

ORIGINAL COMMUNICATION

Determination of the glycaemic index of foods: interlaboratory study

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Objective: Practical use of the glycaemic index (GI), as recommended by the FAO/WHO, requires an evaluation of the recommended method. Our purpose was to determine the magnitude and sources of variation of the GI values obtained by experienced investigators in different international centres.

Design: GI values of four centrally provided foods (instant potato, rice, spaghetti and barley) and locally obtained white bread were determined in 8–12 subjects in each of seven centres using the method recommended by FAO/WHO. Data analysis was performed centrally.

Setting: University departments of nutrition.

Subjects: Healthy subjects (28 male, 40 female) were studied.

Results: The GI values of the five foods did not vary significantly in different centres nor was there a significant centre × food interaction. Within-subject variation from two centres using venous blood was twice that from five centres using capillary blood. The s.d. of centre mean GI values was reduced from 10.6 (range 6.8–12.8) to 9.0 (range 4.8–12.6) by excluding venous blood data. GI values were not significantly related to differences in method of glucose measurement or subject characteristics (age, sex, BMI, ethnicity or absolute glycaemic response). GI values for locally obtained bread were no more variable than those for centrally provided foods.

Conclusions: The GI values of foods are more precisely determined using capillary than venous blood sampling, with mean between-laboratory s.d. of approximately 9.0. Finding ways to reduce within-subject variation of glycaemic responses may be the most effective strategy to improve the precision of measurement of GI values.

European Journal of Clinical Nutrition (2003) **57**, 475–482. doi:10.1038/sj.ejcn.1601551

Keywords: carbohydrates; diet; methods; blood glucose responses

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Contributors: TMSW coordinated the study, did the statistical analysis and drafted the manuscript. TMSW and HHV conceived of the overall study and were responsible for raising funds and planning the studies at their local sites. IB, JBM, FB, JIM and DDR (whose names are listed in alphabetical order) were responsible for raising funds and planning the studies at their local sites. YG, SH, TLP, CV and XW were responsible for implementing the studies at their local sites. All contributors helped with the revision of the paper.

Received 4 October 2001; revised 10 June 2002;

accepted 11 June 2002

Introduction

The glycaemic index (GI) is a classification of the blood glucose raising potential of carbohydrate foods. It is defined as the incremental area under the blood glucose response curve elicited by a 50 g available carbohydrate portion of a food expressed as a percentage of that after 50 g carbohydrate from a reference food taken by the same subject (Wolever *et al*, 1991). Many factors such as food form, particle size, cooking, processing and starch structure affect the GI (Björck *et al*, 1994). There is evidence that low GI foods improve blood glucose control in people with diabetes (Brand *et al*, 1991; Wolever *et al*, 1992a; Frost *et al*, 1994; Järvi *et al*, 1999; Gilbertson *et al*, 2001), reduce serum lipids

in people with hypertriglyceridaemia (Jenkins *et al*, 1987a), prolong endurance during physical activity (Thomas *et al*, 1991), improve insulin sensitivity (Frost *et al*, 1998) and increase colonic fermentation (Jenkins *et al*, 1987b; Wolever *et al*, 1992b). In addition, low GI foods are associated with high HDL cholesterol (Frost *et al*, 1999) and reduced risk for developing diabetes (Salmerón *et al*, 1997a,b) and cardiovascular disease (Liu *et al*, 2000). These effects prompted a recent FAO/WHO consultation to endorse the usefulness of the GI in diet planning (FAO/WHO, 1998).

However, application of the GI is made difficult because the GI value of many common foods is not known. In addition, the GI values reported by different laboratories vary widely for some foods such as potato (Wolever *et al*, 1994; Soh & Brand-Miller, 1999) and rice (Foster-Powell & Brand-Miller, 1995). Differences in GI values of similar foods reported by different investigators could be due to real differences in starch structure or digestibility, variation in methodology, or to the effects of random variation (Wolever *et al*, 1991). It is difficult to know how much each of these effects contributes to the variation of GI values because the performance of GI methodology has not been assessed. Therefore, the main objective of this study was to determine the magnitude of variation of the GI values of the same foods determined by experienced investigators using their usual procedures in different laboratories around the world. The usual methods for taking blood samples and measuring blood glucose and the demographic characteristics and ethnic background of subjects vary in the different participating laboratories. Although these variables are not thought to have a major effect (FAO/WHO, 1998), we were interested to know whether they were associated with any difference in the GI results obtained.

Methods

Coordination of interlaboratory testing

Seven research groups, experienced in GI methodology, participated in this study: Toronto, Canada; Lund, Sweden; Sydney, Australia; Milan, Italy; Dunedin, New Zealand; Trinidad, West Indies; and Potchefstroom, South Africa. The Toronto-based centre was the central laboratory which coordinated the delivery of the test foods, protocol and data analysis. The seven research groups were each sent four identical test foods and the reference food (described below) from the central laboratory. Each laboratory used its usual protocol, which was in line with the procedures recommended by the FAO/WHO (1998). However, these recommendations allow variation in blood sampling (capillary or venous), glucose analysis method and whether glucose is measured in whole blood or plasma.

Test foods

The four centrally provided foods were: instant mashed potato (Idahoan Foods, Lewisville, ID, USA), long grain rice

(Star Brand, Goudas Food Products, Concord, Ontario, Canada), white spaghetti (Unico, Concord, Ontario, Canada) and pot barley (Goudas Food Products, Concord, Ontario, Canada). These foods, selected to provide a wide range of GI values, were purchased in Toronto and sent by courier to the participating laboratories with instructions about portion sizes to use and cooking instructions, which were generally according to package directions (except instant potatoes were prepared with water instead of milk). In addition, each of the research groups determined the GI value of a regular white bread produced in their area. Anhydrous glucose (Sigma Chemical Co., St Louis, MO, USA), dissolved in water was used as the reference food at each research centre. Each of the five test foods and the reference food were fed as portions providing 50 g of available carbohydrate, defined as total carbohydrate by difference minus dietary fibre. Some centres randomized the order of all eight trials, whereas others gave glucose as the first and last trials and randomized the order of the other six trials. Most subjects conducted one trial of each test food and three trials of the reference food. However, one centre studied two different portion sizes of barley (see below), and only repeated the reference food trial twice instead of three times. At another centre, subjects repeated both the glucose and the white bread trials three times (for a total of 10 trials done by each subject).

Portion sizes of instant potato (67.3 g), rice (64.9 g), spaghetti (72.3 g) and barley (79.6 g) were based on proximate and dietary fibre analysis using AOAC methods (AOAC, 1995). In one centre, the total and resistant starch contents of barley were measured using an *in vitro* technique (Åkerberg *et al*, 1998) and an additional trial done using a portion size calculated based on defining carbohydrate as total starch minus resistant starch. This resulted in a portion size of 93.9 g to compensate for the fact that 15.2% of the total starch was resistant starch. The portion size of white bread was based on local food tables.

Subjects

At each research centre, 8–12 normal, healthy subjects without diabetes were recruited to voluntarily participate in the study. Ethical approval for the study was obtained at each participating centre and all subjects gave informed consent to participate.

Experimental procedures

Subjects were studied on eight separate occasions in the morning after a 10–12 h overnight fast. After a fasting blood sample, subjects ate a test meal at a comfortable pace within 15 min and had further blood samples at 15, 30, 45, 60, 90 and 120 min after starting to eat. Test meals were served with a drink of the subject's choice (water, coffee or tea with milk if desired, but no sugar). The drink chosen by each subject remained constant for each of the eight trials.

Blood could be obtained by finger-prick or venepuncture and whole blood or plasma glucose measured by any recognized method. The method of blood sampling and glucose measurement remained standard for the duration of the study at each centre. Two centres measured whole capillary blood glucose using an automatic analyser (YSI Stat2300, Yellow Springs, OH, USA). One centre measured whole capillary blood glucose by glucose oxidase after mixing 1 ml 0.025 M NaOH and 50 µl 0.3 M ZnSO₄ solution with 50 µl whole capillary blood. Four centres measured plasma glucose by a hexokinase method, two obtaining plasma from capillary and two from venous blood samples.

Data analysis

Data were sent to the coordinating centre (Toronto) for statistical analysis. The incremental area under the blood glucose response curve (AUC), ignoring area beneath the baseline, was calculated geometrically (FAO/WHO, 1998).

The mean, s.d. and coefficient of variation (CV = 100 × s.d./mean) of the AUC of each subject's repeated glucose trials (reference food) were calculated. The AUC for each food taken by each subject was expressed as a percentage of the mean AUC for the reference food (glucose) taken by the same subject; the mean of the resulting values was the food GI. To determine the effect of using only a single trial of the reference food in calculating the GI, one glucose trial was selected at random for each subject and the GI values calculated using the AUC for the randomly selected glucose trial. We also explored the effect of calculating the ratio the means, ie 100 × F/R , where F = the mean of all subjects' AUC for the test food, and R = the mean of all subjects' AUC for the reference food, to see whether this would reduce between centre variation.

Theoretical considerations suggest that the distribution of GI values is skewed if large variability of glycaemic response areas exists (Wolever *et al*, 1991). Since some of the differences in methods used in the different centres appeared to affect the variability of the glycaemic responses, the distribution of GI values calculated in different ways was

determined. To remove the variation in GI due to differences between foods, GI values were adjusted for the difference between the median value for each food and instant potato (instant potato was chosen because otherwise the adjustment would have resulted in some negative values). For example, the median GI value for instant potato was 85.7 and that for bread was 70.5; thus, the bread GI values were adjusted by adding 15.2. D'Agostino's test (test statistic is termed 'D') was used to determine whether the values were normally distributed (Zar, 1984).

The data were subjected to analysis of variance (ANOVA) with repeated measures to examine for differences between subjects, centres and foods and the interaction between centre and foods. After demonstration of significant heterogeneity, the significance of differences between individual means was determined with Tukey's test to control for multiple comparisons. The test was modified to compare groups of unequal sizes by using a pooled value for n , eg for the 35 groups (five foods, seven centres) pooled $n = 35/(1/n_1 + 1/n_2 + \dots + 1/n_{35}) = 9.58$. Single and multiple linear regression analyses were performed using Lotus 123 (Lotus Development Corp, Cambridge, MA, USA). For categorical variables (capillary or venous blood, plasma or whole blood glucose, male or female, Caucasian or non-Caucasian), dummy variables (0 or 1) were used in the regression analyses. Differences were considered significant if $P < 0.05$ (two-tailed).

Results

A total of 68 subjects was studied, 28 male and 40 female, median age 25 y (range 19–50) with median body mass index (BMI) 23.0 kg/m² (range 16.8–35.0). The gender distribution of subjects did not differ significantly between centres ($\chi^2 = 9.6$ d.f. = 6, $P = 0.14$), however, differences in age and BMI were significant (Table 1). There were 52 Caucasian subjects (including European and Scandinavian), eight East Indian, two African, two Middle-Eastern, one Chinese, one North American Indian and two mixed. Centres differed significantly with respect to the ethnicity of

Table 1 Characteristics of subjects participating at each centre

Centre	Method ^a	n (M:F)	Age (y)	BMI (kg/m ²)	Reference AUC ^b (mmol × min/l)	Reference CV ^c (%)
A	CBY	4:4	30.1 ± 3.4 ^B	20.8 ± 0.6 ^B	153 ± 17 ^{BC}	28.8 ± 4.9 ^B
B	CBY	5:5	36.3 ± 2.6 ^A	27.8 ± 1.3 ^A	210 ± 16 ^B	23.1 ± 4.3 ^B
C	CPH	5:4	24.9 ± 1.8 ^{BC}	20.9 ± 0.9 ^B	281 ± 27 ^A	25.6 ± 6.0 ^B
D	CPH	3:7	25.2 ± 2.4 ^{BC}	22.6 ± 0.3 ^B	174 ± 18 ^{BC}	19.3 ± 1.8 ^B
E	CBG	6:4	27.7 ± 1.0 ^{BC}	23.8 ± 0.5 ^B	162 ± 17 ^{BC}	21.5 ± 6.2 ^B
F	VPH	5:7	24.3 ± 0.9 ^{BC}	23.8 ± 0.4 ^B	158 ± 21 ^{BC}	53.4 ± 6.1 ^A
G	VPH	0:9	21.0 ± 0.5 ^C	21.6 ± 0.6 ^B	97 ± 16 ^C	61.3 ± 6.5 ^A
All		28:40	27.0 ± 0.9	23.2 ± 0.4	176 ± 9	33.7 ± 2.7

Values are means ± s.e.m.

^{ABC} Means not sharing the same letter superscript differ significantly, $P < 0.05$.

^aMethod: C = capillary, V = venous, P = plasma, B = whole blood, Y = YSI, H = hexokinase, G = glucose oxidase.

^bReference AUC = mean incremental area under blood glucose response curve after the reference food (50 g glucose).

^cReference CV = mean coefficient of variation (100 × s.d./mean) of AUC for repeated reference food tests.

Table 2 Incremental areas under the curve for the test foods determined by the different research centres. All of the test foods, except white bread, were identical between centres

Centre	Potato	Bread	Rice	Spaghetti	Barley	Mean
A	126 ± 45	112 ± 29	83 ± 39	58 ± 24	51 ± 17	86 ^{BCD}
B	188 ± 59	133 ± 39	123 ± 46	88 ± 40	62 ± 37	119 ^B
C	252 ± 101	185 ± 89	237 ± 102	189 ± 45	133 ± 61	199 ^A
D	171 ± 63	120 ± 37	109 ± 36	74 ± 20	42 ± 19	103 ^{BC}
E	142 ± 53	124 ± 50	123 ± 41	65 ± 17	61 ± 20	103 ^{BC}
F	95 ± 72	97 ± 51	90 ± 48	47 ± 30	34 ± 36	72 ^{CD}
G	67 ± 37	75 ± 61	77 ± 63	46 ± 31	40 ± 34	61 ^D
All ^a	147 ± 84 ^X	120 ± 60 ^X	120 ± 73 ^X	80 ± 54 ^Y	59 ± 45 ^Y	

Values are means ± s.d.

^{XY}Means with different letter superscripts differ significantly ($P < 0.05$).

^{ABCD}Means with different letter superscripts differ significantly ($P < 0.05$).

^aMeans for all subjects ($n = 68$). ANOVA effects: food, $F_{4,24} = 50.7$ ($P < 0.001$); centre, $F_{6,24} = 32.5$ ($P < 0.001$); centre × food interaction, $F_{24,237} = 1.77$ ($P = 0.017$).

their subjects with Milan and Potchefstroom having all Caucasian subjects, Trinidad having no Caucasians and the other centres having from one to three subjects of non-Caucasian descent.

There were significant differences between centres for the mean AUC after the reference food (50 g glucose) and the CV of the repeated reference food AUC (CVref; Table 1). There was significantly greater within-subject variation of blood glucose responses in each of the two centres measuring glucose in venous plasma than each of those using capillary blood (Table 1). In the 47 capillary blood subjects, mean CVref, $23.4 \pm 2.1\%$, was significantly less than in the 21 venous blood subjects $56.8 \pm 4.4\%$ ($P < 0.001$). By linear regression analysis, type of blood sampling (capillary or venous) explained 47% of the variation in CVref ($P < 0.001$). Adding the other six variables (age, sex, ethnicity, BMI, plasma or

whole blood glucose, and mean AUC after reference food) to the model only accounted for an additional 3% of the variation of CVref (N.S.).

The mean CV of the AUC of repeated bread trials taken by 10 subjects, 27.7 ± 5.0 , did not differ significantly from that for their repeated glucose trials, 23.1 ± 4.3 .

The median-adjusted GI values for all five foods in all 68 subjects ($n = 340$) were not normally distributed (mean ± s.d., 88.8 ± 32.3 ; median, 85.7; $D = 0.2585$, N.S.). Since use of venous blood sampling was the only variable significantly associated with within-subject variability, the GI data from venous blood samples were excluded. The distribution of the remaining 235 values did not deviate significantly from a normal distribution (mean ± s.d., 91.1 ± 20.5 ; median, 90.1; $D = 0.2780$, $P < 0.05$). When only one trial of the reference food was used to calculate the GI, the resulting mean, s.d. and

Table 3 GI values for the test foods as determined by the different research centres. All of the test foods, except white bread, were identical between centres

Centre	Potato	Bread	Rice	Spaghetti	Barley	Mean
A	86.1 ± 29.7	78.7 ± 28.4	54.8 ± 24.1 ^X	38.7 ± 13.2 ^X	36.0 ± 15.4	58.9
B	93.3 ± 32.5	64.2 ± 15.4	62.6 ± 25.0 ^{XY}	44.1 ± 19.8 ^{XY}	31.4 ± 18.7	59.1
C	89.9 ± 23.9	64.6 ± 21.6	85.0 ± 28.6 ^Y	69.9 ± 18.8 ^Y	46.2 ± 15.4	71.1
D	98.5 ± 20.6	69.4 ± 3.6	63.3 ± 8.1 ^{XY}	43.8 ± 9.2 ^{XY}	24.5 ± 7.3	59.9
E	88.3 ± 21.3	78.9 ± 26.1	76.9 ± 12.9 ^{XY}	42.1 ± 10.8 ^{XY}	39.3 ± 13.1	65.1
F	65.2 ± 44.6	75.8 ± 64.3	68.4 ± 48.0	36.4 ± 35.8	23.2 ± 24.6	53.8
G	74.2 ± 40.3	75.9 ± 50.7	87.0 ± 75.9	57.0 ± 45.3	47.1 ± 49.7	68.2
All ^a	84.5 ± 32.7 ^A	72.5 ± 35.8 ^A	71.1 ± 38.2 ^A	46.9 ± 26.7 ^B	34.7 ± 24.7 ^B	
Venous ^b	69.0 ± 42.0 ^{AB}	75.8 ± 57.5 ^A	76.4 ± 60.5 ^A	45.2 ± 40.4 ^{BC}	33.4 ± 38.4 ^{BC}	
Capillary ^c	91.5 ± 25.1 ^A	71.0 ± 20.7 ^B	68.7 ± 22.6 ^B	47.6 ± 18.1 ^C	35.2 ± 15.6 ^C	

Values are means ± s.d.

^{XY}Values in the same column with different letter superscripts differ significantly ($P < 0.05$).

^{ABC}Values in the same row with different letter superscripts differ significantly ($P < 0.05$).

^aMeans for all subjects ($n = 68$). ANOVA effects: food, $F_{4,24} = 38.8$ ($P < 0.01$); centre, $F_{6,24} = 2.09$ ($P < 0.093$); centre × food interaction, $F_{24,237} = 1.21$ ($P < 0.24$).

^bMeans for subjects from capillary blood centres (A–E; $n = 47$). ANOVA effects: food, $F_{4,16} = 72.1$ ($P < 0.001$); centre, $F_{4,16} = 1.82$ ($P < 0.17$); centre × food interaction, $F_{16,163} = 2.25$ ($P = 0.006$).

^cMeans for subjects from venous blood centres (F and G; $n = 21$). ANOVA effects: food, $F_{4,4} = 4.80$ ($P < 0.002$); centre, $F_{1,4} = 11.06$ ($P < 0.029$); centre × food interaction, $F_{4,74} = 0.29$ ($P = 0.88$).

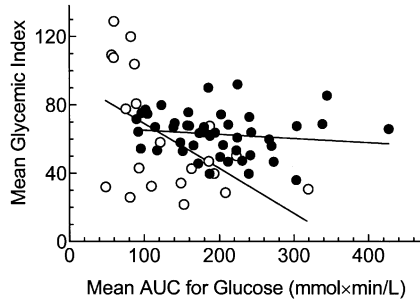


Figure 1 Relationship between mean incremental area under the curve for glucose and the mean GI value for 47 subjects from centres using capillary blood (●; $r = -0.14$, N.S.) and for 21 subjects from centres using venous blood (○; $r = -0.53$, $P = 0.01$). The lines plotted are regression lines.

median values were higher than when GI was calculated using the mean of three reference trials. In addition, the distribution of the resulting GI values was not normally distributed either for all the GI values (mean \pm s.d., 101.7 ± 72.4 ; median, 88.8; $D = 0.1885$, N.S.) or for the centres using capillary blood (mean \pm s.d., 96.4 ± 24.3 ; median 93.8; $D = 0.2755$, N.S.).

The mean incremental areas under the glycaemic response curves (AUC) for each food by centre are shown in Table 2. By ANOVA, AUC was significantly affected by subject, centre, food and food \times centre interaction. The mean GI values for each food, by centre, are shown in Table 3. In one centre the portion size of barley was increased from 79.6 to 93.9 g to adjust for its resistant starch content. This resulted in a non-significant increase in GI from 39.3 ± 13.1 to 44.4 ± 13.1 (mean \pm s.d. $n = 10$). By ANOVA GI was significantly affected by food, but there was no significant difference between centres and no centre \times food interaction. There was a significant difference in GI between subjects ($F_{67,237} = 2.04$, $P < 0.001$), but subject GI was not significantly related to age, sex, BMI, ethnicity or type of blood sampling. GI was not significantly related to AUC for glucose in centres using capillary blood sampling ($r = -0.14$, N.S.), but with venous blood sampling GI was inversely related to AUC for glucose ($r = -0.53$, $P = 0.013$; Figure 1).

Mean GI was not affected by type of blood sampling, but for centres using capillary blood sampling the s.d. were smaller in all cases than centres using venous blood (Table 3). The s.d. of the food GI values were positively related to their means in centres using venous blood ($P = 0.005$) and centres using capillary blood ($P = 0.002$, Figure 2a). The CV of the food GI values were negatively related to their means in centres using venous blood ($P = 0.026$), but the relationship did not reach significance for centres using capillary blood ($P = 0.080$, Figure 2b).

Considering only venous blood data, there were significant differences between foods and centres. Due to high random variation, there was no significant centre \times food interaction, and the only difference in GI between foods

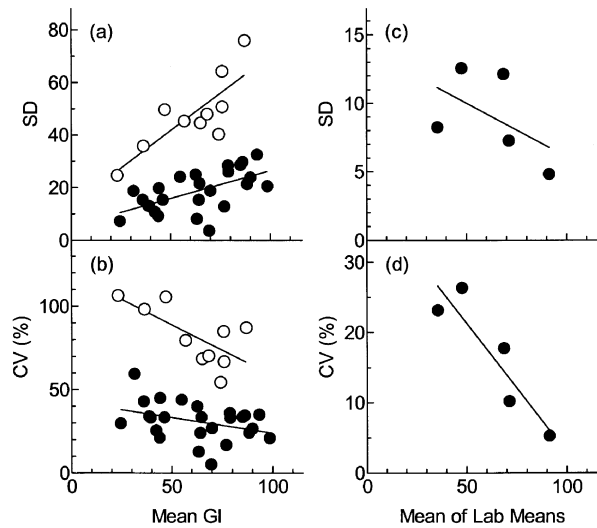


Figure 2 Relationships between mean GI and variation of GI for the five centres using capillary blood (●) and the two using venous blood (○); (a) mean and SD of GI values for five foods from capillary blood centres ($r = 0.597$, $P = 0.002$) and venous blood centres ($r = 0.803$, $P = 0.005$); (b) mean and CV of GI values for five foods from capillary blood centres ($r = -0.357$, $P = 0.080$) and venous blood centres ($r = -0.695$, $P = 0.026$); (c) mean and s.d. of laboratory mean GI values for five foods ($r = -0.518$, $P = 0.37$); (d) mean and CV of laboratory mean GI values for five foods ($r = -0.912$, $P = 0.031$).

was the difference between barley and bread (Table 3). For the capillary blood data, there was no significant difference between centres but there was a highly significant difference between foods (Table 3). The GI of all the foods differed significantly from each other, except for the difference between bread and rice, and the difference between barley and spaghetti. Because of low random variation, there was a significant centre \times food interaction with higher GI values for spaghetti and rice in centre C than in centre A. None of the other differences in GI values for the same food tested in

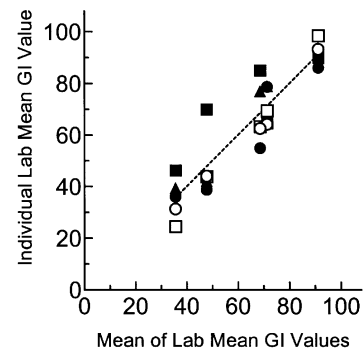


Figure 3 Individual centre mean GI values plotted against the mean of the centre mean GI values for the five foods (centres: A = ●; B = ○; C = ■; D = □; E = ▲). The dotted line is the line of identity.

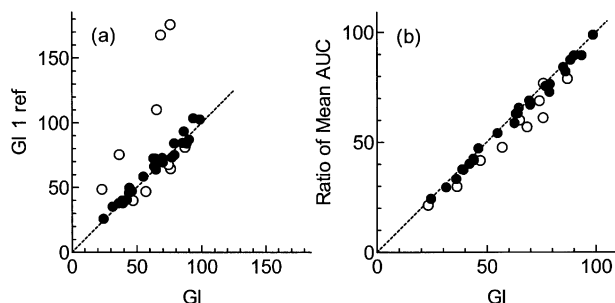


Figure 4 Plots showing individual laboratory mean GI values for the five foods for five centres using capillary blood (●) and two centres using venous blood (○); (a) GI vs GI calculated using one AUC value for the reference food (GI 1 ref); (b) GI vs mean AUC for test food expressed as a percentage of the mean AUC for the reference food (ratio of mean AUC). Dotted lines are lines of identity.

different centres were significant. There was significant heterogeneity between subject's mean GI values ($F_{46,163} = 2.55$, $P < 0.001$) among the 47 subjects from whom capillary blood samples were obtained, with the values being normally distributed and ranging from 35.9 to 91.9. Individual subjects' GI values were not significantly related to age, sex, BMI or ethnicity.

The key question we wished to address was what is the variation in GI determination between centres. Since the use of venous blood sampling appeared to be associated with markedly different within-subject variation, we included in this analysis only the results for the five centres using capillary blood. The mean GI values for the five foods from these five centres are plotted against the mean in Figure 3. The s.d. of the centre mean GI values for the five foods (potato, bread, rice, spaghetti and barley, respectively) are 4.8, 7.8, 12.2, 12.6 and 8.2. These s.d.s were not related to their means (Figure 2c), although their CVs were significantly related to their means (Figure 2d). From these data, therefore, we estimate the average s.d. for GI values estimated in 8–12 subjects by different laboratories is 9.0 resulting in a 95% confidence interval of about 18.

Figure 4a shows the relationship between the centre mean GI values for each food calculated using the mean of three reference trials (GI) vs using one reference trial (GI₁). For capillary blood centres, mean GI₁, 64.9, was 2.1 greater than mean GI, 62.8, with the difference in venous blood centres being 26.7 (87.7 vs 61.0). The mean s.d. for GI₁ values in the five capillary blood centres, 9.1, was slightly greater than the mean s.d. for the GI values, 9.0. Figure 4b shows the relationship between the centre mean GI values for each food vs the ratio of the mean AUC values (F/R). For capillary blood centres, mean F/R (61.3) was 1.5 less than mean GI (62.8), with the difference in venous blood centres being -6.7 (54.4 vs 61.0). The mean s.d. for F/R values in the five capillary blood centres, 8.9, was slightly less than the mean s.d. for the GI values, 9.0.

Discussion

The main purpose of this study was to estimate the magnitude of variation in GI determination between experienced laboratories. Neither type of blood sampling, method of glucose analysis, use of repeated reference food trials, age, sex, ethnicity and BMI of the subjects, type of reference food, nor inclusion of resistant starch within a food's available carbohydrate content appeared to have an appreciable effect on the *mean* GI value obtained. However, several of the variables were associated with a major effect on the *distribution* and *variability* of GI values and hence on the confidence limits of the means.

The major finding of this study was that the average s.d. of laboratory mean GI values for the five foods was 9.0. The magnitude of between centre variation was not reduced by expressing the mean AUC for each food as a percentage of the mean AUC for the reference food (glucose). The s.d. of 9 applies for mean GI values determined in 8–12 subjects. The interlaboratory s.d. was relatively constant across the wide range of mean GI values represented (35–91). The implication of this finding is that when published GI values of the 'same' food determined by different laboratories (particularly those represented here) differ by more than 18, then it is likely that the difference represents a true difference in GI rather than a chance finding. For example, Soh and Brand-Miller (1999) reported GI values for three varieties of mature boiled potatoes of 89, 103, 89 and one variety of mashed potatoes of 93. By contrast, Wolever *et al* (1994) reported GI values of two varieties of mature potatoes to be 59 and 64 when boiled, and 74 when mashed. Since all the values from one lab differ from those of the other by >18 , it is likely that the GI values of Australian potatoes really differ from those Canadian potatoes.

Current recommendations are that capillary blood sampling is preferred for determining the GI, but that it is acceptable to use venous blood sampling (FAO/WHO, 1998). We found that the use of venous plasma was associated with greater within-subject variation of both glycaemic responses and GI values, and non-normal distribution of GI values. The latter is probably related to the former because the GI is the ratio of two independently variable numbers. Increased variation of the numbers will have the effect of skewing the distribution of the resulting ratios (Wolever *et al*, 1991). Thus, the present data are consistent with previous results showing that glycaemic responses measured in venous plasma are lower and more variable than those in simultaneously obtained capillary blood (Wolever & Bolognesi, 1996).

The concentration of glucose in venous blood is lower than that in capillary blood because, as blood flows from the arterial to the venous circulation via the capillaries, peripheral tissues remove some of the glucose. The rise in blood insulin and glucose after eating stimulates glucose removal by tissues; thus, the difference in glucose concentration between arterial and venous blood is greater postprandially than fasting, leading to a smaller glucose rise in venous blood (Jackson *et al*, 1973; Coppack *et al*, 1990).

Venous glucose responses may be more variable than capillary responses for several reasons. Blood glucose concentrations oscillate on a minute-by-minute basis (Abdallah *et al*, 1997), driven, at least in part, by the pulsatile nature of insulin secretion (Matthews *et al*, 1983). Presumably, the oscillations of plasma glucose in different tissues in the body are not in phase with each other, because it takes different lengths of time for the pulses of insulin from the pancreas to reach them. Thus, it is possible that the magnitude of glucose oscillations in forearm venous blood may be greater than those in capillary blood because the vein drains a small volume of tissue with insulin oscillations in phase with each other. However, the glucose oscillations in capillary blood may be damped because arterial blood is derived from all tissues in the body with insulin concentrations oscillating out of phase with each other. Furthermore, there is a small analytical error associated with measuring glucose, and this has a larger proportional effect on the AUC when the rise in glucose is small. For example, a 0.1 mmol/l difference in the fasting glucose concentration results in a 12 mmol min/l difference in the AUC over 2 h, which is 20% of an AUC of 60, but only 6% of an AUC of 200.

The greater validity of using capillary rather than venous blood sampling is also suggested by the lack of relationship, for capillary blood, between the mean GI of individual subjects and the mean AUC for the reference food (Figure 2), but the existence of a significant correlation with venous blood. Correlation between a ratio and its denominator is a common problem of using ratios (Allison *et al*, 1995) and indicates that the ratio does not control adequately for the denominator. This is important because the GI is intended to control for the glycaemic responses of different subjects.

Subject characteristics such as age, sex, BMI and ethnicity may have contributed to the highly significant differences in absolute glycaemic responses between subjects. However, these factors had no significant effect on the GI values obtained in this study. This is consistent with previous data in subjects with diabetes (Wolever *et al*, 1990), which suggests that most of the variation of GI values is due to within-subject variation (Wolever, 1992).

The mean AUC of three trials of the reference food should be used to calculate the GI (FAO/WHO, 1998) because the mean of three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial. Since the reference food response is used to calculate the GI value for every food, an unrepresentative value for the reference AUC affects the GI value of every food tested and may make that subject's mean GI value differ from that of other subjects. A mathematical model showed that, using the mean of 3, compared with one standard trial, to calculate GI values resulted in more normally distributed GI values with reduced variability and a lower mean (Wolever *et al*, 1991). Our results are consistent with this in that the use of one randomly selected AUC value for the reference food to calculate the GI resulted in non-normally distributed GI values with a higher mean and s.d.

Wolever *et al* (1991) have advocated the use of white bread as the reference food instead of glucose because it represents a more physiological meal. However, variation in the composition and digestibility characteristics of white bread with location and time would reduce its usefulness as the reference food. In this study, bread was the only locally obtained food and the portion size was based on local food tables. Despite this, the variability of the GI values for bread was similar to those for the other foods. This suggests that variation in the composition of white bread, at least from the cities represented here, is not large enough to alter its GI value, and thus supports the validity of using bread as the reference food. Nevertheless, glucose is a more logical and easily standardized reference food for international use. GI values based on glucose have been used in Australia for several years (Brand-Miller *et al*, 1996). Thus, for international standardization, we recommend that GI values of foods be expressed relative to glucose. GI values obtained using bread as the reference food can be adjusted to the glucose standard by dividing them by 1.4.

There are different ways of defining and measuring carbohydrate, dietary fibre and resistant starch (RS; FAO/WHO, 1998) in different countries. Thus, the portion size of food containing 50 g carbohydrate may vary in different laboratories depending on the definitions used. Since glycaemic responses are related to the amount of carbohydrate ingested (Wolever & Bolognesi, 1996), use of a smaller portion size will result in a lower GI value. The GI is an index of the blood glucose raising potential of the available or glycaemic carbohydrate in foods. Carbohydrates which do not provide glucose for metabolism, such as non-starch polysaccharides and RS (Englyst *et al*, 1992), should not be included in the portion of carbohydrate tested. However, standardized methods for measuring RS are not available (FAO/WHO, 1998). Since the impact of excluding RS on the GI is not known, we chose barley, a food with a high RS content, to test this. After excluding RS, the smaller portion size contained 44.2 g of glycaemic starch. Increasing carbohydrate intake from 44.2 to 50 g would be expected to increase the glycaemic response by about 8% (Wolever & Bolognesi, 1996). Although the 13% higher GI value found for the higher portion size of barley was similar to the expected difference, the difference was not significant. Over 300 subjects would be needed for an 80% chance of detecting a difference of 8% at $P < 0.05$.

We conclude that the mean of at least three repeated trials of the standard food should be used to calculate the GI. The most precise and accurate determination of the GI may be achieved using capillary rather than venous blood sampling, but prospective studies are needed to confirm this. The estimated between-laboratory standard deviation of GI values is 9.0. Between-laboratory variation in GI appears to be related to random, day-to-day variation of glycaemic responses within subjects. Thus, finding ways to reduce within-subject variation in glycaemic responses may be the most effective strategy to improve the precision of measurement of GI values.

Acknowledgements

Supported by Glycaemic Index Testing Inc., Toronto; Australian Research Council; University of Parma, Italy; Department of Human Nutrition, University of Otago; University of the West Indies; Nestlé South Africa, the Sugar Association of South Africa and the National Research Association of South Africa.

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