Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values

Thomas M. S. Wolever

Department of Nutritional Sciences, University of Toronto, and Glycaemic Index Testing, Inc., Toronto, Ontario, Canada

To evaluate the suitability for glycaemic index (GI) calculations of using blood sampling schedules and methods of calculating area under the curve (AUC) different from those recommended, the GI values of five foods were determined by recommended methods (capillary blood glucose measured seven times over 2·0 h) in forty-seven normal subjects and different calculations performed on the same data set. The AUC was calculated in four ways: incremental AUC (iAUC; recommended method), iAUC above the minimum blood glucose value (AUCmin), net AUC (netAUC) and iAUC including area only before the glycaemic response curve cuts the baseline (AUCcut). In addition, iAUC was calculated using four different sets of less than seven blood samples. GI values were derived using each AUC calculation. The mean GI values of the foods varied significantly according to the method of calculating GI. The standard deviation of GI values calculating using iAUC (20·4), was lower than six of the seven other methods, and significantly less ($P < 0.05$) than that using netAUC (24·0). To be a valid index of food glycaemic response independent of subject characteristics, GI values in subjects should not be related to their AUC after oral glucose. However, calculating GI using AUCmin or less than seven blood samples resulted in significant ($P < 0.05$) relationships between GI and mean AUC. It is concluded that, in subjects without diabetes, the recommended blood sampling schedule and method of AUC calculation yields more valid and/or more precise GI values than the seven other methods tested here. The only method whose results agreed reasonably well with the recommended method (ie. within ± 5 %) was AUCcut.

Glycaemic index: Blood glucose response: Methodology: Dietary carbohydrate

The glycaemic index (GI) is a classification of carbohydrate foods based on their acute blood glucose responses; it should not be used without also considering information about the chemical composition of foods (Jenkins *et al.* 1981). The GI has been recommended to help guide food choice (Food and Agriculture Organization, 1998), because low-GI foods have been shown to improve blood glucose control in people with diabetes (Brand-Miller *et al.* 2003), to increase insulin sensitivity (Frost *et al.* 1998) and β-cell function (Wolever & Mehling, 2002), and to reduce serum triacylglycerol (Jenkins *et al.* 1987). In addition, a low-GI diet has been associated with reduced risk for developing diabetes and CVD in some studies (Salmeron *et al.* 1997a,b; Liu *et al.* 2000), but not all (Meyer *et al.* 2000; Van Dam *et al.* 2000). Widespread use of the GI, as recommended, requires a standardized method for determining the GI of foods that is valid and precise.

The GI is defined as the incremental area under the blood glucose response curve (AUC) after consumption of a 50 g available-carbohydrate portion of a food expressed as a percentage of that after 50 g oral glucose.

For subjects without diabetes, the Food and Agriculture Organization (1998) recommends determining GI by collecting seven blood samples over 2 h and calculating incremental AUC (iAUC; Jenkins *et al.* 1981; Wolever & Jenkins, 1986). Shorter tests and less frequent blood sampling would reduce costs; other methods of calculating AUC have been used (Wolever, 1989; Ha *et al.* 1992), but the effect of these variables on the GI is not known. Thus, the purpose of the present paper was to compare the suitability for GI calculations of using different blood sampling schedules and other ways of calculating AUC than those recommended by the Food and Agriculture Organization (1998).

Methods

Data from a multicentre trial were used, the methods and results of which have been published by Wolever *et al.* (2003). The GI values of five foods were determined in subjects without diabetes in seven centres using the method recommended by the Food and Agriculture Organization (1998). Results from the five centres that measured

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Abbreviations: AUC, area under the curve; AUCcut, incremental area under the curve up to first cut of baseline; AUCmin, incremental area under the curve above minimum glucose; iAUC, incremental area under the curve; netAUC, net incremental area under the curve; GI, glycaemic index.

Corresponding author: Dr Thomas M. S. Wolever, fax + 1 416 978 5882, email thomas.wolever@utoronto.ca

glucose in whole blood or plasma obtained from capillary blood samples were used in this analysis. Results from the two centres that measured glucose in venous plasma were not used because of higher variability of glycaemic responses and GI values (Wolever *et al.* 2003). In brief, subjects without diabetes (twenty-three male, twenty-four female; age 28·9 (SEM 1·2) (range 19–50) years, BMI 23·3 (SEM 0·5) (range 16·8–35·0) kg/m^2) were studied on eight occasions in the morning after a 10–14 h overnight fast. On each occasion, after a fasting blood sample was obtained, subjects consumed a test meal containing 50 g available carbohydrate (defined as total carbohydrate minus dietary fibre), with further blood samples being taken 15, 30, 45, 60, 90 and 120 min after starting to eat. On three occasions (first, middle and last trials), subjects consumed 50 g anhydrous glucose. On the other occasions subjects consumed instant potato, white bread, polished rice, white spaghetti or pearled barley in randomized order. The AUC was calculated for each trial and the AUC after consuming each food was expressed as a percentage of the average AUC after oral glucose taken by the same subject. The average of these values for each food was the GI of the food.

For this analysis, eight different calculations were performed on the same data set and the results compared. Table 1 shows the results of AUC calculations using the different methods on real sets of blood glucose profiles from one subject. The recommended calculation used all seven blood samples with iAUC being calculated. The iAUC includes all area below the curve and above the fasting concentration, with any area beneath fasting being ignored (Wolever & Jenkins, 1986). The iAUC was also calculated on four different combinations of four, five or six blood samples representing a shorter test period, less

frequent blood sampling or both. Net incremental AUC (netAUC) (Gannon *et al.* 1989) includes all incremental area below the curve, including the area below the fasting concentration. Since it is calculated by applying the trapezoid rule to both positive and negative blood glucose increments, the effect is to subtract the area below the fasting level from that above. AUCcut (Ha *et al.* 1992) is calculated in the same way as iAUC, but only includes the area before the blood glucose concentration drops below (cuts) the baseline (fasting concentration); the area after the glucose concentration cuts the baseline is not included. The iAUC above the lowest blood glucose concentration attained (AUCmin) (Vorster *et al.* 1990) is calculated by subtracting the lowest blood glucose concentration attained during the test period from each of the other blood glucose concentrations, and calculating the AUC by applying the trapezoid rule to the resulting increments.

There is no easy way to measure the validity of different methods of calculating the GI. The approach taken here was to determine the correlation between the mean GI (average GI of the five foods) and the mean AUC after oral glucose (average of three trials) for the forty-seven subjects. The rationale for this approach is that the GI is intended to indicate the blood-glucose-raising potential of foods independent of the glycaemic response of the subject. Thus, for valid methods of calculating GI, there should be no correlation between the GI values obtained and the AUC after oral glucose. The presence of a significant correlation between GI and AUC would indicate that the method was not valid, because the GI values obtained depended on the glucose tolerance status of the subject.

The standard deviation of the mean GI values is a measure of precision, which is the degree of variation of values about their mean value. Since the standard deviation

Table 1. Sample calculations (using the same data from one subject for each method)*

		Blood glucose concentrations (mmol/l)								
Time (min)...		0	15	30	45	60	90	120	AUC	GI
Method										
iAUC†	Glucose	3·67	6·11	6·06	4·44	3·17	3·61	4·00	85·8	
	Food	3·94	5·00	5·11	3·44	3·50	3·83	4·33	35·4	41
AUC$_1$	Glucose	3·67	6·11	6·06	4·44	3·17	3·61	–	81·7	
	Food	3·94	5·00	5·11	3·44	3·50	3·83	–	30·8	38
AUC$_2$	Glucose	3·67	6·11	6·06	4·44	3·17	–	–	81·7	
	Food	3·94	5·00	5·11	3·44	3·50	–	–	30·8	38
AUC$_3$	Glucose	3·67	–	6·06	–	3·17	3·61	4·00	69·7	
	Food	3·94	–	5·11	–	3·50	3·83	4·33	34·9	50
AUC$_4$	Glucose	3·67	–	6·06	4·44	–	3·61	–	75·6	
	Food	3·94	–	5·11	3·44	–	3·83	–	23·7	31
netAUC	Glucose	3·67	6·11	6·06	4·44	3·17	3·61	4·00	75·9	
	Food	3·94	5·00	5·11	3·44	3·50	3·83	4·33	18·6	25
AUCcut	Glucose	3·67	6·11	6·06	4·44	3·17	3·61	4·00	81·7	
	Food	3·94	5·00	5·11	3·44	3·50	3·83	4·33	30·8	38
AUCmin	Glucose	3·67	6·11	6·06	4·44	3·17	3·61	4·00	135·9	
	Food	3·94	5·00	5·11	3·44	3·50	3·83	4·33	78·6	58

AUC, area under the curve; GI, glycaemic index; iAUC, incremental area under the curve; AUC$_1$, incremental area under the curve using 0, 15, 30, 45, 60 and 90 min glucose values; AUC$_2$, incremental area under the curve using 0, 15, 30, 45 and 60 min glucose values; AUC$_3$, incremental area under the curve using 0, 30, 60, 90 and 120 min glucose values; AUC$_4$, incremental area under the curve using 0, 30, 45 and 90 min glucose values; netAUC, net incremental area under the curve; AUCcut, incremental area under the curve up to first cut of baseline; AUCmin, incremental area under the curve above minimum glucose.

* For details of procedures and calculations, see pp. 296–297.

† Recommended method (Food and Agriculture Organization, 1998).

of the GI is largely determined by within-subject variation (Wolever, 1992), the CV (CV = 100 × SD/mean value) of the AUC after the repeated trials of oral glucose was calculated.

The mean GI values for each of the five foods from each of the five centres calculated using each of the different methods were compared with those calculated using the recommended method using the Bland & Altman (1986) procedure. They defined the 'limits of agreement' as being the mean value ± 1·96 × SD of the differences between the results from two methods, representing, therefore, the range within which 95 % of the differences between the methods would be expected to fall. In the present study, the analysis involved comparison of twenty-five values (five foods and five centres) for each method *v.* the recommended method. Using the recommended method, foods were classified as high (>69), medium (56–69) or low (<56) GI (Brand-Miller *et al.* 2002), and the number of misclassifications for each alternate method was calculated.

Unless otherwise indicated, results are expressed as mean values with their standard errors. The mean values, standard deviations and CV of the AUC values after the repeated trials of oral glucose taken by each subject were subjected to repeated-measures ANOVA examining for differences between subjects and methods of calculating AUC. Mean values and standard deviations of the GI values were subjected to ANOVA examining for the main effects of food, method of calculating AUC (method), subject, and the food × method interaction. If significant effects were found by ANOVA, the significance of differences between individual mean values was determined using the Newman–Keuls method to adjust for multiple comparisons (Snedecor & Cochran, 1980). Pearson's correlation coefficients between AUC and GI were calculated by the method of least squares (Lotus 123, 1997 edition; Lotus Development Corp., Cambridge, MA, USA). Effects were considered to be statistically significant if two-tailed $P<0.05$.

Results

The mean blood glucose responses after oral glucose and the five foods are shown in Fig. 1. The different methods
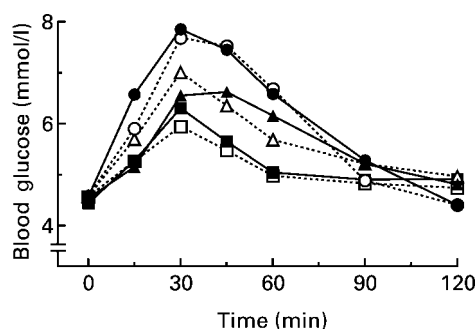
Fig. 1. Blood glucose concentrations of forty-seven subjects after consuming 50 g available carbohydrate from glucose (●), instant potato (○), white bread (▲), polished rice (△), white spaghetti (■) and pearled barley (□). Values are means. For details of subjects and procedures, see pp. 296–297.

of calculating iAUC yielded significantly different mean values. After oral glucose (Table 2), AUCmin was significantly greater ($P<0.05$) than iAUC and AUCcut, which in turn were significantly greater ($P<0.05$) than AUC_1 and AUC_4, which in turn were significantly greater ($P<0.05$) than AUC_2. Mean values for netAUC and AUC_3 were intermediate between iAUC and AUC_1. Statistical analysis of the AUC values after the five foods revealed significant main effects of food ($F_{4,28}$ 240·9, $P<0.001$) and method of AUC calculation ($F_{7,28}$ 26·5, $P<0.001$), and a significant food × method interaction ($F_{28,1794}$ 1·87, $P=0.003$). The significant food × method interaction indicates that the difference in AUC between foods differed significantly depending on the method of calculating AUC. The greatest AUC after the five foods was obtained with AUCmin and the smallest with AUC_2, with the others being intermediate (Table 3).

The degree of within-subject variation, assessed by standard deviations and CV of the repeated glucose trials, varied significantly by method of AUC calculation (Table 2). The largest CV was obtained with netAUC (28·0 %) and the smallest with AUC_2 (20·7 %) and AUCmin (20·5 %), with the CV of iAUC, the recommended AUC calculation method, being intermediate (23·4 %).

Statistical analysis of the GI values calculated using the different AUC methods revealed significant main effects of food ($F_{4,28}$ 649·9, $P<0.001$) and method of AUC calculation ($F_{7,28}$ 6·86, $P<0.001$), but the food × method interaction was not significant ($F_{28,1794}$ 0·86, $P=0.68$). When individual mean values were compared, mean GI calculated using AUCmin, 57·2, was significantly less than that calculated using the other methods (61·4–66·4), which in turn, did not differ significantly from each other (Table 3). The method of calculating AUC significantly

Table 2. Areas under the curves calculated by different methods after repeated trials of oral glucose in forty-seven subjects*

(Mean values, standard deviations and coefficients of variation)

Method	Mean (mmol × min/l)		SD (mmol × min/l)		CV (%)	
	Mean	SD	Mean	SD	Mean	SD
iAUC†	196[b]	11	45·2[abc]	4·7	23·4[abc]	2·1
AUC_1	179[c]	9	39·4[c]	3·9	22·4[bc]	2·0
AUC_2	138[d]	6	26·6[d]	2·2	20·7[c]	2·0
AUC_3	187[bc]	11	47·4[abc]	4·6	26·1[ab]	2·2
AUC_4	177[c]	9	41·9[bc]	3·9	25·3[ab]	2·3
netAUC	184[bc]	11	49·6[ab]	5·0	28·0[a]	2·6
AUCcut	195[b]	11	46·4[abc]	4·8	24·3[abc]	2·2
AUCmin	258[a]	12	52·1[abc]	5·5	20·5[c]	2·0
P‡	<0·001		<0·001		<0·001	

iAUC, incremental area under the curve, AUC_1, iAUC using 0, 15, 30, 45, 60 and 90 min glucose values; AUC_2, iAUC using 0, 15, 30, 45 and 60 min glucose values; AUC_3, iAUC using 0, 30, 60, 90 and 120 min glucose values; AUC_4, iAUC using 0, 30, 45 and 90 min glucose values; netAUC, net incremental area under the curve; AUCmin, incremental AUC above minimum glucose.

[abcd] Mean values with unlike superscript letters were significantly different ($P<0.05$).

* For details of procedures and calculations, see pp. 296–297.

† Recommended method (Food and Agriculture Organization, 1998).

‡ Significance of main effect of method of calculation from ANOVA.

Table 3. Area under the curve (mmol × min/l) and glycaemic index values for each food calculated using different methods*

Method	Potato Mean	SD	Bread Mean	SD	Rice Mean	SD	Spaghetti Mean	SD	Barley Mean	SD	Mean of Means	SD
Area under the curve												
iAUC†	176	77	134	57	135	76	94	56	69	46	122[b]	62[a]
AUC_1	165	65	118	43	117	62	80	45	61	35	108[b]	50[b]
AUC_2	127	40	83	26	89	37	63	33	49	22	82[c]	32[c]
AUC_3	174	78	136	57	136	77	96	56	69	45	122[b]	63[a]
AUC_4	167	64	123	42	124	62	89	55	66	35	114[b]	52[b]
netAUC	165	84	132	58	130	79	93	57	65	49	117[b]	65[a]
AUCcut	176	77	134	57	133	77	92	58	69	47	121[b]	63[a]
AUCmin	221	81	151	58	154	71	102	53	85	44	143[a]	61[a]
Glycaemic index												
iAUC†	91·5	25·1	71·0	20·7	68·7	22·6	47·6	18·1	35·2	15·7	62·8[a]	20·4[ab]
AUC_1	94·0	25·1	68·1	19·0	65·6	23·2	44·6	19·3	34·7	15·2	61·4[a]	20·3[b]
AUC_2	94·8	23·5	63·4	19·5	67·1	24·4	47·6	23·8	37·6	17·1	62·1[a]	21·7[ab]
AUC_3	94·9	27·0	76·9	23·7	72·1	22·5	51·7	20·1	36·4	16·5	66·4[a]	21·9[ab]
AUC_4	96·7	26·1	74·0	24·8	69·8	22·8	50·4	24·7	38·2	18·0	65·8[a]	23·3[ab]
netAUC	90·9	30·7	75·4	23·6	71·0	28·0	50·0	19·6	34·1	17·9	64·3[a]	24·0[a]
AUCcut	92·1	25·3	71·3	21·5	67·6	23·8	46·1	19·2	34·9	16·5	62·4[a]	21·2[ab]
AUCmin	86·8	22·7	60·8	20·2	62·4	24·6	41·2	19·2	34·9	16·0	57·2[b]	20·6[ab]

iAUC, incremental area under the curve; AUC_1, incremental area under the curve using 0, 15, 30, 45, 60 and 90 min glucose values; AUC_2, incremental area under the curve using 0, 15, 30, 45 and 60 min glucose values; AUC_3, incremental area under the curve using 0, 30, 60, 90 and 120 min glucose values; AUC_4, incremental area under the curve using 0, 30, 45 and 90 min glucose values; netAUC, net incremental area under the curve; AUCcut, incremental area under the curve up to first cut of baseline; AUCmin, incremental area under the curve above minimum glucose.
[a,b,c] Mean values with unlike superscript letters were significantly different ($P < 0.05$).
* For details of procedures and calculations, see pp. 296–297.
† Recommended method (Food and Agriculture Organization, 1998).

affected not only the mean GI values, but also the standard deviation of the GI values ($F_{7,28}$ 3·13, $P=0.014$; Table 3). The mean standard deviation obtained using AUC_1, 20·3, was significantly less ($P < 0.05$) than that using netAUC (24·0). The recommended method of calculating AUC, iAUC, had the second lowest mean standard deviation (20·4). Although by the primary statistical analysis mean standard deviation of GI values obtained using AUCcut (21·2), did not differ significantly from that using iAUC, the standard deviation of the GI value for each of the five foods was greater with AUCcut than iAUC, a difference which, though small, was significant by paired t test (0·82 (SD 0·17), $P < 0.01$).

The correlation between AUC and GI was statistically significant for AUC_2 (r −0·506, $P < 0.001$), AUC_4 (r −0·308, $P=0.035$) and AUCmin (r −0·430, $P=0.003$), with those for AUC_1 and AUC_3, −0·23 and −0·21 respectively approaching significance ($P=0.12$ and $P=0.16$ respectively). The correlations between AUC and GI were not statistically significant for iAUC (r −0·139, $P=0.35$), netAUC (r 0·140, $P=0.35$) and AUCcut (r −0·112, $P=0.45$).

The limits of agreement between each method and the recommended method expressed in absolute terms (with % mean GI value calculated using the recommended method in parentheses) were as follows: AUC_1 7·0 (11·2), AUC_2 13·5 (21·4), AUC_3 6·6 (10·5), AUC_4 7·6 (12·1), netAUC 8·9 (14·2), AUCmin 13·0 (20·7), AUCcut 3·3 (5·1). The limits of agreement for the different methods were directly correlated (r 0·966, $P < 0.001$) with the number of foods misclassified using the different methods (n): AUC_1 2 (8 %), AUC_2 4 (16 %), AUC_3 2 (8 %), AUC_4 2 (8 %), netAUC 3 (12 %), AUCmin 7 (28 %), AUCcut 2 (8 %).

Discussion

The present results suggest that the method recommended by the Food and Agriculture Organization (1998), based on taking seven blood samples over 2·0 h and calculating iAUC, is the best method for calculating GI of those foods tested here. Other methods resulted in GI values that either were correlated with the glucose tolerance status of the different subjects or were more variable than the recommended method. Most of the methods yielded GI values that did not agree well with the recommended method, with limits of agreement ranging from ± 10·5 to ± 21·4 %. Only one of the alternative methods, AUCcut, yielded mean GI values 95 % of which were within ± 5 % of the recommended method.

There is no universally 'right' or 'wrong' way to measure blood glucose responses. Different methods are required for different purposes. For example, to determine whether a new treatment for diabetes reduces blood glucose concentrations, total AUC may be preferred over iAUC, since the former is a measure of the average blood glucose concentration over time, whereas the latter is a measure of change over time. Calculating netAUC may be more appropriate for measuring postprandial responses of variables such as NEFA, whose concentrations normally fall after eating. This is because netAUC can have a negative value, whereas the minimum value of iAUC is 0.

The GI is intended to be an index of the relative blood-glucose-raising potential of the available carbohydrate in different foods. For this concept to be valid and useful, the GI value of the same food must be the same in different subjects. Glycaemic responses vary from day-to-day within subjects, and also vary between subjects. To distinguish

between these sources of variation, repeated trials of both the reference and test food must be performed. When blood glucose response trials of two test foods and one reference food were repeated four times in each of twelve heterogeneous subjects with diabetes, there was no significant difference in GI between subjects, despite 4-fold variation in mean iAUC values between subjects (Wolever *et al.* 1990). This is consistent with the present results showing no significant correlation between iAUC and GI. The GI is a ratio of two independently variable values, and the statistical problems associated with the use of ratios have been described by Allison *et al.* (1995). The presence of a significant correlation between a ratio and its denominator indicates that the ratio does not adequately control for the denominator (Allison *et al.* 1995). Thus, methods of AUC calculation resulting in a significant correlation between GI and AUC cannot be considered valid for determining the GI of foods.

Ha *et al.* (1992) studied the glycaemic responses elicited by three different fruits taken by a group of fifteen subjects with impaired glucose tolerance or diabetes and compared the results after calculating the AUC in six different ways. They concluded that iAUC and AUCcut were more appropriate than netAUC, because netAUC resulted in larger standard deviations, and also that less frequent blood sampling increased the standard deviation of glycaemic responses. The results of Ha *et al.* (1992) cannot be compared exactly with the present results because the standard deviations calculated by Ha *et al.* (1992) included both within- and between-subject variation, and no results for GI were presented. Nevertheless, the present results also show that calculating netAUC, and reducing the frequency of blood sampling, tended to result not only in higher within-subject variation of glycaemic responses than other methods, but also higher standard deviations of the GI values.

AUC methods iAUC, AUC_1 and AUC_2 represent the same frequency of blood sampling, but a shorter test period; 2.0 h for iAUC, 1.5 h for AUC_1 and 1.0 h for AUC_2. It is of interest that as the time over which blood glucose was measured decreased, within-subject variation tended to decrease from 23.4 % for 2.0 h to 20.7 % for 1.0 h (Table 2). This suggests that, in normal subjects, blood glucose concentrations during the second postprandial hour are more variable within-subjects from day-to-day than those during the first hour. Although reducing within-subject variation is desirable for the most precise GI results, reducing the time over which blood samples were taken was also associated with a progressively higher correlation between GI and AUC, a factor that tends to make the GI values invalid.

It is not possible to say from the present results what would happen to GI values if the test was carried out for more than 2.0 h. Using tests conducted over 5 h in normal and diabetic subjects, Gannon & Nuttal (1987) showed that the AUC value and the relative glucose area of foods could be markedly affected by the time over which the test was done. As the time of blood sampling increased from 1 to 5 h, AUC values after oral glucose decreased by >50 %, relative glucose area values for legumes increased by approximately 100 %, relative

glucose area values for sucrose, fructose and milk decreased by >50 % and relative glucose area values for potatoes, bread, oats and rice did not change very much. This does not agree with the present results, which showed that as the time of blood sampling increased from 1.0 h (AUC_2) to 2.0 h (iAUC), AUC increased significantly by approximately 40 %, but there was no effect on mean GI. The lack of agreement is because of the different methods used to calculate the AUC. Gannon & Nuttall (1987) used netAUC, in which area below the baseline is subtracted from that above. Since after oral glucose blood glucose tends to undershoot the baseline, the longer the time over which blood glucose is measured, the more time the blood glucose is below baseline, the more area below the baseline there is to subtract, and the lower the netAUC. On the other hand, iAUC cannot decrease as the time of the test is extended, because area beneath the baseline is ignored.

It is concluded that the blood sampling schedule and method of AUC calculation recommended by the Food and Agriculture Organization (1998) for subjects without diabetes results in more valid or more precise GI values than the other methods tested here. The only method with an acceptable degree of agreement ($\leq \pm 5$ %) with the recommended method was AUCcut. These conclusions may not necessarily apply to the assessment of GI in subjects with diabetes.

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