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

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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.

Methods

Thirteen healthy subjects (seven females, six males; age 27·3 (SEM 2·5) years; BMI 22·7 (SEM 0·6) kg/m²) 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,
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. 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, 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. The 95 % CI (CI 1.96 x SD / n) of mean AUC calculated from FBG0 was ± 29.8 (n 13). To obtain the same CI for FBG0 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 x 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 / n, where CV is the random variation and n is the number of measures taken. Since sources of

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**Fig. 1.** Blood glucose concentrations after glucose alone (+), glucose plus fat and protein (○), white bread alone (△) and white bread plus fat and protein (▲). Values are means, with their standard errors represented by vertical bars. 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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References


