

## Original Research Article

# The Metabolic Effects of Two Meals with The Same Glycaemic Index But Different Slowly Available Glucose Parameters Determined *In Vitro*: a Pilot Study

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**Received:** July 14, 2014; **Accepted:** July 30, 2014;**Published:** July 31, 2014**Abstract**

Some foods with a similar Glycaemic Index (GI) are known to have different metabolic impacts in terms of blood glucose and insulin responses. This difference could be explained by the difference in the rapidly and slowly Available Glucose (RAG and SAG) values for the foods determined *in vitro*. This study was set up to investigate the metabolic impact of two meals with essentially the same GI and macronutrient content but with different SAG values. Twelve healthy male subjects were recruited from the University of Surrey postgraduate student population. RAG and SAG values were measured for each meal based on enzymatic hydrolysis of carbohydrate content. The postprandial glucose, insulin, Triacylglycerol (TAG) and Non-Esterified Fatty Acid (NEFA) responses to high and low SAG meals were determined in two randomised occasions. The incremental Area Under the Curve (iAUC) for glucose and insulin was  $106.3 \pm 14.46$  and  $26550 \pm 3266$ , respectively for the low SAG meal. The high SAG meal produced a lower glucose and insulin response with an iAUC of  $88.1 \pm 10.67$  and  $23701 \pm 3065$ , respectively; although this difference did not reach statistical significance ( $p = 0.21$  and  $0.33$  respectively). This work has demonstrated that small differences can occur in the metabolic response for low and high SAG meals in terms of the glucose and insulin levels. This highlights that RAG and SAG values may be an important adjunct to the GI of foods in determining metabolic response.

**Keywords:** Glucose; Insulin; Available glucose; Glycaemic index**Abbreviations**

GI: Glycaemic Index; CHO: Carbohydrate; RAG: Rapidly Available Glucose; SAG: Slowly Available Glucose; TAG: Triacylglycerol; NEFA: Non-Esterified Fatty Acid; iAUC: Incremental Area Under The Curve; II: Insulinaemic Index; ELISA: Enzyme Linked Immunosorbant Assay; BMI: Body Mass Index; g: Gram; KJ: Kilojoule; UK: United Kingdom; CV: Coefficient of Variation

**Introduction**

Carbohydrates (CHOs) are important sources of stored energy providing between 40 and 55 % of the energy supplied by typical Western diets. In some population groups, however, particularly those that exist in the tropics, CHOs contribute up to 85 % of dietary energy [1,2]. CHOs exist in a wide range of forms which are for the most part heterogeneous in terms of their physical and chemical characteristics [3]. Nevertheless, in order to understand further the dietary impact of this important macronutrient it is necessary to classify CHOs in a manner appropriate to their effects. It has been suggested that CHOs should be classified, on the basis of their molecular size, into three groupings, namely i) monosaccharides and disaccharides, ii) oligosaccharides and iii) polysaccharides [4]. However, classifying CHOs based simply on their chemical structure is not a reliable indicator of their physiological effects [5,6]. As such, the approach of classifying foods according to their physiological effects can be considered a more useful method in terms of understanding the

health effects of diets containing CHOs. Indeed, a concept, known as the Glycaemic Index (GI), has been introduced which allows foods to be ranked according to their effects on blood glucose levels [7-9].

Alternatively, the *in vitro* method for starch hydrolysis has been proposed as a faster and more cost effective method for predicting the GI of starchy foods [5,6]. As such, the terms Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG) have been devised relatively recently and are used as a direct measurement of absolute CHO which is available for absorption in the human small intestine [10-13].

RAG and SAG measurements are based solely on the hydrolysis of dietary CHOs *in vitro* using a mixture of enzymes resembling those present in the GI. The measurements of RAG give values for glucose that are likely to be absorbed in the human small intestine and, thus likely to influence blood glucose and insulin responses [12,13]. The relationship between the food RAG values and GI has been studied and it has been found that there is a significant positive correlation between these values for starchy foods ( $r = 0.76$ ,  $p < 0.001$ ). It is therefore apparent that using the RAG but also the SAG measurements of CHO containing foods may be used as a supplement to the GI approach and may provide further information which could be useful in understanding the impact of different foods containing CHO on blood glucose and insulin levels [11].

As highlighted, RAG and SAG measurements may provide a

useful index to help understand the metabolic impact of CHOs *in vivo*. However, before this can be put into place greater attention needs to be paid in terms of understanding the metabolic impact of CHOs on the basis of their RAG and SAG values in combination with the GI approach. As such, we hypothesised that studying the effect of two CHO rich meals one with a high value of SAG and the other with a low SAG could help us to understand the differences in the metabolic effects of different dietary CHOs on blood glucose and insulin levels *versus* GI values. Therefore, this study examined the metabolic impact of two meals with similar GI and macronutrient content but different (high and low) SAG values.

## Subjects and Methods

### Subjects

Twelve healthy subjects were recruited from the postgraduate student population of the University of Surrey, UK, by the distribution of both e-mails and posters between July and September 2009. Inclusion criteria included: male gender, non-smoking status and age between 20 and 65 y. Subjects also needed to have a normal weight in relation to their height, normal resting blood pressure and normal fasting plasma glucose levels. Subjects who were either overweight (BMI > 25), or had abnormal blood pressure or abnormal fasting plasma glucose were excluded from the study. All subjects gave informed written consent. Weight, height, blood pressure, fat mass and fasting plasma glucose were measured at baseline.

### Study design

A randomised controlled crossover trial of a single meal (either low SAG meal followed by high SAG meal or the reverse) was employed. Each subject was randomly assigned to receive one of these two meals first. The study design received a favourable ethical opinion from the University of Surrey Ethics Committee (EC/2007/78/FHMS) and approved as being in accordance with the Helsinki II declaration. Subjects were asked to consume the meal at their breakfast time (at 0830 h).

### *In vitro* measurement of RAG and SAG

The *in vitro* procedure was based on the enzymatic hydrolysis of the food CHO using the method of Englyst [14]. Portions of the food samples (3 g) were weighed into 50 ml centrifuge tubes (polypropylene tubes from Corning Inc, NY 14831) to the nearest  $\pm 1$  mg and incubated with a mixture of hydrolytic enzymes (amyloglucosidase from Englyst Carbohydrate Services Ltd (Southampton, UK), amylase (heat-stable) and pancreatin from Sigma Chemical Co. Ltd., Poole UK) under controlled conditions of temperature (37 °C), pH (5.2) and viscosity. Subsamples were collected from the incubation mixture at specific time points (20 and 120 min) and measured for glucose which was then used to calculate the RAG and SAG values, respectively. The released glucose from each of the samples was determined colorimetrically using Glucose Oxidase/Peroxidase Reagent (Sigma Chemical Co. Ltd.).

Two reference samples, namely potato starch (Sigma Chemical Co. Ltd) and Cornflakes® (Kellogg's, UK) were included in every batch analysed and the inter-assay CVs were calculated to be less than 10 % for reference 1 and 5 % for reference 2.

### Meal design

In order to test the hypothesis, the two near-identical meals were

designed to achieve as large a difference as possible in the content of SAG, but with no significant differences between the two meals regarding the overall GI, energy content, absolute available CHO, fat or protein levels. All the RAG and SAG values for the two meals were obtained using the above method [14]. The low SAG meal consisted of Hassawi rice (previously characterised by Al-Mssallem *et al.* [15], a plain yoghurt drink and Arabic dates (also previously characterised by Al-Mssallem *et al.* [16]. This meal had a total SAG of 11.8 g and an overall GI of 47 (Table 1). The high SAG meal contained Uncle Ben's rice, a plain yoghurt drink and Arabic dates; with a total SAG of 20.2 g and an overall GI of 46. The GI values were based on glucose as the standard reference and taken from previous studies [15,16], and were calculated for the two meals from the individual foods [17]. All foods were prepared in the kitchen unit of the Clinical Investigation Unit at the University of Surrey, UK.

### *In vivo* study. Blood samples collection

Participants arrived at the Clinical Investigation Unit at the University of Surrey at 0830 h on the day of the study after an overnight fast (10-12 h). Subjects were cannulated via the antecubital forearm vein prior to sampling. Blood samples of 5 ml were drawn fasting and at 15, 30, 45, 60, 90, 120, 180 minutes after consuming either the low or high SAG meal and analysed for glucose, insulin, free fatty acids and triglyceride levels. Blood samples were collected into 5 ml dipotassium EDTA polystyrene tubes for insulin, Non-Esterified Fatty Acids (NEFA) and Triacylglycerol (TAG) (Teklab Ltd, Durham, UK) and 2 ml fluoride oxalate polystyrene tubes (Teklab Ltd.) for glucose, and were immediately centrifuged at 3000  $\times$  g for 10 min. The centrifuged plasma was transferred into separate 500  $\mu$ l plastic plain microvette tubes (Alpha Laboratories Ltd, Eastleigh, Hampshire, UK). The tubes were then frozen and kept in the freezer at -20 °C until analysis (within 4 weeks).

### Glucose, NEFA and TAG measurement

The enzymatic automated colorimetric method on the ILab 650 (Instrumentation Laboratory, UK) was applied to measure plasma glucose, TAG and NEFA concentrations using glucose GOD/PAP test kit, NEFA kit and TAG kit along with two quality control (QC) samples (Randox Laboratories Ltd., County Antrim, UK). The intra-assay coefficient of variation of the level 1 and 2 QCs for glucose was less than 2 % (1.34 and 0.49 % respectively). For the TAG and NEFA, the precision gave CVs for QC1 and QC2 within acceptable limits (0.58 & 0.28 %, and 0.25 & 1.45 %, respectively).

**Table 1:** High and low SAG meal composition.

	High SAG meal	Low SAG meal
Weight (g)	492	492
RAG (g)	49.6	50.0
SAG (g)	20.2	11.8
Energy (KJ)	2145 (517 Kcal)	2118 (510 Kcal)
CHO (g)	86.6	84.7
Fat (g)	12.2	12.4
Protein (g)	14.5	14.9
GI	46	47

RAG= rapidly available glucose, SAG = slowly available glucose, CHO = carbohydrate, GI = glycaemic index.

### Insulin measurement

An enzyme linked immunosorbant assay (ELISA) was used for measuring plasma insulin concentrations (MLT, Cardiff, UK). The quality controls, standards (Invitron Ltd, Monmouth, UK) and samples (25 µL per each) were incubated with the labelled antibody solution (Invitron Ltd, Monmouth, UK) at 37 °C for 2 h and unbound labelled antibodies were removed using the wash buffer (Invitron Ltd) according to the manufacturer’s instructions. The insulin was then measured using the microtitre plate luminometer (Luminescent plate reader Centro LB 960, Berthold, Germany). All readings obtained from the luminometer were multiplied by 6 to convert the units (mU/l) into pmol/l. Two quality controls were employed and their CVs were 9 % and 6 %, respectively.

### Statistical analyses

Results were expressed as means ± one standard error of the mean (SEM) and checked for normality using the Kolmogorov-Smirnov test (K-S test). Comparison within the two groups was analysed statistically using paired *t*-test (SPSS 16.0 for Windows; Copyright (c) 2009 SPSS Inc.). All data were examined using a two-tailed approach with a level of *p* < 0.05 being considered as significant.

## Results

### In vitro measurement of RAG and SAG levels of the two meals

Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG) values for the low and high SAG meals are shown in Table 1. For the high SAG meal, the RAG and SAG values were 49.6 ± 0.58 and 20.2 ± 1.86 g, respectively; and 50.0 ± 1.2 and 11.8 ± 0.63 g, respectively in the low SAG meal. The SAG value in the latter meal was found to be significantly lower than that present in the high SAG meal (*p*=0.002). However, there was no significant difference between the two meals in terms of their content of RAG (*p*= 85).

### In vivo study

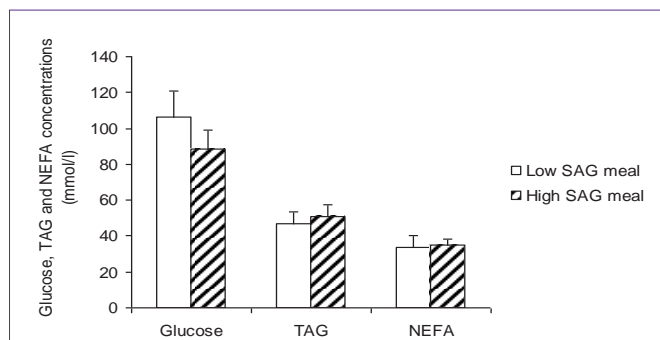
Subjects’ characters are displayed in Table 2. They had normal BMIs, resting blood pressure values and fasting blood glucose levels.

### Glucose, insulin, TAG and NEFA

The incremental Area Under the glucose Curve (iAUC) was calculated for the low and high SAG meals and was 106.3 ± 14.46 and 88.1 ± 10.67 mmol/l/min, respectively (Figure 1). These figures showed that the iAUC for the glucose in the low SAG meal was higher than that in the high SAG meal although the difference between the

**Table 2:** Characteristics of volunteers (n=12, mean ± SEM).

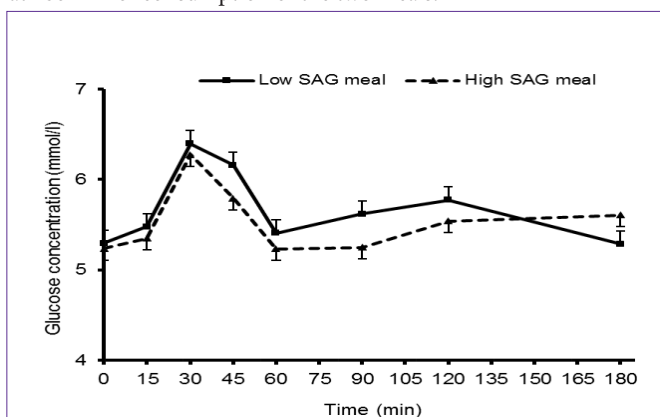
Subjects’ characteristics	Mean ± SEM
Age (year)	26.5 ± 1.3
Weight (kg)	68.1 ± 3.8
Height (m)	1.74 ± 0.02
BMI (kg/m <sup>2</sup> )	23.7 ± 0.6
Blood pressure (diastolic) mm Hg	70.7 ± 1.7
Blood pressure (systolic) mm Hg	119.9 ± 2.1
Fat (%) (normal range 8-20 %)	17.04 ± 1.10
Fat mass (normal range 5-14 kg)	12.56 ± 1.11
Fasting plasma glucose (mmol/l)	4.3 ± 0.06



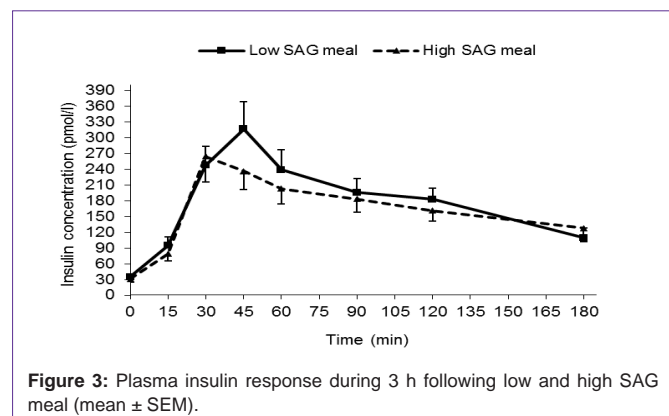
**Figure 1:** The iAUC for the glucose, TAG and NEFA in the low and high SAG meals (mean ± SEM). iAUC = incremental area under the curve, SAG = slowly available glucose, TAG = triacylglycerol, NEFA = non-esterified fatty acid.

responses to the two meals was not significant (*p* = 0.21). The highest peak glucose concentration was observed at the 30 min point after meal consumption. The glucose concentration then fell to the base line level at 60 min. In the high SAG meal, however, the plasma glucose concentration rose again and then levelled off for the next 1 h after the consumption of the meal. In the low SAG meal the plasma glucose concentration also rose at 120 min and then declined again reaching the baseline at 180 min after the consumption the low SAG meal (Figure 2 and 3).

Responses of the other metabolic parameters measure also showed some small differences. For the insulin, TAG and NEFA measurements the iAUC were 26550 ± 3266 pmol/l/min, 46.9 ± 6.62 and 33.5 ± 7.19 mmol/l/min respectively in terms of the responses to the low SAG meal and 23701 ± 3065 pmol/l/min, 50.6 ± 6.50 and 34.8 ± 3.78 mmol/l/min, respectively in terms of the responses to the high SAG meal (Figure 1,2 and 3). The insulin response to the low SAG meal was higher than that observed for the high SAG meal, however; this difference was not significant (*p* = 0.33). On closer scrutiny of the time course of insulin concentrations it was observed that at 45 min the low SAG meal produced an insulin response that was considerably higher than that observed with the high SAG meal (*p* = 0.053). The consumption of the high SAG meal resulted in a lower insulin peak at 30 min, while low SAG meal peaked at 45 min. Then the insulin concentration started to fall gradually to reach the baseline at 180 min of consumption of the two meals.



**Figure 2:** Plasma glucose response during 3 h following low and high SAG meal (mean ± SEM).



**Figure 3:** Plasma insulin response during 3 h following low and high SAG meal (mean ± SEM).

The fasting TAG level was  $1.1 \pm 0.09$  mmol/l for subjects prior to the low SAG meal and it was  $1.2 \pm 0.17$  mmol/l prior to the high SAG meal ( $p = 0.52$ ). The mean fasting NEFA concentration was  $0.44 \pm 0.06$  and  $0.44 \pm 0.05$  mmol/l ( $p = 0.96$ ) for subjects prior to the low and high SAG meals, respectively. There was a similar increase in the postprandial TAG concentration response to both of the meals. As expected, the plasma NEFA concentrations were suppressed after the consumption of both meals and remained suppressed throughout the postprandial period.

## Discussion

The main target of this study was to investigate the metabolic effect of two meals with similar GI but with different SAG values. It is clear that some foods with similar GI can have different metabolic impacts on the blood glucose and insulin responses due to differences in their *in vitro* CHO digestibility [18]. This dissimilarity in these responses could be explained by small differences in the RAG and SAG values of the foods.

In this study the low SAG meal was shown to produce higher glucose and insulin responses despite the fact that the two meals had similar GI values. Although this difference was not significant ( $p = 0.21$  and  $0.33$ , respectively). It is evident that the differences are in the direction that would be expected. Foods with a high content of SAG contain an amount of available glucose that is likely to be absorbed more slowly, and thus delay the elevation of blood glucose and insulin levels. The two meals (high and low SAG) were well designed meals with similar GI (46 and 47, respectively) and similar macronutrient content (Table 1), but they were significantly different in their SAG value ( $p = 0.002$ ).

It has been previously observed that GI is positively correlated ( $r = 0.50$ ;  $p = 0.01$ ) with RAG values and negatively ( $r = -0.42$ ;  $p = 0.04$ ) with SAG values for plain sweet biscuits [19]. Similar results have been reported for 23 cereal products [11] and starchy foods [6]. Similarly, there was positive correlation between insulinaemic index (II) and RAG values and an inverse correlation between II and SAG values [11,19]. The inverse correlation between the SAG and glycaemic and insulinaemic indices suggests that aiming for a high SAG content can be an alternative way to lower the glucose and insulin responses [19]. Our findings are consistent with that stated above where we observed that a high SAG meal elicited lower glucose and insulin responses; however, this is particularly interesting given the fact that the GI values of the two meals were almost the same.

The RAG and SAG measurements provided values that could be used as a supplement to the GI approach which may be useful in the understanding of the impact of CHO on blood glucose and insulin levels [10-13]. There was, as expected, a higher increase in the blood glucose and insulin levels in the low SAG meal compared to high SAG meal. On the other hand, there was no significant difference in the iAUC for TAG ( $p = 0.61$ ) and NEFA ( $p = 0.77$ ) between low and high SAG meals. The plasma NEFA concentration fell rapidly after consuming both meals and reached lower concentrations with value of 0.12 mmol/l after consuming low SAG meal compared to 0.20 mmol/l in subjects who consumed high SAG meal ( $p < 0.001$ ).

A finding such as this supports the idea that a reductive effect on blood glucose and insulin responses can be achieved by increasing the consumption of foods with a high content of SAG [11]. A dietary preference for foods with a slow-release CHO may provide health benefits, especially for those with diabetes [11,20]. It is well established that RAG and SAG values are good indicators in predicting the metabolic impact of CHOs on blood glucose and insulin profiles. It is important to know that the RAG and SAG values relate to the food as eaten and are expressed as g/100 g which allowed food to be compared on an equal weight basis [6,15]. This is consistent with the American Diabetes Association (2014) recommendations which focus on the total of the CHOs consumed rather than only their source [21]. For people with diabetes, it is critical for them to know the quantity and quality of their intakes of dietary CHOs, therefore it has been suggested that the RAG value should be used with the GI value where RAG value indicates the amount of glucose likely to be rapidly absorbed in the human small intestine [6]. In addition, it has been found that RAG and SAG explained more of the variation in GI and II than macronutrient composition of the starch foods [19]. And we found that RAG and SAG values have shown to be a good indicator for the postprandial glucose and insulin responses.

To our knowledge, this study is the first of its kind for investigation the metabolic impact of CHOs on the basis of their RAG and SAG contents. It was not possible to ascertain with certainty the responses that would be achieved on the two meals. Further studies are needed to understand more about the effects of high SAG foods on postprandial blood glucose and insulin levels in comparison with low SAG foods. Also, an intervention clinical trial should be carried out to investigate the long term impact of consumption of high SAG meal on blood glucose and insulin levels in people with diabetes.

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