Do Different Fractions of Digestible Carbohydrates in Foods Influence the Glycaemic Responses?

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ABSTRACT

The Glycaemic Index (GI) concept ranks foods according to the postprandial glycaemic responses. The *in vivo* procedure used to determine GI values of food is very laborious and time consuming. Thus, the aim of this study was to analyze the *in vitro* rate of release of glucose from foods of Sri Lankan origin with the intention of identifying the relationships of the *in vitro* data with the published *in vivo* glycaemic responses. The fractions of glucose released and starch hydrolyzed in the *in vitro* method were classified as rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS) and slowly digestible starch (SDS) depending on the time taken for digestion and release of glucose. Basic foods (n=15) prepared with wet and dry heat process were analyzed to determine above fractions. The glucose released from basic foods were then used to calculate the RAG, RDS, SAG, and SDS fractions present in the mixed meals (n=2) containing more than one carbohydrate source. Significant positive correlations were observed with GI and RAG (r=0.513, p=0.025), RDS (r=0.597, p=0.011) and significant negative correlation with SAG/RAG (r=-0.511, p=0.036) ratio of both basic foods and mixed meals. The foods with high GI contain a higher percentage of carbohydrates in a form that can be rapidly hydrolyzed to yield glucose and correspondingly increased postprandial glucose levels.

Key words: Glycaemic index, *in vitro* glucose release, rapidly available glucose, slowly available glucose

INTRODUCTION

The starch is digested in the human small intestine at different rates producing a wide range of glycaemic responses (Jenkins et al., 1981). Thus, the concept of Glycaemic Index (GI) was introduced to rank starchy food items depending on their rates of digestion and absorption (Jenkins et al., 1981). GI is measured as a ratio of the incremental area under curve (IAUC) of the blood glucose response of a test food to that of the standard food (glucose or white bread) (Wolever et al., 1991, Brouns et al., 2005) containing the same amount of available carbohydrate. The starchy foods are categorized as low, medium, or high GI foods according to the rates at which they are digested and absorbed (Beals, 2005). The digestion and absorption of starch in the human small intestine is affected by various intrinsic factors (i.e., food processing/preparation methods, physical and chemical characteristics of the food or the meal) (Bjorck et al., 1994, Holt and Brand-Miller, 1994, Arvidsson-Lenner et al., 2004) and extrinsic factors like extent to which food is chewed, transit time through the gut and the degree of the insulin response (Urooj and Puttaraj, 2000, Leinonen et al., 1999).

However, the *in vivo* procedure of determination of GI is very laborious (Granfeldt et al., 1992) as it requires the corporation of supportive volunteers, time consuming and needs estimation of the available carbohydrate of the meals (as eaten). Thus several *in vitro* methods had been developed (as alternatives to *in vivo* method) mainly by focusing on the carbohydrate sensitivity to the action of digestive enzymes (Englyst and Cummings, 1985, 1986, 1987). These *in vitro* studies have indicated a close relationship between the rate of starch digestion and glycaemic response *in vivo* (O'Dea et al., 1980, O'Dea and Nestle, 1981).

The *in vitro* method used in the present study measured the glucose released at timed incubations using digestive enzymes as encountered *in vivo*. The

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fractions analyzed were rapidly available glucose (RAG), slowly available glucose (SAG), rapidly digestible starch (RDS) and slowly digestible starch (SDS). These fractions have been classified according to the rate and extent of digestion of starchy foods *in vivo* (Englyst et al., 2000).

The aim of the present study was to analyze the above parameters depending on the rate of release of glucose from commonly consumed basic foods of Sri Lankan origin. As a novel approach the data of the above fractions of basic foods were used to calculate the glucose and starch fractions present in mixed meals containing more than one carbohydrate source. The relationships between the *in vitro* data of the present study and the *in vivo* GI values of the foods from previously published data of our research group will be identified to study the applicability of the above mentioned fractions in influencing the GI values [Author, Author, Corresponding author].

METHODOLOGY

Enzymes/Chemicals

The enzymes were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Roche Diagnostics (Mannheim, Germany). All the chemicals used were of analytical grade and purchased from BDH (Poole, England) unless otherwise specified. Glucose oxidase kit (GOD-PAP) was from BIOLABO (Maizy SA, France).

Food Items

All the foods were prepared by standard recipes (Table 1). Basic foods were selected to cover cereals, legumes, tubers and vegetables (n=15). Mixed meals (n=2) that contained more than one carbohydrate source were selected.

Preparation of Flour

Food items were dried at 35–45 °C and milled (0.315 mμ) using the analytical mill (A 11 Basic, IKA® Works do Brazil Ltd, Estrada do Guerengue, Taquara**,** Brazil). The flour samples were stored at –20 ºC until analyzed.

Enzyme Mixture - (For 2 samples)

Enzyme concentrations were optimized using Kelloggs' corn flakes (Reference sample).

Pancreatin (4.0 g, EC. No. 232-468-9) was mixed with distilled water (12 ml) and the solution was centrifuged at 1500 *g* for 10 min. Supernatant (8.2 ml) was pipetted into a test tube. Amyloglucosidase (1800 µl from 14 mg/ml stock, EC 3.2.1.3) and invertase (80 mg, EC 3.2.1.26) were added to the above tube and mixed well. The enzyme mixture was prepared each day of analysis immediately before use.

Estimation of Rapidly Available Glucose (RAG), Slowly Available Glucose (SAG) and Slowly Digestible Starch (SDS) Fractions

Modified method of Englyst et al., 2000, was used to analyze the above mentioned fractions. This modified procedure used flour (0.5 g) without correcting for moisture or starch as the most of the Sri Lankan foods analyzed contained high carbohydrate levels.

Blank and standards were prepared with 50 mg of guar gum powder added to sodium acetate buffer (20 ml) and glucose standard (20 ml from 25 mg/ml) respectively. Samples (0.5 g) were weighed and pepsin-guar solution (0.05 g/ 10 ml 0.05 M HCl) was added to each beaker, mixed and incubated at 37 ºC for 30 min. After the incubation two glass balls and 10 ml of 0.25 mol l^{-1} sodium acetate were added to each sample beaker. Contents were mixed and beakers replaced in the water-bath at 37 ºC to equilibrate. Sodium acetate buffer (5 ml) was added to blank, standard beakers and kept in the shaking (linear strokes – 150/min) water bath (time – zero). Samples were removed from the 37 ºC water-bath and above prepared enzyme mixture (5 ml) was added at timely intervals to each sample. The contents were mixed and left at 37 ºC. Blank, standard

Table 1: Food items analyzed and methods of processing

Processing method	Food	Preparation method
Wet heat	1. String hopper (wheat)	Wheat flour (500 g) was first steam cooked, flour was mixed with water and used for string hopper preparation. Individual string hoppers prepared were again steam cooked.
	2. *Pittu* (wheat)	*Wheat flour (100 g) was mixed with coconut scrapings (100 g) and steam cooked.
	3. Red rice	Rice variety AT 353 was obtained from Rice Research Institute, Batalegoda, Sri Lanka. Red rice (500 g) was boiled with sufficient amount of water in a rice cooker.
	4. String hopper (red rice flour)	Red rice (500 g) was soaked in excess water overnight, powdered and roasted. The rice flour was mixed with water and string hoppers were prepared by again steam cooking.
	5. Chick pea, 6. Cowpea, 7. Mung beans,	*All (100 g) were soaked overnight in excess water and boiled with sufficient amount of water.
	8. Lentil curry	Lentils (100 g) were boiled with water (200 ml) and condiments. The curry was prepared by adding coconut milk.
	*9. Manihot esculenta (*Manioc)	Manioc (200 g) was cut in to pieces of about 1 $inch^3$ and boiled with 200 ml water.
	10. *Artocarpus altilis* (Bread fruit)	*Bread fruit was cut into pieces of about 1 $inch^3$, boiled for 45 min. Excess water drained.
Dry heat	11. White sliced bread	Bread was bought from reputed retail outlets.
	12. Whole meal bread	
	13. *Roti* (wheat)	*Roti were prepared by mixing the flour (50%) with coconut scrapings (50%) and cooked on a pan by turning the sides.
	14. *Roti* (atta)	
	15. *Roti* (rice: wheat - 1:1)	
Mixed meals	16. Whole meal bread with lentil curry	Whole meal bread (64% starch), Lentils curry (36% starch).
	17. Red rice with lentil curry	Red rice (82% starch), Lentils curry (18% starch).

* Source: [Corresponding author].

and samples were removed from the water bath exactly at 20 min after addition of the enzyme mixture. Hydrolysis was stopped by adding sample (0.5 ml) into 20 ml of 66% ethanol (G20 portion – RAG content). The sample was returned to the water bath immediately. A second 0.5 ml from beakers was transferred to 20 ml of 66% ethanol after two hours (G120 portion). The G20 and G120 fractions were centrifuged at 500 g for 5 minutes and assayed for glucose (using GOD-PAP kit). SAG content was calculated by taking the difference between G120 and G20 fractions and SDS content by multiplying the SAG content with a conversion factor of 0.9.

Estimation of Rapidly Digestible Starch (RDS)

For the estimation of RDS content, the free sugar contents (FSG) of the food items were determined as given below.

Sodium acetate buffer (25 ml) was used as the blank and standards (25 ml)

were 25 mg/ml glucose standard. Samples (0.50 g) and two glass balls were added to beakers (250 ml). Sodium acetate buffer (25 ml of 0.1 mol l^{-1}) was added to each beaker and the contents were mixed vigorously. The beakers were incubated in a boiling water-bath for 30 min. Contents were mixed vigorously and cooled to 37 °C. Invertase (40 mg) was added and incubated at 37 °C for 30 min while shaking. Each sample/standard solution (1 ml) was transferred into tubes containing 2 ml of absolute ethanol. The contents were centrifuged at 500 *g* for 5 min and 1 ml of the supernatant was transferred into 5 ml of distilled water. In the case of the standard 1 ml of the supernatant was transferred into 20 ml of water. RDS was calculated as the difference between G20 and FSG multiplied by the conversion factor of 0.9.

Statistical Analysis

The GI values are presented as mean ± standard error of mean (SEM) and glucose, starch fractions as mean ± standard deviation (SD). The results were analyzed and correlations studied using Microsoft Excel (2003) and Minitab, USA (Version 14).

RESULTS AND DISCUSSION

The RAG, SAG, RDS and SDS fractions per 100 g edible portions of basic foods (n=15) are presented in table 2. The amount of glucose released within 20 minutes was classified as rapidly available glucose (RAG) and glucose released between 20-120 minutes as slowly available glucose (SAG). The rapidly digestible starch (RDS) comprises of starch hydrolyzed to glucose during first 20 minutes of digestion. Slowly digestible starch (SDS) refers to the amount of starch hydrolyzed to glucose after 20 minutes taking in to consideration that all the glucose that are freely available and converted from sucrose had been accounted and omitted when estimating RDS content.

According to the results, except for *Artocarpus altilis* (bread fruit) all other foods had more RAG contents compared with the corresponding SAG fractions. The RAG contents of white wheat bread and whole meal bread are reported to be 42 and 36 (g/100 g edible portion) respectively (Englyst et al., 2000). The bread varieties analyzed in the present study contained 39 and 38 (g/100 g edible portion) RAG contents. The legumes are reported to contain RAG contents of 5-11 (g/100 g edible portion) and SDS contents in the range of 0.8-9.8 (g/100 g edible portion) (Englyst et al., 2000). The values of legumes analyzed in the present study are within the reported range (RAG – 7-11 g/100g edible portion, SAG -7-8 g/100 g edible portions).

The *in vitro* procedure was extended to analyze the glucose and starch fractions of mixed meals (containing more than one carbohydrate source) as this had not been attempted previously. The individual glucose or starch fractions of basic foods were used to arrive at the final RAG, SAG, RDS and SDS fractions of meals. The present study attempted this approach with a) red rice mixed meal (red rice & lentil curry) and b) whole meal bread mixed meal (whole meal bread & lentil curry) (Table 3).

When the results of *in vitro* glucose and starch contents in the portions given for determination of GI of both basic foods and mixed meals were correlated with the observed *in vivo* glycaemic responses published by our research group [Author, Author, Corresponding author], significant correlations ($p<0.05$) were observed with *in vivo* and *in vitro* data (Figure 1). The RAG and RDS contents of both basic foods and mixed meals showed significant positive correlations with *in vivo* GI values ($r = 0.513$, $p = 0.025$ and $r = 0.597$, $p = 0.011$) [Figure 1(a), Figure 1(b)] and incremental area under the blood glucose response curves (IAUC) ($r = 0.701$, $p = 0.002$, $r = 0.740$, $p = 0.001$) respectively. The foods with high GI contained a higher percentage of carbohydrates that can be rapidly hydrolyzed to yield glucose giving rise to an increased postprandial glucose levels. Significant correlations between the RAG contents of basic foods with either GI or IAUC had been reported previously (Garsetti et al., 2005). The SAG contents of

Table 2: Glucose values, starch fractions of basic foods (g/ 100 g edible portions), GI

No.	Food item	RAG[1]±SD	SAG[1]±SD	RDS[1]	SDS[1]	GI±SEM
1.	String hopper (wheat flour)	26.9±0.8	9.9± 0.5	24.2	8.9	104±7[2]
2.	String hopper (red rice flour)	28.1±0.5	10.9±0.2	25.3	9.8	103 ±11[2]
3.	*Pittu* (wheat flour)	35.0±0.9	3.8±0.4	31.5	3.4	102±10[3]
4.	Red rice	18.8±0.6	10.5±0.3	16.9	9.5	99±10[2]
5.	Chick pea	9.5±0.7	8.2±0.4	8.5	7.4	29±5[3]
6.	Mung beans	10.7±0.3	7.5±0.4	9.6	6.8	57±6[3]
7.	Cow pea	7.1±0.4	7.5±0.4	6.4	6.8	49±6[3]
8	Lentil curry	8.9±0.4	7.3±0.5	8.0	6.6	ND
9.	Manioc	21.5±0.5	9.8±0.2	19.3	8.8	120±9[2]
10	Bread fruit	6.8±0.3	18.4±0.3	6.1	16.5	64±9[3]
11.	White sliced bread	38.5±0.4	7.1±0.7	34.6	6.4	100[4]
12.	Whole meal bread	38.0±0.9	9.9±0.6	34.2	8.9	103±11[4]
13.	*Roti* (wheat flour)	38.3±0.3	1.0±0.4	34.5	0.9	72±6[3]
14.	*Roti* (Atta flour)	37.8±0.3	13.3±0.3	34	12.0	67±9[3]
15.	*Roti* (Rice: Wheat)	35.4±0.9	7.5±0.2	31.9	6.8	69±7[3]

[1]Values are expressed as average of four samples; SEM – Standard error of mean; ND- Not determined. [2]-Source- [Author]; [3]-Source - [Corresponding author]; [4]- source – [Author]

Table 3: Glucose, starch fractions of the mixed meals (g / per 50 g available carbohydrate portion) and GI values

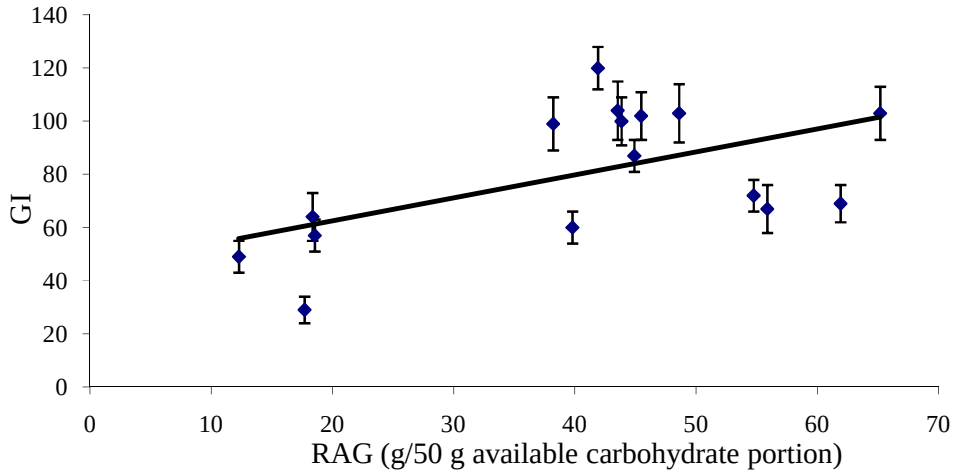
No	Meal	RAG	SAG	RDS	SDS	GI± SEM
1.	Whole meal bread meal	44.9	19.2	40.4	17.3	87 ± 6[4]
2.	Red rice meal	39.8	22.9	35.7	21.7	60 ± 5[2]

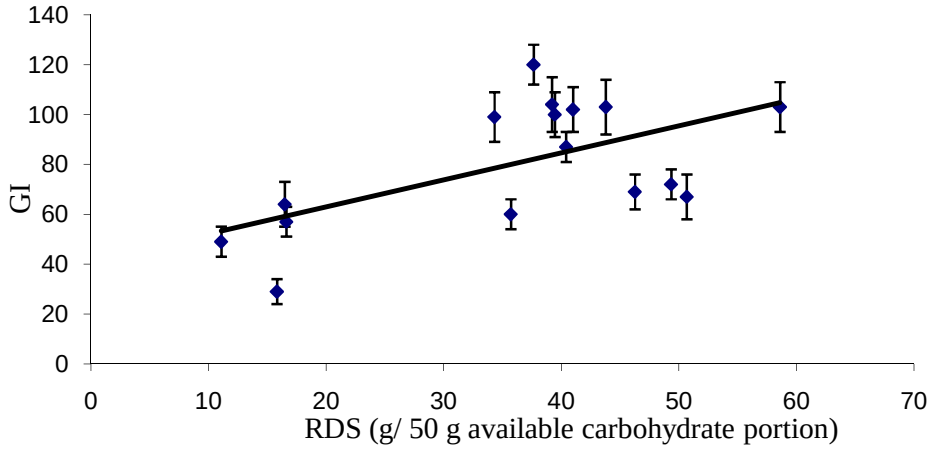
SEM – Standard error of mean; [2]-Source- [Author]; [4]- Source – [Author]

the foods ranged from 1.0 -18.4 (g/100 g edible portion) with 75% of the foods having SAG levels above 7.5 g/100 g edible portion (Table 2). However, significant relationships ($p<0.05$) were not observed among *in vivo* GI, IAUC and SAG nor the SDS contents, contradicting the findings of another study (Garsetti et al., 2005). High levels of SAG contents of foods are available with less gelatinization. The starch of the basic foods analyzed except for *roti* preparations (dry heat process) are gelatinized (Unpublished data) and generally contain more RAG levels compared with the SAG contents. Thus, the influence of SAG on the glycaemic response of the foods studied will be minimal.

A negative correlation was obtained in the present study with SAG/RAG ratio of the 50 g available carbohydrate portions and GI ($r = -0.511$, $p = 0.036$) [Figure 1(c)], IAUC ($r = -0.626$, $p = 0.007$) for both basic foods and mixed meals. A Chilean study had shown a significant positive correlation between ratio of RAG/SAG and GI ($r=0.425$, $p<0.001$) (Araya et al., 2002).

Thus, apart from RAG, RDS fractions, the SAG/RAG ratio can also be taken as important food related determinant of the glycaemic response of basic foods as well as meals containing different sources of carbohydrates. Among the fractions analyzed, the RAG contents will be more useful in predicting the glycaemic responses as this can be measured within a very short period.





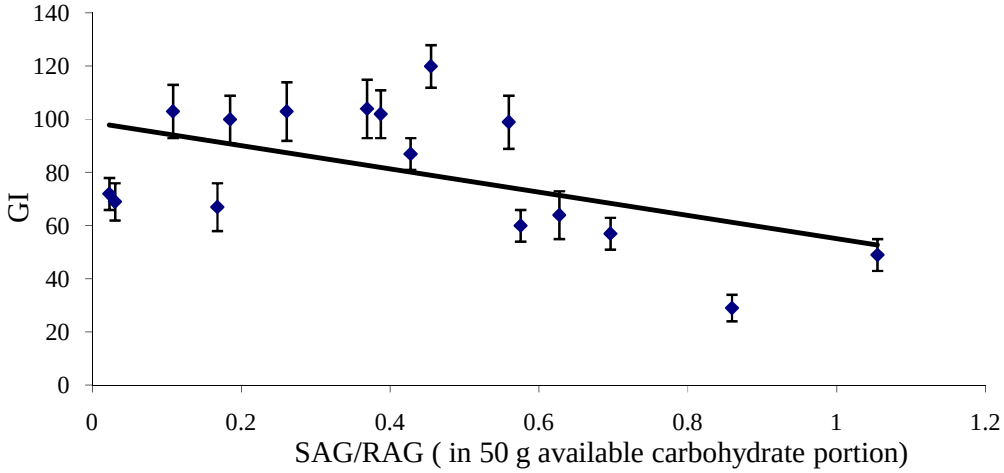


Figure 1: The correlations between the *in vivo* GI values and the RAG, RDS values, SAG/RAG ratio (of the portions given in determination of GI) of the foods.
(a) GI and RAG; (b) GI and RDS; (c) GI and SAG/RAG

CONCLUSION

The RAG, RDS contents and the ratio SAG/RAG (of the portions given in determination of GI) of the basic foods and mixed meals (calculated using the basic food data an approach not attempted previously) elicited significant correlations with GI indicating that this could be a successful method for predicting the *in vivo* GI where necessary.

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