# Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response<sup>1,2</sup>

Klaus N Englyst, Hans N Englyst, Geoffrey J Hudson, Tim J Cole, and John H Cummings

#### ABSTRACT

The American Journal of Clinical Nutrition

**Background:** A chemically based classification of dietary carbohydrates that takes into account the likely site, rate, and extent of digestion is presented. The classification divides dietary carbohydrates into sugars, starch fractions, and nonstarch polysaccharides, and groups them into rapidly available glucose (RAG) and slowly available glucose (SAG) as to the amounts of glucose (from sugar and starch, including maltodextrins) likely to be available for rapid and slow absorption, respectively, in the human small intestine.

**Objective:** We hypothesize that RAG is an important food-related determinant of the glycemic response.

**Design:** The measurement of RAG, SAG, and starch fractions by an in vitro technique is described, based on the measurement by HPLC of the glucose released from a test food during timed incubation with digestive enzymes under standardized conditions. Eight healthy adult subjects consumed 8 separate test meals ranging in RAG content from 11 to 49 g.

**Results:** The correlation between glycemic response and RAG was highly significant (P < 0.0001) and a given percentage increase in RAG was associated with the same percentage increase in glycemic response. After subject variation was accounted for, RAG explained 70% of the remaining variance in glycemic response.

**Conclusions:** We show the significance of in vitro measurements of RAG in relation to glycemic response in human studies. The simple in vitro measurement of RAG and SAG is of physiologic relevance and could serve as a tool for investigating the importance of the amount, type, and form of dietary carbohydrates for health. *Am J Clin Nutr* 1999;69:448–54.

#### **KEY WORDS**

Carbohydrate classification, glycemic index, glycemic response, rapidly available glucose, RAG, slowly available glucose, SAG, starch

## INTRODUCTION

Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine, depending on their botanical source and the physical form of the food (1). It has been suggested that diets that contain large amounts of rapidly digested carbohydrates, which elevate blood glucose and insulin responses, may be detrimental to health. In 2 large, recently published epidemiologic studies, an increased relative risk of type 2 diabetes mellitus of 2.17 for men and 2.5 for women was seen with a combination of high glycemic load and low cereal fiber intake (2, 3). It has been suggested also that diets rich in slowly digested carbohydrates may protect against chronic disease (4). Clinical studies of persons with diabetes have found improved glycemic control with such diets (5–8). The potential health implications of altering the quality of carbohydrates in the diet highlight the requirement for a classification of dietary carbohydrates that takes this aspect into account.

The proposed classification of dietary carbohydrates (**Table 1**), which was developed in conjunction with human studies, characterizes carbohydrates with respect to both chemical composition and likely site, rate, and extent of digestion (9–15). Starch is divided into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch. Here we describe a technique, with an HPLC endpoint, for the in vitro measurement of these starch fractions as well as rapidly available glucose (RAG) and slowly available glucose (SAG). The designations RAG and SAG are designed to reflect the rate at which glucose (from sugars and starch, including maltodextrins) becomes available for absorption in the human small intestine.

#### SUBJECTS AND METHODS

# Materials

The polyethylene tubes used (50 mL) were from Falcon (Oxford, United Kingdom). Glass balls (1.5-cm diameter) were from Magnet Wholesale (Halesworth, United Kingdom). The shaking water bath was a Grant Instruments Ltd model SS-40-2 (Cambridge, United Kingdom). The bath was fitted with clips to hold 50-mL tubes exactly horizontal, fully immersed in the water, with the long axis of each tube in the direction of movement. The HPLC system was from Dionex (UK) Ltd, (Camberley, United Kingdom) and is described in detail below. Reagents

<sup>&</sup>lt;sup>1</sup>From the MRC Dunn Clinical Nutrition Centre and the MRC Dunn Nutritional Laboratory, Cambridge, United Kingdom.

<sup>&</sup>lt;sup>2</sup>Address reprint requests to HN Englyst, MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH, United Kingdom. E-mail: hans.englyst@mrc-dunn.cam.ac.uk.

Received March 13, 1998.

Accepted for publication September 4, 1998.

IADLE I
---------

Classification of the carbohydrates in plant foods<sup>1</sup>

Class and component	Comments			
Sugars				
Mono- and disaccharides and their alcohols	Physiologic response depends on identity and rate of release			
	Free glucose + glucose from sucrose = $FSG$			
Short-chain carbohydrates				
Maltodextrins	Measured as RDS			
Resistant short-chain carbohydrates (nondigestible oligosaccharides)	Fermented in the large bowel and may stimulate growth of bifidobacteria			
Starch				
RDS	RDS + rapidly released FSG = RAG			
SDS	SDS + slowly released FSG = SAG			
RS	Escapes digestion in the small intestine			
Nonstarch polysaccharides				
Plant cell wall nonstarch polysaccharide (dietary fiber)	Encapsulate and slow absorption of other nutrients;			
	marker for naturally high-fiber diets for which			
	health benefits have been shown;			
	fermented in the large bowel to different extents			
Other nonstarch polysaccharides	Food additives; minor components of the human diet;			
	fermented in the large bowel to different extents			

<sup>1</sup> FSG, free sugar glucose; RDS, rapidly digestible starch; RAG, rapidly available glucose; SDS, slowly digestible starch; SAG, slowly available glucose; RS, resistant starch.

were from Sigma (Poole, United Kingdom) or Merck (Poole, United Kingdom) unless stated otherwise.

The internal standard solution was 40 g arabinose/L in water with 50% saturated benzoic acid. The stock sugar mixture was 50 g glucose/L and 25 g fructose/L in water with 50% saturated benzoic acid. Sugars were dried to constant weight at reduced pressure over phosphorus pentoxide before use.

The enzymes used were pepsin from Sigma (catalog no. P-7000; St Louis), amyloglucosidase from Novo Nordisk (AMG 400 L type LP; Bagsvaerd, Denmark), pancreatin from Sigma (catalog no. P-7545), and invertase from Merck (catalog no. 390203D). The enzyme mixture was prepared on the day of use. For 18 samples, 3.0 g pancreatin was weighed into each of 6 centrifuge tubes and a magnetic stirring bar and 20 mL water was added to each. The pancreatin was suspended by vortex mixing and then mixed for 10 min on a magnetic stirrer. The tubes were centrifuged at  $1500 \times g$  for 10 min; 15 mL of the cloudy supernate from each tube (90 mL total) was removed into a flask and 4 mL amyloglucosidase and 6 mL invertase were added and mixed well.

#### Analytic protocol

The American Journal of Clinical Nutrition

The method described here is modified from that of Englyst et al (12), which uses a colorimetric endpoint. The method described here is made more reproducible by the introduction of an HPLC endpoint, which allows the use of an internal standard and has the potential advantage of measuring sugars other than glucose. The method yields CVs of 3.7% intraassay ( $n = 3 \times 5$ ) and 6.6% interassay ( $n = 3 \times 6$ ) for the measurement of RAG, 2.2% and 3.9% for the sum of RAG and SAG ( $G_{120}$ ), and 1.6% and 2.5% for total glucose for the 3 reference samples, respectively.

It is important that samples are analyzed in a form that resembles the food "as eaten." The spaghetti and pearled barley were first boiled in water, for 11 and 30 min, respectively, then drained and while still hot passed through a hand-operated mincer with holes 0.9 cm in diameter. The minced samples were kept at 60-70 °C until analyzed. The corn flakes were crushed and the white bread was broken up.

#### In vitro measurement of free sugar glucose and fructose

Samples of food (containing <0.6 g carbohydrate) were weighed to the nearest milligram into 50-mL polypropylene centrifuge tubes and 5 mL internal standard (arabinose), 20 mL water, and 5 glass balls were added. The tubes were capped and the contents vortex mixed vigorously. The tubes were placed into a boiling water bath and left for 30 min. The tube contents were then vortex mixed vigorously to completely disrupt the sample. The tubes were cooled to 37 °C, 0.3 mL invertase was added, and the tubes placed into a shaking water bath at 37 °C and left for 30 min. Then 0.2 mL of each sample was added to 4 mL absolute ethanol and vortex mixed; this was the free sugar glucose (FSG) portion. Values for FSG (the sum of free glucose and glucose from sucrose) and for free sugar fructose (FSF; the sum of free fructose and fructose from sucrose) were obtained from this portion.

# In vitro measurement of RAG, SAG, total glucose, and starch fractions

Samples of food (containing < 0.6 g carbohydrate) were weighed to the nearest milligram into 50-mL polypropylene centrifuge tubes. Internal standard solution (5 mL of 40 g arabinose/L) and 10 mL freshly prepared pepsin-guar gum solution (5 g pepsin/L and 5 g guar gum/L in 0.05 mol HCl/L) was added. The tubes were capped and the contents were vortex mixed and placed into a water bath at 37°C for 30 min to allow hydrolysis of proteins by pepsin. Five milliliters 0.5 mol sodium acetate/L (equilibrated to 37 °C) was added to each tube to form a buffer at pH 5.2. Five glass balls were added and the tubes were capped and shaken gently to disperse the contents and then placed in the 37°C water bath to equilibrate for a few minutes. In the shaking water bath, the glass balls function to mechanically disrupt the physical structure of the samples during the main incubation. The guar gum standardizes viscosity, keeps the sample in suspension, and prevents its sedimentation and excessive disruption by the glass balls.

One sample tube was removed from the 37 °C water bath and 5 mL enzyme mixture added. The tube was immediately capped and the contents mixed gently by inversion before it was secured hor-

The American Journal of Clinical Nutrition

izontally in the 37 °C shaking water bath. The shaking action of the water bath was started at this time, which was taken as time zero for the incubation and was not interrupted until all the  $G_{120}$  portions were collected (*see* below). The enzyme mixture was added to the rest of the sample tubes at 1-min intervals, to aid timing of incubations, and they were placed into the shaking water bath. Each tube was removed from the bath exactly 20 min after the enzyme mixture was added and 0.2 mL of the contents was added to 4 mL absolute ethanol and vortex mixed to stop the hydrolysis; this was the  $G_{20}$  portion. The tube was returned to the shaking water bath immediately after the sample was taken. After another 100 min (a 120-min incubation), another 0.2 mL was added to 4 mL absolute ethanol and vortex mixed; this was the  $G_{120}$  portion.

When all the  $G_{120}$  portions had been collected, the tube contents were vigorously vortex mixed to break up any large particles and the tubes were placed together into a boiling water bath and left for 30 min. The tube contents were vortex mixed again and the tubes cooled in ice water for 15 min.

Potassium hydroxide (10 mL of 7 mol/L) was added, the tubes capped, and the contents mixed by inversion. The tubes were secured horizontally in a shaking water bath containing ice water and shaken for 30 min. Tubes were removed singly from the ice water and 0.2 mL of the contents was added to 1 mL of 1 mol acetic acid/L. Amyloglucosidase solution (40  $\mu$ L amyloglucosidase diluted 1:7 with water) was added to these tubes, the contents mixed, and the tubes placed into a 70 °C water bath for 30 min followed by 10 min in a boiling water bath. The tubes were cooled to room temperature before the addition of 12 mL absolute ethanol; this was the total glucose portion.

Potato starch and white wheat flour were included as reference materials (**Table 2**) in each batch of samples analyzed and 2 reagent blanks, one containing 4 mL stock sugar mixture, were included to correct for the sugar content of the enzyme preparations. The hydrolysis conditions were calibrated with potato starch, white wheat flour, and corn flakes reference materials (Table 2). (Reference materials and enzymes are available from Englyst Carbohydrate Services Ltd, United Kingdom). Potato starch (air-dried, from Kartoffelmel Centralen, Herning, Denmark) has a high resistant starch content and was used to establish the optimum speed of the shaking water bath. If the  $G_{120}$ value for the potato starch was too high (Table 2), the strokespeed was decreased and vice versa.

#### HPLC measurement of sugars

Two sugar standards were used for calibration. Standard 1 was 1 mL and standard 2 was 10 mL of the stock sugar mixture, each made to 20 mL with water, to which 5 mL of the internal standard solution was added and mixed well; 0.2 mL of this mixture was then removed and added to tubes containing 4 mL absolute ethanol.

Before HPLC analysis, all the ethanolic fractions were centrifuged for 5 min at  $1500 \times g$  at room temperature. The amount taken for analysis varied according to sugar content: 70 µL for the sugar standards and the  $G_{20}$  and  $G_{120}$  portions, 200 µL for the total glucose portions, and 70–120 µL for the FSG portions, depending on the expected free glucose content of the sample. The samples were placed into HPLC vials, 1 mL deionized water was added, and they were vortex mixed.

An autoinjector (model AS3500; Dionex) was used to inject 20  $\mu$ L of the diluted ethanolic fractions. Sugar separation was achieved with an anion-exchange analytic column (Carbopac

TABLE	2
-------	---

Target values for the 5 reference samp	mples	sam	reference	3	the	for	values	Target
--	-------	-----	-----------	---	-----	-----	--------	--------

$G_{20}$	G <sub>120</sub>	Total glucose
	% by wt	
4	27	89
35	70	72
77	80	81
	G <sub>20</sub> 4 35 77	$\begin{array}{c ccc} G_{20} & G_{120} \\ & & & \\ $

 $^{I}$   $G_{\rm 20 \ and}$   $G_{\rm 120,}$  glucose measured after 20 and 120 min incubation with enzymes, respectively.

PA100; Dionex) and guard column (Carbopac PA10; Dionex) by using a gradient pump (model GP40; Dionex). Column switching and an anion-exchange guard column (Aminotrap; Dionex) were used to prevent amino acids and peptides from reaching the analytic column. The eluents, high-purity water and 200 mol NaOH/L (16 mL 50% NaOH solution/L high-purity degassed water), were degassed. The flow rate was 0.8 mL/min and the elution conditions are shown in **Table 3**. Monosaccharide detection was achieved with an electrochemical detector (model ED40; Dionex) with the following pulse potentials (*E*) and durations (*t*):  $E_1$ , 0.05 V;  $t_1$ , 400 ms;  $E_2$ , 0.75 V;  $t_2$ , 200 ms;  $E_3$ , 0.15 V; and  $t_3$ , 400 ms. The response time was 1 s, and the output on the detector was used to integrate and plot the results.

Values for RAG, SAG, RDS, SDS, resistant starch, and total starch were calculated from the measured FSG,  $G_{20}$ ,  $G_{120}$ , and total glucose values (*see* Calculations and statistics). Values for the starch fractions were expressed as polysaccharides by using a conversion factor of 0.9.

#### Study design

Eight healthy, nonobese volunteers (3 men aged 24–57 y, 5 women aged 23–56 y) were recruited for the study. Ethical permission for the study was obtained from the Dunn Ethical Committee. On test days, after an overnight fast following a day on which the subjects were required to abstain from alcohol and excessive exercise, each subject arrived at the Dunn Clinical Nutrition Centre at 0800 without undue physical exertion.

Fasting blood glucose was determined as the average from a finger-prick sample taken at 0815 and another taken at 0830, immediately before the test meal was eaten. Subjects ate the test meals in different orders; they were required to consume the test meal within 10 min and to avoid undue physical exertion for the 2 h of the test. Additional finger-prick blood samples were taken at 15, 30, 45, 60, 90, and 120 min after the start of the test meal.

Sequence of elution	conditions for	or the HPLC	measurement of	of sugars

Switch		
position <sup>1</sup>	NaOH	Time
	mmol/L	min
А	10	0-3.5
В	70	3.6 - 14.0
В	200	14.1-15.0
В	10	15.1 - 20.0

<sup>1</sup> In switch position A, the flow is from Aminotrap (Dionex UK Ltd, Camberley, United Kingdom) to guard column to separation column. In switch position B, the flow is from guard column to separation column to Aminotrap. Sample injection is at time 0.1 min. Whole-blood glucose was measured by a hexokinase method.

#### Test foods and meals

The 4 test foods were 1) corn flakes, 2) white bread, 3) cooked white spaghetti, and 4) cooked pearled barley. These foods contain various proportions of RAG and SAG, and the carbohydrate content is almost exclusively starch. The details of the carbohydrate components of each cooked food are shown in **Table 4**.

There is evidence to suggest that the glycemic response is likely to be dose dependent for amounts  $\leq 50$  g RAG (16, 17) and the test meals were designed to lie within this range. Each of the test meals was calculated to provide 25 g (small portion) or 50 g (large portion) glucose available for absorption in the small intestine ( $G_{120}$ ). The RAG content of the test meals ranged from 11 g (small portion of pearled barley) to 49 g (large portion of white bread).

The small portion weights were 117 g pearled barley, 85 g spaghetti, 31.5 g corn flakes, and 59 g white bread. The large portions were double these weights. Water was provided such that the total weight of each meal was 500 g.

#### **Calculations and statistics**

RAG, SAG, and the various starch fractions were calculated as follows:

$$RAG = G_{20} \tag{1}$$

$$SAG = G_{120} - G_{20}$$
(2)  
RDS = (G<sub>20</sub> - FSG) × 0.9 (3)

$$SDS = (G_{120} - G_{20}) \times 0.9$$
(3)  
$$(3)$$

Total starch = (total glucose 
$$-FSG$$
) × 0.9 (5)

Resistant starch = (total glucose  $-G_{120}$ ) × 0.9 (6)

The glycemic response was calculated as the incremental area under the curve above the fasting glucose concentration, ignoring any area below the fasting value, according to the equation given by Wolever et al (18). Regression analysis of the observed glycemic response data was performed for individual subjects and for the mean of all subjects.

To further investigate the relation between RAG intake and glycemic response, we fitted the data to a model. The starting point for the analysis was that for each subject, glycemic response was assumed to be proportional to RAG intake. This can be written as follows:

$$Glycemic response = KRAG$$
(7)

where K is the constant of proportion. K may be different for each subject, and this is taken into account in the following for-

#### TABLE 4

#### Sugar and starch composition of the test foods<sup>1</sup>

	Fru	FSG	TG	RAG	SAG	RS	TS		
		% by wt of cooked food							
Corn flakes	3.0	3.3	82.3	75.7	3.7	2.6	71.1		
White bread	0.1	0.1	44.5	41.8	0.7	1.8	40.0		
Spaghetti	0.0	0.1	30.8	17.5	12.0	1.2	27.6		
Pearled barley	0.0	0.0	26.1	9.4	12.0	4.2	23.5		

<sup>1</sup> Fru, fructose; FSG, free sugar glucose; TG, total glucose; RAG, rapidly available glucose; SAG, slowly available glucose; RS, resistant starch; TS, total starch. For calculations, *see* the text.

mula:

 $Glycemic response = KS_i RAG$ (8)

where *K* is an overall constant and  $S_i$  is a constant for the *i*th subject (*i* takes values between 1 and 8). To avoid ambiguity, *K* is the constant for the first subject and  $S_i$  is scaled appropriately, so  $KS_i$  is the constant for the *i*th subject (*i* = 2...8) and  $S_1 = 1$ .

The RAG content of each food differs and can be represented in the formula by  $F_j$ , where j = 1...4. As for the subjects, the first food level,  $F_{1}$ , is set to 1. In addition, each food is provided at 2 doses, and the assumption is that this increases the response by a constant factor. The 2 dose effects are represented as  $D_k$ , and again, the first is fixed at 1. The complete formula is then

$$Glycemic response = KS_i F_i D_k$$
(9)

where K is the response for subject 1 eating food 1 at dose level 1. The predicted response for other combinations of subject, food, and dose is obtained by multiplying it by the appropriate constants. For example, the fifth subject eating the third food at the high dose has a response predicted by

Glycemic response = 
$$KS_5F_3D_2$$
 (10)

The statistical problem is how to estimate the values of K,  $S_i$ ,  $F_j$ , and  $D_k$ . The coefficients are simple to estimate by using analysis of variance or regression analysis if the effects are added rather than multiplied together, and this is achieved with a logarithmic transformation. If the natural logarithm of each term is taken, the formula becomes the following:

$$\ln(\text{Glycemic response}) = \ln K + \ln S_i + \ln F_i + \ln D_k \qquad (11)$$

The logarithmic transformation assumes that the random error is proportional to the mean. This compensates for the heteroskedasticity seen in the original data, where the variability is greater for foods with a larger mean response. The random error is additive on the logarithmic scale and multiplicative on the original scale.

The formula represents the 8 meals in terms of 2 doses (2 portion sizes) of each of 4 foods. Assuming a proportional relation between RAG and glycemic response, the food and dose terms can be replaced in the formula by the measured RAG content of the meals. This leads to the following simplified formula:

$$\ln(\text{Glycemic response}) = \ln K + \ln S_i + C \ln RAG \qquad (12)$$

where *C* is the regression coefficient of ln(glycemic response) on lnRAG. The value of *C* ought to be close to 1 because this corresponds to the formula glycemic response =  $KS_i$ RAG on the untransformed scale.

To test the validity of the assumptions on which the formula is based, the simpler and the extended versions of the formula can be compared in terms of their fit to the data. The simpler formula estimates 1 coefficient (*C*) instead of 7 (3  $F_j$ , 1  $D_k$ , and 3 for their interaction), but, inevitably, it fits less well. If the loss of fit is matched by the saving of 6 degrees of freedom, so that the residual variation is not significantly greater, this is evidence that the glycemic response–RAG relation is proportional, as assumed. Other tests of the model are that *I*) the interactions between subject, meal, and dose are not significant; 2) the effect of the high-dose  $\ln D_2$  is not significantly different from  $\ln 2 = 0.69$ , being a doubling of the low dose; and 3) the coefficient (*C*) for lnRAG is not significantly different from 1.

# RESULTS

The American Journal of Clinical Nutrition

The mean change in blood glucose concentration for all subjects in response to consumption of the 8 test meals, namely, the 2 portion sizes of each of the 2 high-RAG foods (white bread and corn flakes) and the 2 low-RAG foods (pearled barley and spaghetti), is shown in **Figure 1**. Linear regression analysis was performed for the observed glycemic response values for individual subjects and the RAG content of the test meals. For 7 of the 8 subjects, the correlation coefficient was significant (for each subject, n = 8; P < 0.05) and the regression coefficient was extremely variable between subjects, ranging from 1.83 to 5.27. The highly significant correlation (r = 0.981, P < 0.0001) between the mean glycemic response values for all the subjects and the RAG content of the test meals is shown in **Figure 2**.

Two extreme outliers (glycemic response ~ 0) were excluded from the analysis of variance, which had the effect of reducing the residual variation and making the model more severe. Fitting the remaining 62 glycemic response values to the model shown in equation 11 revealed that the subject, food, and dose terms were all significant (P < 0.0001). (Fitting the data to a model, not shown, that included subject, food, and dose interaction terms showed that none was significant, and these interaction terms were excluded from all further models.) Linear regression of ln(glycemic response) on lnD yielded a coefficient of 0.78 (SE: 0.09), which was not significantly different from 0.69, indicating a 2-fold increase in glycemic response between the 2 doses, as expected.

Replacing the 4 food terms with their RAG values explained the difference in glycemic response between the foods, and replacing both the food and dose terms with RAG as a continuous variable in the simplified formula shown in equation 12yielded a value for C of 1.04 (SE: 0.09, P < 0.0001). This regression coefficient is not significantly different from 1, indicating that under the conditions of this study, a given percentage change in RAG is associated with the same percentage change in glycemic response. After subject variation was accounted for, RAG content per meal explained 70% of the remaining variance (**Table 5**).

#### DISCUSSION

The human diet contains many types of carbohydrates, each of which contributes to different physiologic responses (1). Starch is considered by many to be digested slowly, resulting in a modest glycemic response. However, the rate and extent to which starch is digested and absorbed, and the resulting glucose and insulin responses, vary considerably depending on the source and degree of food processing (9–11, 19–22).

Present knowledge of the variation in glycemic response to carbohydrate-containing foods comes largely from measurements of glycemic index (GI). In principle, the GI is calculated as the measured glycemic response to a portion of test food that contains 50 g "available" carbohydrate (which includes fructose) expressed as a percentage of the glycemic response to the same amount of "available" carbohydrate from a standard food eaten by the same subject (16, 18). GI values have been published for a wide range of foods (23) and have been used in several studies to design low-glycemic-load diets for diabetic subjects (5–8). However, no simple in vitro term is available that defines the carbohydrate in a food in such a way as to characterize its digestion in the gut.

The in vitro technique described here determines RAG, SAG, and starch fractions by measuring the amount of glucose released from a test food during timed incubation with digestive enzymes under standardized conditions. GI is a direct measure of the glycemic response to a food, and thus reflects all the mechanisms that can influence the glycemic response. For most carbohydrate foods, however, the RAG content is almost certainly a major determinant of the magnitude of the GI, and we have shown a strong correlation between published GI values and



**FIGURE 1.** Glycemic response ( $\bar{x} \pm$  SEM increase in blood glucose over fasting concentrations) to 8 test meals containing different amounts of rapidly available glucose (RAG). Low-RAG foods spaghetti and pearled barley and high-RAG foods corn flakes and white bread were each fed in portions containing 25 g (—) and 50 g (----) available glucose.

 TABLE 5

 ANOVA results for the final model for the association between rapidly available glucose (RAG) and the glycemic response

Source	df	Sum of squares	Mean square	F ratio	Р
Subject (S)	7	11.31	1.62	12.95	< 0.0001
RAG	1	15.37	15.37	123.2	< 0.0001
Error	53	6.61	0.12		
Total	61	33.28			

RAG values for a wide range of starchy foods (24).

This study presents measurements of glycemic response to test foods for which RAG and SAG have been measured. The demonstration that a given percentage change in RAG (achieved by altering the type of food or the amount of food consumed) is associated with the same percentage change in glycemic response supports the hypothesis that RAG intake is a major determinant of the magnitude of the glycemic response, under the conditions of this study. Once between-subject variation is accounted for, 70% of the remaining variance in glycemic response is explained by differences in the RAG content of the test meals.

Both GI and RAG measurements show how food type and food processing can influence the physiologic properties of dietary carbohydrate. White bread, corn flakes, and spaghetti are all examples of highly processed foods. The starch in bread and in corn flakes is fully gelatinized and thus likely to be rapidly digested and absorbed (Figure 1) and these foods have high RAG values. Spaghetti, however, has a low RAG value (Figure 1), due to a dense food matrix, which hinders enzymatic hydrolysis of the starch. In many plant foods, such as legumes and minimally processed cereal grains (eg, pearled barley), nutrients are encapsulated within cell walls (dietary fiber), which retard the release and hence digestion and absorption of starch and sugars, and these foods have low RAG values.

National dietary guidelines for the general public advocate a decreased intake of fat and an increased intake of carbohydrate, with the recommendation that both can be achieved by increased consumption of fruit, vegetables, legume seeds, and minimally



**FIGURE 2.** Correlation of rapidly available glucose (RAG) content and glycemic response to 8 test meals by least-squares analysis of the incremental area under the glucose response curve (iAUC  $\pm$  SEM). The numerals indicate small and large portions, respectively, of the test meals: 1 and 3, pearled barley; 2 and 6, spaghetti; 4 and 7, corn flakes; 5 and 8, white bread.

processed cereal foods. In this type of naturally high-fiber, lowfat diet, which is associated with a wide range of health benefits, the encapsulation of starch and sugars within plant cell walls (dietary fiber) slows their release; hence, much of the available carbohydrate in these foods is likely to be SAG.

Potentially detrimental effects have been associated with diets containing large amounts of fructose (25), suggesting that fructose intakes should be assessed separately. A value for fructose, including that from sucrose, is obtained by the HPLC technique described here.

The results of this study show the usefulness of the in vitro measurement of RAG and SAG for characterizing the test foods with respect to glycemic response. To determine the relation between dietary carbohydrate intake and health, the proposed classification scheme (Table 1) and associated measurements are being applied in ongoing epidemiologic studies.

# REFERENCES

- Cummings JH, Roberfroid MB, and members of the Paris Carbohydrate Group. A new look at dietary carbohydrates: chemistry, physiology and health. Eur J Clin Nutr 1997;51:417–23.
- Salmerón J, Ascherio A, Rimm EB, et al. Dietary fiber, glycemic load, and risk of NIDDM in men. Diabetes Care 1997;20:545–50.
- Salmerón J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA 1997;277:472–7.
- FAO/WHO. Carbohydrates in nutrition. Report of a Joint FAO/WHO Expert Consultation, Rome, 14–18 April 1997. Rome: FAO, 1997. (FAO Nutrition Paper 66.)
- Brand JC, Colagiuri S, Crossman S, Allen A, Roberts DCK, Truswell AS. Low-glycemic index foods improve long-term glycemic control in NIDDM. Diabetes Care 1991;14:95–101.
- Wolever TMS, Jenkins DJA, Vuksan V, Jenkins AL, Buckley GC, Wong GS. Beneficial effect of a low glycemic index diet in type 2 diabetes. Diabet Med 1992;9:451–8.
- Fontvielle AM, Rizkalla SW, Penfornis A, Acosta M, Bornet FRJ, Slama G. The use of low glycemic index foods improves metabolic control of diabetic patients over five weeks. Diabet Med 1992;9:444–50.
- Frost G, Wilding J, Beecham J. Dietary advice based on the glycemic index improves dietary profile and metabolic control in type 2 diabetic patients. Diabet Med 1994;11:397–401.
- Englyst HN, Cummings JH. Digestion of the polysaccharides of some cereal foods in the human small intestine. Am J Clin Nutr 1985;42:778–87.
- Englyst HN, Cummings JH. Digestion of the carbohydrates of banana (*Musa paradisiaca sapientun*) in the human small intestine. Am J Clin Nutr 1986;44:42–50.
- Englyst HN, Cummings JH. Digestion of the polysaccharides of potato in the small intestine of man. Am J Clin Nutr 1987;45:423–31.
- Englyst HN, Kingman SM, Cummings, JH. Classification and measurement of nutritionally important starch fractions. Eur J Clin Nutr 1992;46(suppl):S33–50.
- Englyst HN, Kingman SM, Hudson GJ, Cummings JH. Measurement of resistant starch in vitro and in vivo. Br J Nutr 1996;75:749–55.
- Silvester KR, Englyst HN, Cummings JH. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. Am J Clin Nutr 1995;62:403–11.
- Englyst HN, Hudson GJ. The classification and measurement of dietary carbohydrates. Food Chem 1996;57:15–21.
- Jenkins DJA, Wolever TMS, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981;34:362–6.
- Wolever TMS, Bolognesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. J Nutr 1996;126:2798–806.
- 18. Wolever TMS, Jenkins DJA, Jenkins AL, Josse RG. The glycemic index:

Downloaded from ajcn.nutrition.org by guest on May 29, 2016

methodology and clinical implications. Am J Clin Nutr 1991;54:846-54.

- Jenkins DJA, Wolever TMS, Taylor RH, et al. Rate of digestion of foods and postprandial glycaemia in normal and diabetic subjects Br Med J 1980;281:14–7.
- Snow P, O'Dea K. Factors affecting the rate of hydrolysis of starch in foods. Am J Clin Nutr 1981;34:2721–7.
- Heaton KW, Marcus SN, Emmett PM, Bolton CH. Particle size of wheat, maize, and oat test meals: effects on plasma glucose and insulin responses and on the rate of starch digestion in vitro. Am J Clin Nutr 1988;47:675–82.
- Larsen HN, Christensen C, Rasmussen OW, et al. Influence of parboiling and physiochemical characteristics of rice on the glycaemic index in non-insulin-dependent diabetic subjects. Eur J Clin Nutr 1996;50:22–7.
- Foster-Powell K, Miller JB. International tables of glycemic index. Am J Clin Nutr 1995;62(suppl):S871–93.
- Englyst HN, Veenstra J, Hudson GJ. Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycemic response. Br J Nutr 1996;75:327–37.
- McDonald RB. Influence of dietary sucrose on biological aging. Am J Clin Nutr 1995;62(suppl):S284–93.

Downloaded from ajcn.nutrition.org by guest on May 29, 2016