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Short Communication

Measuring glycaemic responses: duplicate fasting samples or duplicate measures of one fasting sample?

Thomas M. S. Wolever*, Blanche Ip and Elham Moghaddam

Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 3E2

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The precision with which glycaemic responses, expressed as incremental area under the curve (AUC), can be measured may be improved by using the average of several measures of fasting blood glucose (FBG). To see if taking two fasting blood samples would increase the precision of AUC, the glycaemic responses elicited by four test meals (50 g glucose; 50 g glucose plus 10 g fat and 10 g protein; 100 g white bread; 100 g white bread plus 10 g fat and 10 g protein) were determined in thirteen overnight-fasted healthy subjects. Two fasting blood samples were taken 5 min apart (−5 min and 0 min before starting to eat) with glucose measured three times in each sample. AUC was calculated using different estimates of FBG derived from the three measures of glucose in the two fasting blood samples and each set of AUC values subjected to ANOVA. Unexpectedly, the results were more precise when AUC was calculated from mean glucose in the 0 min blood sample (FBG0) than from mean glucose in the two different fasting blood samples. The 95 % CI of the AUC calculated using FBG0 in thirteen subjects was ±29·8; to obtain the same CI using the mean of the two fasting blood samples would require fourteen subjects. These results suggest that taking two fasting blood samples does not necessarily improve, and may even reduce, the precision of AUC as a measure of glycaemic response. Further studies are needed before requiring that two fasting blood samples be taken for determining glycaemic index.

Human glycaemic responses: Carbohydrates: Glucose: Glycaemic index: Methodology

There is much interest in measuring the glycaemic responses elicited by foods because high postprandial glucose or diets with a high glycaemic load are associated with increased risk for CVD (Coutinho *et al.* 1999; Liu *et al.* 2000), diabetes (Salmerón *et al.* 1997) and cancer (Augustin *et al.* 2001; Higginbotham *et al.* 2004). In addition, diets with a low glycaemic index (GI) or low glycaemic load improve glycaemic control (Brand-Miller *et al.* 2003), increase β-cell function (Wolever & Mehling, 2002) and insulin sensitivity (Frost *et al.* 1998), and may influence mood, memory (Benton & Nabb, 2003) and body-weight regulation (Ebbeling *et al.* 2003). Glycaemic responses are commonly measured as incremental area under the curve (AUC). The blood sampling schedule and way of calculating AUC influence the results obtained (Wolever, 2004). AUC may also be affected by the precision of the estimate of fasting blood glucose (FBG) concentration. The estimate of FBG may be made more precise by averaging several measures; thus, it has been suggested that two fasting blood samples should be taken for determining the GI of foods (Standards Australia, 2005). However, the effect of using two fasting blood samples on the precision of the resulting AUC values is not known (Brouns *et al.* 2005). Therefore, the purpose of the present study was to see if taking two fasting blood samples improved the power to detect significant differences in AUC between different test meals compared with measuring glucose two or three times in a single blood sample.

Methods

Thirteen healthy subjects (seven females, six males; age 27·3 (SEM 2·5) years; BMI 22·7 (SEM 0·6) kg/m^2) were studied on four separate mornings after 10–14 h overnight fasts. On each occasion, subjects consumed one of four different test meals within 15 min according to a Latin square design with the variables being carbohydrate source (50 g glucose or 50 g available-carbohydrate from white bread) and the presence or absence of 10 g fat and 10 g protein. The four test meals consisted of about 100 g white bread plus 250 ml water (meal WB); meal WB plus 11·6 g margarine (Becel; Unilever Canada, Toronto, ON, Canada) and 83·3 g cottage cheese (1% fat) (Gay Lea Nordica; Gay Lea Foods Corp. Ltd, Weston, ON, Canada) (meal WBFP); 50 g anhydrous glucose (Sigma Chemical Co., St Louis, MO, USA) dissolved in 250 ml water (meal G); or meal G plus 10 g maize oil (Mazola; ACH Food Co. Ltd, Memphis, TN, USA) and 11·1 g 90 % soya protein powder (Supro® Brand; Swiss Herbal Remedies,

Abbreviations: AUC, area under the curve; FBG, fasting blood glucose; GI, glycaemic index.

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

Ltd, Richmond Hill, ON, USA) mixed in 250 ml water using a blender (meal GFP). The order of the test meals was randomised.

On each morning fasting capillary finger-prick blood samples were obtained 5 min (FBG-5) and immediately (FBG0) before the subjects began to consume the test meal. Further blood samples were obtained at 15, 30, 45, 60, 90 and 120 min after starting to eat. Blood (two to three drops) was placed into fluro-oxalate tubes, mixed by rotation and frozen before analysis of whole-blood glucose using a YSI Model 2300 STAT analyser (Yellow Springs, OH, USA). Glucose concentrations were measured three times in each fasting blood sample and the results labelled sequentially for comparison. The glucose concentration in the remaining blood samples was measured only once.

The results of repeated determinations of FBG were subjected to ANOVA, dividing the sources of FBG variation into analytical, minute-to-minute, day-to-day (within subject) and between subjects using methods described by Kringle & Johnson (1986). The CV was SD expressed as a percentage of the mean.

Incremental areas under the glycaemic response curves (AUC), ignoring area beneath the baseline, were calculated geometrically (Wolever *et al.* 1991) using four different estimates of FBG termed $FBG0_1$, FBG-5, FBG0 and FBG-5,0. $FBG0_1$ was the first measure of glucose in the 0 min sample, which represents our usual practice of measuring glucose once in each blood sample. FBG-5 and FBG0 were taken to be the average of the first two measures of glucose in the −5 min and 0 min blood samples, unless the difference between them was >0·2 mmol/l, in which case the average of the closest two measures was used. FBG-5,0 was the average of FBG-5 and FBG0. Each set of AUC values was subjected to ANOVA examining for the effects of carbohydrate source, presence of fat and protein and subjects.

The protocol for the present study was approved by the Research Ethics Board of the University of Toronto (Canada), and all subjects gave written consent to participate.

Results

Mean values (n 52; thirteen subjects, 4 d) of the first, second and third glucose determinations of FBG-5 were 4·247 (SD 0·445), 4·242 (SD 0·464) and 4·243 (SD 0·450), and of FBG0 were 4·281 (SD 0·425), 4·277 (SD 0·426) and 4·251 (SD 0·414) mmol/l, respectively; these six means did not differ significantly from each other (F(5,255) 1·38; P=0·23). The mean values of FBG-5, FBG0 and FBG-5,0, respectively, were 4·251 (SD 0·447), 4·275 (SD 0·411) and 4·263 (SD 0·421) mmol/l. The SD of analytical variation of FBG was 0·066 mmol/l (CV 1·54 %); corresponding values for minute-to-minute, day-to-day (within subject) and between-subject variation were: 0·111 (CV 2·61 %), 0·216 (CV 5·07 %) and 0·379 mmol/l (CV 8·91 %), respectively.

Mean glycaemic responses elicited by the four test meals are shown in Fig. 1, and the AUC calculated using different measures of FBG in Table 1. The mean AUC values for each test meal were very similar for the different measures of FBG. There were significant main effects of carbohydrate source and presence of fat and protein on AUC, and no significant interaction, whatever the method used to determine FBG (Table 1). Compared with $FBG0_1$, using FBG0 to calculate AUC reduced margin of error, increased F and reduced the P values (Table 1). However, when FBG-5,0 was used to calculate AUC, the margin of error was larger, F smaller, and the P values larger than for $FBG0_1$. The 95 % CI (CI 1·96 × SD/$\sqrt{n}$) of mean AUC calculated from FBG0 was ±29·8 (n 13). To obtain the same CI for $FBG0_1$ would require 13·3 subjects, and for FBG-5,0 would require 13·6 subjects.

Discussion

The results showed that using the average of two measurements of glucose in the 0 min fasting blood sample, instead of only one, increased power to detect differences in AUC between test meals. Surprisingly, however, using the average glucose from two fasting blood samples taken 5 min apart to calculate AUC tended to reduce the statistical power. This suggests that taking more than one fasting blood sample is not necessarily an effective strategy for improving the power to detect differences in glycaemic response between different test meals. These data also suggest that, for best results, there should be as short an interval of time as possible between the fasting blood sample and the start of test meal consumption.

Based on the statistical principle that variances are additive, small analytical errors in metabolite concentrations result in larger errors in values derived from calculations involving the results of several measurements (Kringle & Johnson, 1986). AUC is calculated from multiple measures of blood glucose and is particularly dependent on the value of FBG because FBG is subtracted from every other blood glucose value. A FBG difference of 0·1 mmol/l (about 2 %) could result in an AUC difference of up to 12 mmol × min/l over a 2 h period; this represents 10 % of the average AUC elicited by 100 g white bread in sixty-eight normal subjects (Wolever *et al.* 2003). If variances are additive, it follows, therefore, that improving analytical precision of FBG by even a small amount should improve the precision of AUC values, which, in turn, would be reflected in more power to detect differences in AUC.

Taking the average >1 measurement of FBG will improve precision by a factor of CV/$\sqrt{n}$, where CV is the random variation and n is the number of measures taken. Since sources of

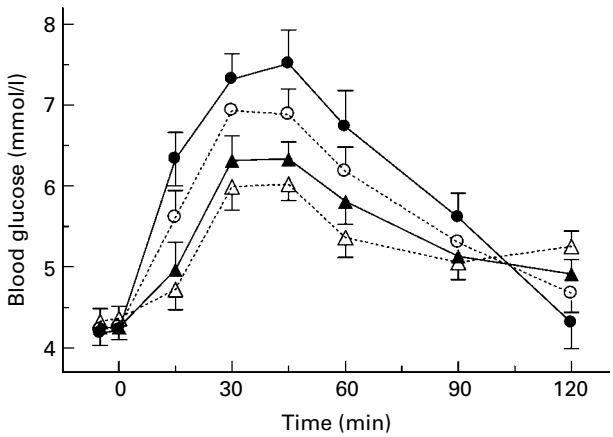

Fig. 1. Blood glucose concentrations after glucose alone (•), glucose plus fat and protein (○), white bread alone (▲) and white bread plus fat and protein (Δ). Values are means, with their standard errors represented by vertical bars. For details of diets and procedures, see p. 799.

Table 1. Incremental areas under the glucose response curves (mmol × min/l) elicited by oral glucose (G) or white bread (WB) alone or with fat and protein (FP) calculated using different values for fasting blood glucose (FBG)

(Mean values with their standard errors)

	Test meal								ANOVA*						
	G		WB		GFP		WBFP		Carb		FP		Subj		
FBG†	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*F*	P	*F*	*P*	*F*	*P*	ME
$FBG0_1$	228	28	185	23	145	14	112	7	25·71	$1{\cdot}2 \times 10^{-5}$	6·15	0·018	3·00	0·005	30·2
FBG0	230	28	183	23	145	15	113	7	26·31	$1{\cdot}0 \times 10^{-5}$	6·61	0·014	3·20	0·003	29·8
FBG-5	234	29	187	22	146	14	118	7	23·45	$2{\cdot}4 \times 10^{-5}$	5·28	0·028	2·38	0·022	31·7
FBG-5,0	232	28	185	22	145	14	116	6	25·39	$1{\cdot}3 \times 10^{-5}$	6·01	0·019	2·79	0·009	30·4

* *F* values from ANOVA for main effects of carbohydrate source (Carb; glucose *v.* white bread), fat and protein (FP; Carb alone *v.* Carb plus FP) and subjects (Subj). ME is margin of error (half the width of the 95 % CI) calculated from the pooled SD from ANOVA.

† Fasting blood taken at −5 min and 0 min with glucose measured three times in each blood sample. $FBG0_1$ is the first measure of glucose in the 0 min sample; FBG-5 and FBG0 are the average of the first two measures of glucose in the −5 min and 0 min samples, respectively, unless the difference between them was >0·2 mmol/l, in which case the average of the closest two measures was used; FBG-5,0 is the average of FBG-5 and FBG0.

For details of diets and procedures, see p. 799.

variation in FBG include analytical and minute-to-minute variation, variation can be reduced by measuring glucose more than once in a single blood sample or by measuring glucose in more than one blood sample. Since the magnitude of minute-to-minute variation, CV 2·6 %, was 70 % greater than that for analytical variation, CV 1·5 %, taking two blood samples would have been expected to reduce variation of AUC more than measuring glucose twice in a single sample. However, this was not the case. How can this be explained?

Measuring glucose at 1 min intervals reveals the existence of approximately sinusoidal fluctuations with amplitude ±0·05–0·20 mmol/l about the mean and frequency 6–12 per h (Abdullah *et al.* 1997; Melanson *et al.* 1999). Presumably the time blood glucose starts to rise after eating is related to the time of starting to eat, i.e. time 0 min, rather than at some other time, such as −5 min. Thus, using average blood glucose in several fasting blood samples may be a less precise measure of the true baseline and yield a less precise estimate of AUC than the blood glucose concentration just before eating. The implication of this is that, for most precise measurement of AUC, multiple fasting blood samples should not be taken and as little time as possible should elapse between taking the fasting blood sample and starting to eat the test meal. However, if analytical variation of glucose is greater than minute-to-minute variation this conclusion may not hold, and it may be useful to obtain several fasting blood samples.

The results of the present study are relevant to the recent draft proposal for an official method for determining the GI of foods (Standards Australia, 2005), in which it is specified that two fasting blood samples shall be taken within 5 min of each other and the average result used as the baseline blood glucose concentration for the purposes of calculating GI. Taking an extra blood sample increases costs, which could only be justified if the results were improved. However, the present study showed that using the mean of two fasting blood samples resulted in less statistical power than a single blood sample just before starting to eat. It should be noted that the present results do not necessarily apply to GI, since each food was only tested once in each subject, and, therefore we cannot calculate valid GI values from the data. Nevertheless, since GI is calculated from AUC, the present results suggest that taking two fasting blood samples may not necessarily reduce, and may even increase, the variability of GI values. Thus, taking two fasting blood samples should not be a requirement for GI testing, at least until it has been shown to reliably improve the results of GI testing.

It is concluded that taking two fasting blood samples does not necessarily improve, and may even reduce, the precision of AUC as a measure of glycaemic response. Further studies are needed before requiring that two fasting blood samples be taken for determining GI.

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