Development of Low Glycemic Index Foods and Their Glucose Response in Young Healthy Non-Diabetic Subjects

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ABSTRACT: Development of low glycemic-foods is important in the prevention and management of type 2 diabetes. In this context, we prepared four test foods (TFs) (two mixed mini-meals and two breakfast items) with low glycemic-components and assessed their glycemic index (GI) in young healthy non-diabetic volunteers with mean age of 29 yr, body mass index of 24 kg/m², and fasting plasma glucose levels less than 4.62 mmol/L. Volunteers were given 50 g of glucose, as a reference food (RF) on the first day, and TFs, i.e. TF1 (mixed mini meal: roti made of wheat flour and chana dal+ curd), TF2 [mixed mini meal made of wheat, pearl barley, and Bengal gram flour (besan) mix with chana whole (un-husked chana+curd)], TF3 (pearl barley rawa upma), and TF4 (wheat rawa upma) were given 2-day intervals in the same order. Glucose levels at fasting conditions and after the consumption of RF and TFs at different time intervals (15, 30, 45, 60, 90, and 120 min) were measured, and the incremental area under curve (IAUC) for glucose and GI of the TFs were calculated. The glucose IAUC values at different time points were highest for TF2 (GI=71.9±7.4), while all other TFs had comparable GI in the range of 53.7~54.9. Among the various TFs, TF1, TF3, and TF4 exerted low to moderate glycemic response, and thus can be classified as low glycemic-foods. Nevertheless, these foods need to be tested for their efficacy in controlling and/or managing hyperglycemia and glucose over-load in diabetic subjects.

Keywords: glycemic index, diabetic, diet, insulin

INTRODUCTION

During the past two decades due to globalization, there has been a rapid rise in the prevalence of chronic degenerative diseases, especially type 2 diabetes across the world, and India is no exception (1). This has been attributed to increased consumption of refined foods and simple sugars in place of complex carbohydrates (2). Therefore, to contain the pandemic rise of this metabolic disease, consumption of complex carbohydrates with low glycemic index (GI) has been recommended.

As early as 1981, Jenkins et al. (3) have introduced the concept of GI and developed this as an alternate ranking system for carbohydrate foods based on their ability to raise plasma glucose levels. Low GI foods elicit lower glucose response in contrast to high GI foods. Further, the amount of food consumed determines the postprandial hyperglycemia, and thus the glycemic load (GL) takes into account the amount of food consumed along with GI. Despite controversy over the usefulness of the GI and GL (4) and their relevance to disease development, several agencies such as the Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), American Diabetes Association, Canadian Diabetes Association, and Diabetes UK, concerned with health promotion, have extended qualified support to this concept and a few countries like Australia, Sweden, and France have incorporated GI into their dietary guidelines. This has lead to the development international tables of GI and GL for individual food items such as different types of bread, boiled rice, noodles, breakfast cereals (corn flakes and different porridges), fruits and fruit products, vegetables, dairy products, legumes, snack products, and sugars (5).

However, these tables cannot be translated to Indian context, as the type of food consumed in India is complex, consisting of various components with marked differences in carbohydrate source. Importantly, the source
of carbohydrate which determines its quality shows regional differences. For instance, wheat is consumed in large quantity in northern India, whereas rice is more common in southern India (6,7). Moreover, in India the practice of eating mixed meals consisting of cereals (whole wheat flour roti), pulses in the form boiled legumes/dal and milk products (curd/yogurt) is very common. Some commonly consumed food patterns in different parts of India are available in scientific reports (8,9) and information on food composition values of commonly consumed foods are available on the webpage of our Institute (10). If GI data for these food preparations are made available, it would help the people to make better food choices for optimal health.

Therefore, to contain the pandemic rise of this metabolic disease, consumption of complex carbohydrates with low GI, based on the food composition, has been recommended. However, GI and GL for ingested foods/recipes have not been thoroughly studied. Thus, the main aim of this study was to develop recipes using various cereal-based ingredients, and to assess their GI and GL in young, healthy non-diabetic volunteers.

**MATERIALS AND METHODS**

**Subjects**
The current study was conducted using internationally recognized GI methodology (11-13). An information session was held for interested individuals. Volunteers, who were willing and having body mass index (BMI) <25 kg/m², fasting plasma glucose <5.55 mmol/L, and devoid of thyroid and other endocrine disorders or food allergies, were enrolled into the study. Twelve normal, healthy, young, and non-diabetic male subjects (working in the Institute) aged between 20~35 years with mean BMI 24 kg/m² participated in the study. The study was approved by the Institutional Ethical Committee (12/II/2014), National Institute of Nutrition, Hyderabad, Telangana, India. Informed written consent was obtained from all subjects. The study was conducted according to the guidelines laid down in the declaration of Helsinki (14).

**Experimental protocol**
At the initiation of the study, height, weight, and blood pressure of the subjects were measured. Prior to the initiation of the experiment, the taste and acceptability of foods were assessed. All foods were found to be highly acceptable. In the presence of a dietitian, subjects were asked to complete a ‘GI test questionnaire’. Through this questionnaire, information regarding the time when the last night’s meal was eaten, details about the type and quantity of food consumed, state of the subject’s health, type of physical activity on the previous day, duration of sleep, any unusual events, and the mode of transportation were collected. The subjects were also asked to keep pre-test conditions as uniform as possible, with regard to the previous day’s activities and timing and composition of evening meals and snacks. If there was any deviation, the appointment was rescheduled. The subjects visited the centre in the morning after a 12-h overnight fast and fasting blood samples were collected. The reference food (RF) and test foods (TFs) were given to the subjects on different days. Further, from the first bite of either RF or TFs consumption, blood was drawn at various time intervals such as 15, 30, 45, 60, 90, and 120 min. The RF was consumed on the first day, and the TFs were randomized and designated as TF1, TF2, TF3, and TF4. The TFs were consumed within 10~15 min, along with 200 mL of water. The two mini meals (TF1 and TF2) were matched with respect to available carbohydrate (50 g), dietary fiber (11 g), protein (13 g), and fat (6 g), provided 350 kcal, and were prepared under identical conditions like dry roasting for roti and pressure cooking for dal preparation. Similarly, the two breakfast items TF3 (pearl barley rawa upma) and TF4 (wheat rawa upma) were prepared by boiling and had comparable available carbohydrate (50 g), dietary fiber (11~12 g), protein (9~10 g), and fat (7 g), provided 310~345 kcal (Table 1). The test sessions were separated by at least two days. The experimental methods were as described by Woliver et al. (15).

**Body composition analyses**
Body composition was determined by the bio-impedance method, using a segmental body composition analyzer.
(Tanita BC-418, Tanita India Pvt Ltd., Mumbai, India). This instrument directly provides basal metabolic rate, fat percent, fat mass, fat free-mass, total body water, and trunkal fat percent.

**RF**

Fifty grams of glucose (glucose monohydrate; glucon-D, Heinz India Pvt Ltd., Mumbai, India) dissolved in 200 mL water were used as the RF.

**TF composition and preparation**

Typical South Indian mini meals, which consist of cereal and pulse in 5:1 proportion, along with curd (fermented milk/yogurt) and breakfast items, upma made of wheat or pearl barley rawa were provided. The mini meals were prepared with 50 g of available carbohydrate to assess the GI, which is equivalent to the RF; glucose of 50 g. Available carbohydrate was calculated using food composition tables (16) where dietary fiber was subtracted from total carbohydrate content.

TF1, the mixed mini meals was prepared as roti using 72 g whole wheat flour and given with split dehusked chana (chick pea/Bengal gram; 15 g) dal and curd (50 mL). Two rotis were prepared using 72 g of wheat flour by mixing with water (75 mL) to make dough and rolled into two rotis and dry-roasted on a heated pan. Dehusked split chana dal (15 g) was pressure-cooked, salt, pepper, and 2.5 g oil were added. Volunteers were asked to consume two rotis along with dal and curd (which provided 50.2 g of available carbohydrate) within 15 min.

Similarly, a second TF2 was also a mixed mini-meal, prepared as roti, by replacing the whole wheat flour with mixed flour of wheat, pearl barley and chick pea with the respective proportions of 40 g, 27 g, and 6 g. Further, whole chick pea/chana was provided instead of dehusked chana (chick pea) along with curd.

TF3 and TF4 (breakfast items) namely barley upma and wheat upma were prepared using only pearl barley rawa and wheat rawa, respectively. TF3, the pearl barley rawa upma was prepared by using 77 g of pearl barley rawa (coarse flour) and 5.7 g groundnut oil and salt. The methodology reported by Brouns et al. (12). This has also taken into consideration the elegant study of Wolever et al. (15) for measuring inter-laboratory variability in GI measurements and final recommendation that ten subjects is sufficient to provide reasonable degree of power and precision for most purposes of measuring GI with reasonable cost. Based on this, the difference in GI that can be detected with 80% statistical power at the level of $P<0.05$ (two tailed) by number of subjects and mean GI, we have arrived at a number of ten, giving a margin for possible dropouts, 12 subjects were enrolled into the study.

**Glucose measurement and GI calculation**

Blood glucose levels were measured using glucose-strips (Accu-Chek, Roche Diagnostics GmbH, Mannheim, Germany), and plasma glucose levels were quantified using a commercially available kit (Biosystem, Barcelona, Spain). Duplicate readings were accepted, if the difference between two glucose values was less than 0.3 mmol/L for each time point. The two or three similar (i.e. within 0.3 mmol/L) readings were then averaged to obtain the glucose response for that time point. A two-hour glucose response curve was constructed and the incremental area under glucose response curve (IAUC) was calculated geometrically, using the trapezoid rule (FAO/WHO) (2).

For each subject, the GI value for each TF was calculated by expressing each subject IAUC after the TF ingestion, as a percentage of the same subject’s mean RF IAUC.

GI and GL for TFs were calculated using the formula:

$$
\text{GI value for the TF (\%) = } 100 \times \frac{\text{IAUC glucose value for the TF}}{\text{mean IAUC value for the same available carbohydrate portion of the RF}} 
$$

$$
\text{GL=IAUC glucose value}\times\frac{50}{100} 
$$

**Plasma insulin assay**

Plasma insulin levels were measured in a sub-sample of the five subjects after TF4 consumption by radioimmunoassay kit method (Bhabha Atomic Research Centre, Mumbai, India).

**Statistical methods**

**Sample size:** Sample size was calculated according to GI methodology reported by Brouns et al. (12). This has also taken into consideration the elegant study of Wolever et al. (15) for measuring inter-laboratory variability in GI measurements and final recommendation that ten subjects is sufficient to provide reasonable degree of power and precision for most purposes of measuring GI with reasonable cost. Based on this, the difference in GI that can be detected with 80% statistical power at the level of $P<0.05$ (two tailed) by number of subjects and mean GI, we have arrived at a number of ten, giving a margin for possible dropouts, 12 subjects were enrolled into the study.

**Statistical analysis:** Data are presented as mean±standard error of mean (SEM). The significance of difference among
various groups was tested by ANOVA (Analysis of Variance) and post-hoc least significant difference test with significance at $P \leq 0.05$. Pearson correlation between plasma glucose values with blood glucose values was performed to validate the methods. IBM SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, USA) was used for data analyses. Inter-individual and intra-individual variations were calculated considering the fasting plasma glucose values of 12 subjects measured before the consumption RF and four TFs and expressed as CV%.

RESULTS

The demographic and clinical characteristics of the study-subjects are given in the Table 2. The mean age of the subjects was 29 yr, the BMI was 24 kg/m², and fasting plasma glucose levels were lower than 4.6 mmol/L.

Plasma glucose and incremental glucose values at different time points over 2 h (IAUC) after the consumption of various TFs are given in Fig. 1 and Table 3, respectively. TF1, TF2, TF3, and TF4 induced varied glycemic responses in terms of raising blood glucose levels (Fig. 1). Of the four TFs, only TF2 induced higher glycemic response (>3.9 mmol/L) followed by TF1, TF3, and TF4. The mean fasting plasma glucose levels were comparable

Table 2. Demographic and clinical characteristics and body composition of male subjects

| Variables                  | Data are mean±SEM (n=12) |
|----------------------------|--------------------------|
| Age (year)                 | 29.2±1.40                |
| Height (cm)                | 167.8±2.13               |
| Weight (kg)                | 67.8±2.43                |
| BMI (kg/m²)                | 24.1±0.81                |
| BMR (kJ)                   | 6,635±198                |
| Fat (%)                    | 20.8±1.02                |
| Fat mass (kg)              | 14.3±1.09                |
| Far free mass (kg)         | 53.5±1.64                |
| Total body water (kg)      | 39.2±1.20                |
| Truncal fat (%)            | 24.0±1.15                |
| Fasting plasma glucose (mmol/L) | 4.6±0.13            |

BMI, body mass index; BMR, basal metabolic rate.

Data are mean±SEM (n=12).

Fig. 1. Effect after reference food (RF) and test foods (TFs) consumption on plasma glucose levels at various time points. Plasma glucose concentrations at each time point are depicted as vertical bars are mean±SEM (n=12). Data were analyzed by ANOVA and post-hoc least significant difference test. Vertical bars bearing different letters (a-c) are significantly different from each other ($P<0.05$) at that time point compared to RF. RF, 50 g glucose; TF1, wheat roti mixed mini meal; TF2, barley roti mixed mini meal; TF3, pearl barley raw upma; TF4, wheat rawa upma.

Table 3. Plasma incremental area under curve (IAUC) for glucose after reference food (RF) and different test food (TF) ingestion (unit: mmol/L)

| Time intervals (min) | IAUC-glucose |
|----------------------|--------------|
| 15                   | 30           | 45           | 60           | 90           | 120          |
| RF                   | 10.5±1.59a   | 37.0±3.23a   | 55.5±4.12a   | 49.9±6.75a   | 68.3±15.60a  | 37.8±9.96a   |
| TF1                  | 3.1±2.87b    | 14.6±3.20c   | 32.1±0.39b   | 30.7±4.64b   | 38.7±5.77b   | 16.9±3.10b   |
| TF2                  | 3.8±0.69bc   | 18.4±3.03bc  | 37.4±2.96b   | 36.2±2.45ab  | 52.6±6.96ab  | 35.0±6.11a   |
| TF3                  | 5.5±1.03bc   | 18.4±2.70bc  | 26.9±3.73b   | 22.9±3.91b   | 33.3±5.10b   | 28.7±4.80ab  |
| TF4                  | 5.6±0.56bc   | 23.5±2.95bc  | 37.2±4.11b   | 30.5±5.49b   | 32.8±6.22bc  | 11.6±3.42c   |

Data are mean±SEM (n=12).

Data were analyzed by one way ANOVA and post-hoc least significant difference test. Values bearing different letters (a-c) are statistically significant at $P \leq 0.05$. RF, 50 g glucose; TF1, wheat roti mixed mini meal; TF2, barley roti mixed mini meal; TF3, pearl barley raw upma; TF4, wheat rawa upma.
in the subjects consuming TF1 and TF2, while lowered significantly on TF3 and TF4. In general, glucose response was lower due to the consumption of various TFs, which was significantly lower than that of RF (glucose) at $P<0.05$ at 15 and 30 min. Further, TF3 was significantly lower than that of TF2 at 30 min. At 45 and 60 min, compared to RF and TF2, TF3 consumption resulted in lower plasma glucose levels. At 90 min, after TF2 consumption, plasma glucose levels were not different from that of RF, TF1, and TF3. On the other hand, TF4-induced glucose response was significantly lower than all the other foods (RF, TF2, and TF3) except TF1 (Fig. 1). Even at 120 min, the TF2 induced significantly higher glucose response (in terms of plasma glucose concentration), which was comparable to RF and higher than TF1 and TF4.

The intra-individual variation for repeated measurements of fasting glucose (6%) and inter-individual variation of the fasting glucose values were observed to be 9%.

The incremental plasma glucose values, at different time points over two hours, are given in Table 3. The increment in plasma glucose concentration, for that particular time point, was given by difference between the levels at that time point minus previous time point’s value according to the method given by FAO/WHO (2). At 15 and 30 min, the incremental plasma glucose concentrations of various TFs were lower than that of RF. TF4 induced larger increase in plasma glucose concentration, which was significantly lower than that of RF, but not different from that of TF2 and TF3, which were significantly higher than TF1. At 45 min, the incremental plasma glucose concentrations due to consumption of all the TFs were comparable and significantly lower than that of RF. At 60 min, the observed increments in plasma glucose concentrations of TF1, TF3, and TF4 were significantly lower than that of RF. However, TF2-induced increment in plasma glucose concentration was not different from that of RF and the rest of the foods (TF1, TF3, and TF4). At 90 min, the same trend continued. However, at 120 min, the increment in plasma glucose concentrations of TF1 and TF4 were significantly lower than that of RF. TF2 exhibited increment in plasma glucose concentration, which was on par with that of TF3 and higher than that of TF4. On the other hand, TF2 and TF3 displayed significantly higher increments in plasma glucose concentrations than those of TF1 and TF4, and were comparable to RF. TF3 had significantly higher glucose increments compared to TF4 and comparable with those of RF, TF1, and TF2. Of all the TFs, at 120 min, TF4 consumption resulted in the least increment, which was lower than that of RF and TF3, and comparable to TF1 and TF2.

Fig. 2. The impact of test foods (TFs) on glucose response. (A) Incremental area under curve (IAUC), (B) glycemic index (GI), and (C) glycemic load (GL). Values are mean±SEM (n=12). (D) Regression between plasma glucose and blood glucose ($r=0.758$). RF, 50 g glucose: TF1, wheat roti mixed mini meal; TF2, barley roti mixed mini meal; TF3, pearl barley rawa upma; TF4, wheat rawa upma.
significantly lower than all other foods (Table 3).

In Fig. 2A, B, and C, the IAUC, GI, and GL of the four TFs are presented, respectively, and the TFs were also classified as low or high GI foods. There were no significant differences among the various foods with regard to these parameters. However, based on GI units, TF2 was classified as high GI food and TF1, TF3, and TF4 were identified as low GI foods.

Plasma insulin levels of the subjects on one of the low GI TFs, i.e. TF4, were also measured. The plasma insulin and glucose concentration at different time points over 2 h period of the subjects, who consumed TF4, are given in Table 4 and Fig. 1 respectively. Plasma insulin concentrations at 15 min were significantly lower than that of RF. Further, plasma glucose concentrations of the subjects on TF4 were lower than that of RF at 15, 30, and 45 min. The association between glucose measurement in capillary blood sample and plasma has been established by Wolever et al. (23). In line with this, the regression analysis revealed that the glucose measurement by strips and enzymatic method for blood and plasma respectively correlated at all time points with r value of 0.76 (Fig. 2D).

**DISCUSSION**

Controlling post-prandial glucose level is considered to be one of the key strategies in the management of type 2 diabetes (17). In this context, we developed new recipes of Indian mixed mini meals and breakfast items (in a way that are consumed by the population) and tested their glycemic response in healthy and non-diabetic volunteers. Notably, all the TFs that are prepared in the study contain low GI ingredients and high dietary fiber. Although, the GI of various TFs did not differ, barley-based mixed mini meal i.e. TF2 had induced higher glycemic response (GI>70) and can be classified as a high GI food. On the other hand, the rest of the TFs elicited moderate and comparable glycemic responses, thereby suggesting that wheat-based mini mixed meal, wheat rawa upma, and pearl barley rawa upma having coarse particles, are low GI foods. This observation is of great relevance in the context of the dietary habits of the Indian population, who subsist on high carbohydrate diets, with rice and wheat as staple foods (8,9). In general, high carbohydrate diets induce high GL. Recently, it has been shown that GL is an independent risk factor for type 2 diabetes (17). Beside post-prandial hyperglycemia, hyperinsulinemia, and insulin resistance are important components of metabolic syndrome (18) and therefore, we measured the insulin levels, particularly after consumption of the low GI breakfast item (TF4). However, the plasma insulin data suggest that it did not have any impact on insulin response, except at 15 min.

The concept of the present study was to develop TFs by replacing the commonly consumed cereals of Indian foods by other cereals and assessment of their GI. Thus, the present study is different from the study of Radhika et al. (19), wherein whole wheat flour-based rotis were provided as a single food item. However, when the results were compared with that of Chaturvedi et al. (20), the wheat-based mini meals (TF1) of our study had lower GI (54 units), against their combination diet consisting of wheat flour chapathi served with bottle gourd and tomato curry (GI of 66 units). Urooj and Puttaraj (21) have reported two extreme GI values (44∼81 units) for chapathi, made of wheat flour with green gram dal. The observed differences in GI of the TFs could be attributed to the variations in gluten content of wheat and pearl barley (22,23), methods of processing (24,25), preparation, and the types of fat/oil used (26). Further, dry roasting, which was used for the preparation of chapathi or roti, is known to increase the resistant-starch content; various other factors such as the presence of salt in making dough, anti-nutrients (especially inhibitors of α-amylase), their chemical nature, and stability at the temperature of preparation, may contribute to the differences in the GI values (27-29).

Interestingly, in the present study, the mixed mini meals (TF1), which consists of the rotis made up of whole wheat flour alone (when eaten with dehusked split chana dal and curd) had lower GI than the mixed mini

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**Table 4. Plasma insulin levels after reference food (RF) and test food 4 (TF4) ingestion** (unit: μU/mL)

| Time intervals (min) | 0    | 15   | 30   | 45   | 60   | 90   | 120  |
|----------------------|------|------|------|------|------|------|------|
| RF                   | 7.4±2.2 | 53.6±6.5 | 109.6±15.3 | 114.4±17.5 | 114.2±6.0 | 78.4±17.4 | 30.2±9.2 |
| TF4                  | ND      | 27.8±7.3* | 97.0±4.4  | 90.6±14.7  | 60.6±20.3  | 41.6±9.5   | 34.0±9.6  |
| Mann Whitney’s test  | 0.094 | 0.036 | 1.00 | 0.459 | 0.093 | 0.175 | 0.754 |

Data are mean±SEM (n=5).

*P<0.05.

RF, 50 g glucose; TF4, wheat rawa upma.
ND, not detected.
meals (TF2) consisting of rotis (wheat flour in combination with barley flour and besan) consumed with whole (unhusked) chana and curd. Despite comparable available carbohydrate, protein, fat, and dietary fiber contents in these two mixed mini meals, differences in the gluten content and the formation of the resistant starch, due to the presence or absence of anti-nutrients, could have influenced their GI. Further, the α-amylase inhibitors present in wheat can withstand cooking temperatures and may effectively decrease blood glucose response (24), which could also have contributed to the observed low glycemic response by consumption of wheat-based mixed mini meals (TF1). On the other hand, the inhibitors of α-amylase of fine flour obtained from pearl barley (with small particle size) perhaps were labile at dry roasting condition and could have resulted in higher glycemic response, despite being eaten with other dietary items such as whole (unhusked and unsplit) chana dal and curd. Furthermore, upma made up of wheat rawa and pearl barley rawa had comparable GI, which may be due to several factors such as particle size (as gelling properties differ between coarse and fine particle), method of cooking, absence of other foods, gluten content, and the dietary fiber, despite the fact that the staple (cereal) ingredients are entirely different between these food items (TF3 and TF4). Though an increase in protein content is known to decrease GI (30), in the present study, the two mixed mini meals (TF1 and TF2) having comparable protein contents (13 g) behaved differently. Further, TF1, which had higher content of protein than the breakfast items, displayed higher GI than the TF3 and TF4, having 9.0 ~ 10.0 g protein. This observation suggests that the differences in the GI of mixed mini meal and breakfast items cannot be attributed to protein content. Overall, besides chemical composition (including phenolic compounds, anti-nutrients, and anti-oxidants), the method of processing (milling, grinding, and polishing), preparation (boiling, pressure cooking, steam cooking, and dry-roasting), and other consumed dietary components are key determinants of glycemic response of the foods. Limitations of the present study include: women were not included, RF and TFs were given only in one session each, and insulin levels were not measured in the subjects after the consumption of all the TFs.

In conclusion, among newly developed recipes; upma (prepared with wheat rawa or barley rawa) and mixed mini meals (whole wheat flour roti and chana dal with curd) ingestion in young healthy non-diabetic volunteers showed moderate glycemic response and therefore classified as low GI food. Further, these newly developed low GI recipes may be useful in ameliorating hyperglycemia and glucose-over load associated with diabetic conditions. Nevertheless, further studies are required to address their efficacy in diabetic subjects.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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