent and availability.

Participants for this study were chosen based on criteria that included having a normal BMI for their age, weight, and height. Factors such as WC, HC, and WHR were considered to ensure subjects were considered in the normal range. According to the results of bio-clinical evaluations conducted on 14 randomly selected participants from this healthy human CTU, the mean values of FBS and 2h-BG tests were 85.7 ± 6.5 mg/dL and 70.6 ± 19.1 mg/dL respectively. Blood glucose levels were within the normal range in both FBS (74-92 mg/dL) and 2h-BG (50-106 mg/dL), which indicates the overall well-being of the subjects. Furthermore, healthy subjects in GI clinical trials help prevent complications and obtain accurate and precise test results.

Determination of *In Vivo* Glycaemic Response in the Developed Human CTU

Chemical analyses of chosen rice varieties

The first step in determining the *in vivo* GR involved calculating the available carbohydrate content of the chosen rice varieties. This was achieved by deducting the dietary fiber from the total carbohydrate content. The data for total carbohydrate, available carbohydrate, and dietary fiber content as g /100 g of chosen rice varieties are presented in Table 5. Substantial variations were observed in the nutrient content of the analyzed foods concerning total carbohydrates, available carbohydrates, and dietary fiber across the four rice varieties. Carbohydrate analysis showed that the Super Kernel rice variety had the highest available carbohydrate level at 76.3 g/100 g, whereas Ceylon Purple had the least level at 71.2 g/100 g. Dietary fiber analysis revealed that the Ceylon Purple rice variety had the highest dietary fiber level at 7.6 g/100 g, while Red Fragrant had the least level at 5.0 g/100 g. The fat and ash level were present in higher amounts in the Red Fragrant rice variety than in other chosen rice varieties. Super Kernal rice variety had the least level of ash content at 0.69 ± 0.01%. The Ceylon Purple rice variety had the highest protein level at 7.4 ± 1.3% and the lowest level of moisture and fat at 11.8 ± 0.8% and 1.0 ± 0.2%, respectively.

Preparation of rice samples for GI studies

The GI study relied on 50 g of available carbohydrates in every rice variety, which was calculated as the total carbohydrate content minus the dietary fiber. Therefore, preliminary studies of selected rice varieties were conducted to determine the appropriate cooking time and the ratio of water to uncooked rice for each variety. The results of the preliminary studies aimed at establishing the serving portion of every test food containing 50 g of available carbohydrates were studied (Table 6).

Table 5: **Proximate composition of selected rice varieties**

Rice composition	Rice varieties (mean ± SD)			
	Super Kernel	**Rathu Suduru**	**Ceylon Purple**	**Red Fragrant**
Moisture content (d.b %)	12.8 ± 0.3^{ab}	14.0 ± 0.2^{a}	11.8 ± 0.8^{b}	12.9 ± 0.3^{ab}
Ash content (d.b %)	0.69 ± 0.01^{d}	0.84 ± 0.01^{c}	0.98 ± 0.02^{b}	1.01 ± 0.01^{a}
Protein content (d.b %)	3.7 ± 0.6^{b}	3.7 ± 0.3^{b}	7.4 ± 1.3^{a}	4.9 ± 0.2^{b}
Fat content (d.b %)	1.1 ± 0.2^{b}	1.3 ± 0.3^{ab}	1.0 ± 0.2^{b}	1.8 ± 0.2^{a}
Total carbohydrate content (g/100g)	81.7 ± 0.7^{a}	80.3 ± 0.2^{b}	78.8 ± 0.4^{c}	79.3 ± 0.1^{bc}
Dietary fiber content (g/100g)	5.4 ± 0.2^{c}	6.6 ± 0.3^{b}	7.6 ± 0.2^{a}	5.0 ± 0.2^{c}
Available carbohydrate content (g/100g)	76.3 ± 0.4^{a}	73.7 ± 0.1^{c}	71.2 ± 0.2^{d}	74.3 ± 0.1^{b}

All values are expressed as the mean ± SD of three replications. Means in a given row marked with the same superscript are not statistically significant ($P > 0.05$).

Table 6: Weight of uncooked and cooked rice per person and cooking time of the test rice

Test rice	Uncooked rice (g)/ person[a]	Cooking time (min)[b]	Water: rice	Rice (g)/Person [c]
Super kernal	66.0	45	1.5:1	170
Rathu suduru	68.0	40	1.5:1	171
Ceylon Purple	70.0	40	1.5:1	175
Red fragrant	67.0	40	1.5:1	182

a Amount of uncooked rice (g) containing 50 g of available carbohydrate per person

b Cooking time (min) for 16 serving portions

c Serving portion of cooked rice (g) per person

It is important to note that these portion sizes may vary depending on the quantity of available carbohydrates in each food. The serving sizes of the test foods varied between 170 g for Super Kernel and 182 g for Red Fragrant.

Determination of glycaemic index

Glucose was used as the standard food sample, and it contained 50 g of available carbohydrates. The mean blood glucose response of the developed healthy human CTU is presented in Table 7 as three clusters at different time points observed after consuming the reference food, glucose. According to Table 7, the reference food (100% pure glucose) reached its peak blood glucose value (147.7 ± 25.0 mg/dL) in cluster 2 at 45 min, while in clusters 1 and 3, it reached peak blood glucose values of (146.5 ± 19.4 mg/dL) and (146.0 ± 21.9 mg/dL), respectively, at 30 min. Figure 1 illustrates the blood glucose responses of the three clusters of the developed human CTU, based on the glucose test results using the reference food, conducted following the ISO 26642:2010 (E) standard.

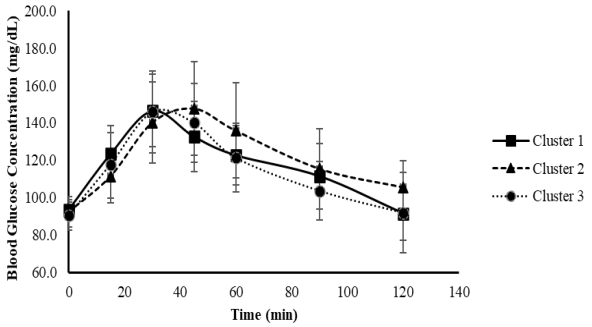

Figure 1: Average blood glucose response curves of healthy human individuals of three clusters

Based on the findings, there is no statistical significance (P>0.05) between the developed three clusters as measured by Tukey's post-hoc HSD test. However, the blood glucose response can be influenced by various other factors, including the physical composition of seemingly similar foods, the food processing or preparation, and the pattern of meal consumption (whether meals are consumed individually or as combinations). These factors contribute to the significant variation observed both between individuals and within individuals over time in terms of GR (Matthan

Table 7: The mean blood glucose response (mg/dL) of the developed healthy human CTU at different time points observed after consuming the reference food (glucose) as mean ± SD of N study participants of three clusters

Time (min)	Cluster 1 (N=14)	Cluster 2 (N=14)	Cluster 3 (N=14)
0	93.3 ± 4.5[a]	92.4 ± 8.3[a]	90.7 ± 8.3[a]
15	123.3 ± 11.6[a]	111.5 ± 11.8[a]	117.9 ± 20.5[a]
30	146.5 ± 19.4[a]	140.3 ± 21.7[a]	146.0 ± 21.9[a]
45	132.6 ± 18.7[a]	147.7 ± 25.0[a]	140.1 ± 21.1[a]
60	122.7 ± 15.9[a]	135.9 ± 25.5[a]	121.4 ± 18.5[a]
90	111.5 ± 17.6[a]	115.4 ± 21.5[a]	103.6 ± 15.8[a]
120	91.4 ± 14.4[a]	105.5 ± 14.2[a]	91.9 ± 21.6[a]

All values are expressed as the mean ± SD of three replications. Means in a given row that are marked with the same superscript are not statistically significant (P>0.05).

et al., 2016). Variations in GR levels, both within and between individuals, are apparent even when assessed under standardized conditions. (Bellmann *et al.*, 2018).

The PPGR for each rice variety are presented in Figure 2. The reference food reached its peak blood glucose value (142.9 ± 17.4 mg/dL) at 45 min, coinciding with Super Kernel (124.3 ± 15.6 mg/dL), exhibiting its peak blood glucose response at 45 min. Additionally, the reference food attained its peak blood glucose value (149.8 ± 20.4 mg/dL) at 30 min, while Red Fragrant (137.8 ± 21.1 mg/dL) reached its peak blood glucose response at 45 min. Furthermore, the reference food peaked at (156.1 ± 18.6 mg/dL) at 30 min, and Ceylon Purple (130.5 ± 12.5 mg/dL) also exhibited its maximum blood glucose value at 30 min. Similarly, the reference food reached its peak blood glucose value (151.6 ± 16.6 mg/dL) at 30 min, with Rathu Suduru (130.5 ± 24.2 mg/dL) showing its maximum blood glucose value at 30 min.

The mean values for GI and GL of each rice variety and their classification are presented in Table 8. The results indicate that GI values of tested four rice varieties in the range of 40 to 61, classifying them as low and medium GI rice. The Super Kernel rice variety showed the lowest GI value at 40 ± 5.5 (low GI), while the Rathu Suduru rice variety showed the highest GI value at 61 ± 5.5 (medium GI). The GI values for Ceylon Purple and Red Fragrant were 47 ± 4.9 (low GI) and 54 ± 3.8 (low GI), respectively. Additionally, the GL values refer to four rice varieties varied from 8 to 12, placing them into low and medium GL categories. Rathu Suduru showed the highest GL value of 12 (medium GL), and Super Kernel showed the lowest GL value of 8 (low GL), while Ceylon Purple and Red Fragrant showed GL values of 9 (low GL) and 10 (low GL), respectively.

Validation of the Healthy Human Clinical Trial Unit

The comparison of GI test results for selected rice varieties with GI data obtained by local and international GI testing laboratories for the same rice varieties is shown in Table 9.

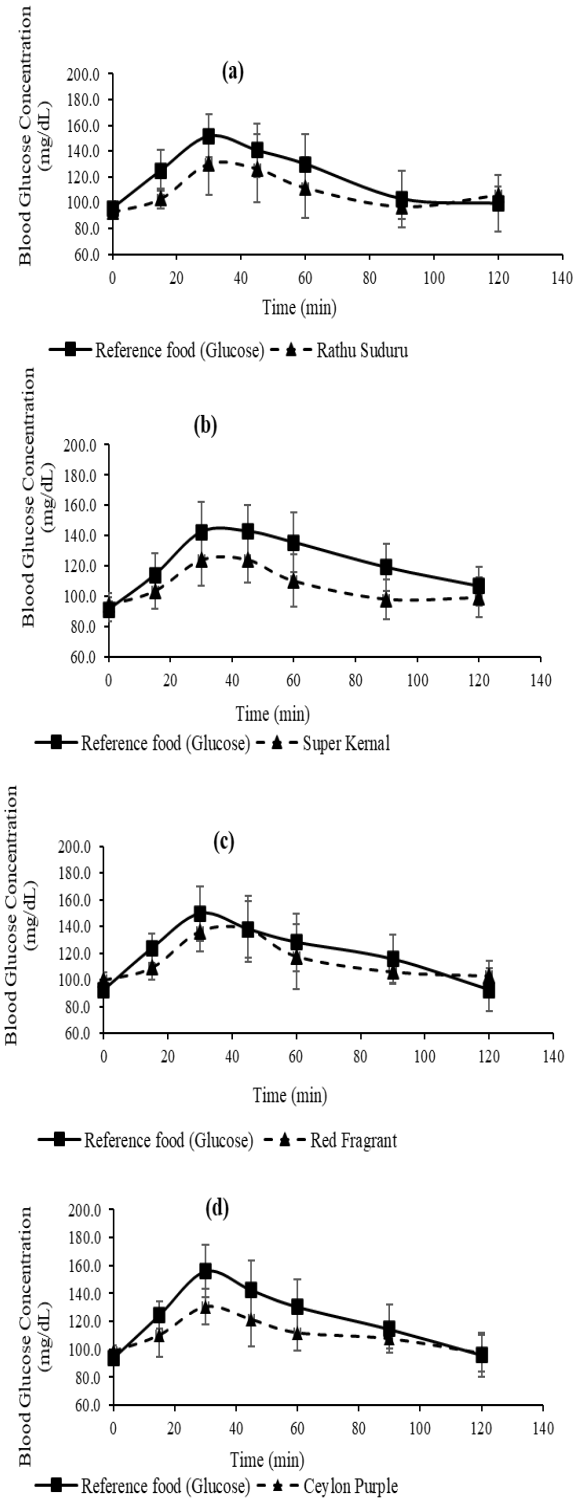

Figure 2: Postprandial blood glucose response (mg/dL) of each rice variety. (a) Rathu Suduru, (b) Super Kernel, (c) Red Fragrant, (d) Ceylon Purple

Table 8: Glycaemic index and glycaemic load of the selected rice varieties

Test rice	N	GI ± SE (%)	Classification	Portion size (g) *	Recommended serving size (g) **	GL	Classification
Super Kernel	16	40 ± 5.5[b]	Low	170	65	8	Low
Rathu Suduru	11	61 ± 5.5[a]	Medium	171	65	12	Medium
Ceylon Purple	10	47 ± 4.9[ab]	Low	175	65	9	Low
Red Fragrant	10	54 ± 3.8[ab]	Low	182	65	10	Low

N = Number of participants SE= Standard error
Values within a column marked with common superscript letters are not statistically significant at P>0.05, as determined by Tukey's post-hoc HSD test.
* Portion size for an individual which contains 50 g available carbohydrates
** Recommended serving size of rice per individual (Ministry of Health, 2021)

Table 9: Inter-laboratory validation of glycaemic index values

Rice variety	Laboratory	Mean GI ± SE (%)
Rathu Suduru	**Nutrition Laboratory, DFST, UoP**	**61 ± 5.5[a] (Medium)**
	CIC Food and Nutrition Research Laboratory, Dambulla, Sri Lanka	57 ± 7.9[a] (Medium)
Ceylon Purple	**Nutrition Laboratory, DFST, UoP**	**47 ± 4.9[a] (Low)**
	CIC Food and Nutrition Research Laboratory, Dambulla, Sri Lanka	44 ± 0.9[a] (Low)
		47 ± 0.9[a] (Low)
		41 ± 5.3[a] (Low)
Red Fragrant	**Nutrition Laboratory, DFST, UoP**	**54 ± 3.8[a] (Low)**
	CIC Food and Nutrition Research Laboratory, Dambulla, Sri Lanka	52 ± 5.3[a] (Low)
	Glycaemic Index Research Unit, Temasek Analytical Services Facility, Singapore	55 ± 5.0[a] (Low)

DFST: Department of Food Science and Technology, UoP: University of Peradeniya, GI: Glycaemic index, SE: Standard error.
Values within a column marked with common superscript letters are not statistically significant at P>0.05, as determined by Tukey's post-hoc HSD test

There was no statistical significance (P>0.05) among the GI value of the Rathu Suduru rice variety obtained from the healthy human CTU and the GI data obtained by CIC Food and Nutrition Research Laboratory, Dambulla. Both GI values fall into the medium GI category. Similarly, there was no statistical significance (P>0.05) among the GI value of the Ceylon Purple rice variety obtained from the healthy human CTU and the GI data obtained by CIC Food and Nutrition Research Laboratory, Dambulla. All GI values for the same variety fall into the low GI category.

Moreover, there was no statistical significance (P>0.05) among the GI value of the Red Fragrant rice variety obtained from the healthy human CTU and the GI data obtained by CIC Food and Nutrition Research Laboratory, Dambulla. The Red Fragrant rice variety had also been tested in the Glycaemic Index Research Unit, Temasek Analytical Services Facility, Singapore, and all the GI data showed no statistical significance (P>0.05) in GI scores. However, there was no previously available data obtained by local and international GI testing laboratories for Super Kernal rice variety for validation purposes.

Establishing and Maintaining a Healthy Human Panel for Long-Term GI Analysis

A comprehensive documentation of the entire process, including recruitment, screening, training, and the methodology for GI analysis, has been compiled into a guidebook (ISBN 978-624-94637-0-7). The research outcome provides a harmonized and standardized procedure for research investigators and other stakeholders in different academic institutions to advance PPGR research in educational activities or regulatory services.

DISCUSSION

The establishment of the GI healthy human CTU at the University of Peradeniya, Department of Food Science and Technology, represents a significant stride in addressing

the nutritional needs of Sri Lanka. The recruitment of 301 participants, including a diverse mix of 202 females and 99 males, underscores the robustness of this initiative. While the exclusion of certain participants based on age and ongoing medical treatments ensures a focused and relevant cohort for GI studies, it does raise questions about the broader applicability of the findings to diverse demographic groups, as noted by Stuart *et al*., (2011).

The strategic selection criteria, which prioritized participants with normal BMI and low WHR values, demonstrate a methodical approach in forming a suitable group for in-depth GI investigations, as per the guidelines of Kramer *et al*., (2012). This choice aligns with prevailing health standards (Li *et al.*, 2020), though the exclusion of individuals at moderate or high metabolic risk poses challenges in extrapolating the results to populations with varying health profiles (Kivimäki *et al*., 2023).

The formation of the healthy human CTU, comprising 42 participants, embodies a pragmatic method for long-term GI analysis, maintaining a representative sample within normal health ranges. The emphasis on thorough bio-clinical testing prioritizes participant safety and the validity of the study (Kumar *et al.*, 2022), despite potential implications on the generalizability of blood glucose responses due to normal FBS and 2h-BG levels (Dekkers *et al.*, 2008). It also offers challenges to the generalizability of the findings. Therefore, researchers are required to balance the need for essential testing to produce results that apply to a wider population (Geijselaers *et al.*, 2017).

Analyzing the GI and GL values of rice varieties offers crucial insights into their impact on blood sugar levels, distinguishing between low and medium GI foods (Kumar *et al.*, 2022). Moreover, it provides benefits to manage personalized nutrition, dietary planning and chronic diseases management (Kumar *et al*., 2024). Therefore, the observed GI and GL values suggest potential health benefits, including reducing the chance of getting cardiovascular diseases and type-2 diabetes (Kaur *et al.*, 2021).

The validation of GI results with local and international laboratories underscores the reliability of the healthy human CTU (RamyaBai *et al.*, 2019). Therefore, the highlighted evidence is when conducting GI testing with high CHO foods under standardized methods; The SD of GI values among different laboratories is about nine (Atkinson *et al.*, 2021). Furthermore, the validation of GI data for the Super Kernel rice variety needs to be addressed through future investigations. Documenting the entire process in a guidebook furthers the standardization of PPGR research, extending its utility to regulatory services and educational sectors. Moreover, the guidebook facilitates collaborative research among different institutions and countries and compares data from many studies, which lead to a wider range of applications. The recommendation for regular inter-laboratory and intra-laboratory validations using the established CTU aligns with best practices for accuracy and precision in test results. This step is critical in paving the way for an accredited GI testing laboratory in Sri Lanka, boosting the credibility of future research endeavours.

Moreover, the commitment to periodic bio-clinical evaluations and fitness assessments of participants highlights ethical research practices (Crabu, 2021). Investigating age-related impacts on GI values and exploring inter-individual and intra-individual variability in GR among healthy individuals will contribute to a more comprehensive understanding of the study outcomes, aligning with the evolving landscape of nutritional research.

CONCLUSION

In summary, this research marks the successful establishment of a healthy human CTU within the Faculty of Agriculture at the University of Peradeniya. The CTU, divided into three distinct subject clusters as cluster 1, cluster 2 and cluster 3, emerges as a crucial resource for future investigations into the GI

of various foods. Its role extends beyond academic research, offering potential commercial applications that could foster income generation within the academic setting in Sri Lanka.

The establishment of such a GI centre carries significant implications for the food industry. It provides essential facilities for the analysis of GI and GL values in commercially available food products, thereby playing a crucial function in the food labelling procedure. The recommendations of this study highlight the necessity of continuous use and enhancement of the CTU. Regular analysis and validation of GI values, both within and between laboratories, are imperative to ensure the accuracy and precision of the results. Such measures not only bolster the credibility of the CTU but also lay the foundation for creating an accredited GI testing laboratory in Sri Lanka.

Emphasizing geographical specificity in validation processes and the importance of periodic bio-clinical evaluations of CTU participants, the study upholds high standards of robustness and ethical research practices. Future research endeavours should focus on understanding the age-related effects on GI values and the variability of GR among individuals. These efforts will deepen our understanding of GI outcomes and drive continuous methodological advancements in the field.

In essence, this study's accomplishments and forward-looking recommendations underline the pivotal role of the healthy human CTU. It stands as a dynamic and versatile instrument, propelling research, industry practices, and public health initiatives forward in Sri Lanka.

AUTHOR CONTRIBUTION

NMAIN designed the study, gathered and analysed data, and developed the manuscript draft. GMS designed the concept of the study, planned experiments, guided the study, and reviewed and revised the manuscript. SDM designed experiments and supervised the study. DCSG planned the experiments and supervised the study. APW conducted experiments. TND reviewed and revised the manuscript. NBLS read and edited the manuscript. HKATK and KDUM performed the human clinical studies procedure. DSDZA reviewed and revised the manuscript. AC reviewed and revised the manuscript. BDRP reviewed and revised the manuscript. All authors reviewed and accepted the manuscript.

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