Diabetic patients have for many years made use of the carbohydrate exchange lists to plan their meals.1 Foods with approximately the same amount of a given nutrient are grouped together. However, all starches in equal amounts do not produce the same effect on blood glucose concentration.2 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 the area under the curve after 50 g carbohydrate from a standard food tested by the same subject.2 Many factors such as food form, particle size, cooking, processing and starch structure affect the GI.3 There is evidence that low-GI foods improve blood glucose control in people with diabetes,4,5 reduce triglycerides in patients with hypertriglyceridaemia,4 prolong endurance during physical activity,7 and improve insulin sensitivity8.9 and colonic fermentation.10 In addition, low GI foods are associated with elevated high-density lipoprotein cholesterol,11 and reduced risk for developing type 2 diabetes.12 There is also indication of improvement in fibrinolytic activity. A low-GI diet was therefore found to normalise plasminogen activator inhibitor-1 (PAI-1) levels in subjects with type 2 diabetes.9 The finding that a low-GI diet reduces fasting insulin levels in parallel to weight loss in obese women is also noteworthy.13 The GI of foods and food products could potentially be labelled on food products in order to guide consumers in their choice of particular carbohydrate-containing foods.

However, several publications highlight the controversy surrounding the validity of the GI in a clinical setting. Although a recent Food and Agricultural Organisation/World Health Organisation (FAO/WHO)14 consultation endorsed the use of the GI in diet planning, Laine et al.15 concluded that the diabetic exchange lists are a more accurate predictor of postprandial response to carbohydrate-containing foods eaten as part of a mixed meal than the GI of foods. More recently the American Diabetes Association (ADA)16 recommended that priority should be given to the total amount of carbohydrate consumed rather than the source of carbohydrate.5 One of the issues raised is the large variation in glycaemic response within the same subject.3,17,18

Little is known about the reproducibility of glycaemic responses after the standards used to determine the GI (white bread or oral glucose). Wolever et al.19 found that starchy test Intra- and inter-individual variation in glucose response to white bread and oral glucose in healthy women

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Objectives. To determine within- and between-individual variation in plasma glucose and serum insulin after the intake of the two standards, glucose and white bread, used to calculate the glycaemic index (GI) of foods, and to determine the number of subjects necessary for GI testing.

Design and methods. Nine healthy female volunteers randomly took four repeats each of 50 g glucose or 50 g available carbohydrate from white bread. Venous blood samples were drawn before, and every 15 minutes for 1 hour after the test meals, and thereafter every 30 minutes for 1 hour. The incremental areas under the glucose and insulin response curves were calculated using two different methods (AUCmin and AUC0). Coefficients of variation (CV) were measured for each time interval and for the AUCs. The GI of bread, taken on four occasions by the same subjects, was calculated using the mean of four glucose responses as standard. Power calculations were done to determine the number of subjects necessary for determining the GI of foods.

Results. No significant differences (p > 0.05) for between- or within-subject variation in plasma glucose were found at any time interval. After glucose, mean plasma glucose was significantly greater at the 15-minute time interval, and the incremental AUCs were significantly greater for glucose. The mean GI of bread varied from 46% to 77%. Power calculations, based on this variation, indicated that if a 10% range for the GI of a food is sought with 80% confidence, between 24 and 90 subjects should participate in GI testing.

Conclusion. Patients and clients should be made aware that responses in the same person may differ from week to week, and that ranges for low, medium and high GI foods, rather than specific values, best describe the glycaemic response to foods.
meals (white bread) varied less (2 - 3 times less) compared with oral glucose. They suggested that starchy test meals may allow a more precise assessment of carbohydrate tolerance than glucose. Therefore it was relevant to investigate the magnitude of the variation of the two standards in this study.

A number of different methods have been used to calculate the area under the blood glucose response curve. For most glycaemic data, the area under the curve has been calculated as the incremental area under the curve, ignoring the area beneath the fasting concentration (AUC). When a blood glucose value falls below the baseline, only the area above the fasting level is included. However, the sharp rise in the glucose curve when oral glucose is administered results in a hypoglycaemic response at approximately 90 minutes in a large number of healthy subjects, which represents a physiologically undesirable state and which is not reflected in the size of the area if ignored. The method in which incremental areas are calculated with the lowest glucose value reached during the test as baseline (AUCmin) gives results which reflect the actual curves. In this study both methods were used for calculation of AUCs.

**Subjects and methods**

Nine healthy female student volunteers were recruited to participate in the study. The group was homogeneous with regard to age (21.1 ± 1.1 years), weight (59.8 ± 4.7 kg), height (165.8 ± 5.5 cm) and body mass index (BMI 21.8 ± 1.7 kg/m²). Each subject had to undergo a screening glucose tolerance test to ensure normal glucose tolerance before inclusion in the study. This was done by means of a finger prick test after consumption of a 50 g pure glucose drink.

The subjects were studied after a 10-hour fast on eight mornings over a 9-week period. Subjects consumed a standard pre-evening test meal (50% of the total kilojoules from carbohydrates, 34% from fat, 16% from protein) to optimise carbohydrate metabolic enzyme induction and to standardise potential ‘second-meal’ effects. On the test day subjects consumed either a 50 g glucose drink made up in 250 ml water or a 50 g available carbohydrate portion of white bread. Test meals were consumed with 250 ml water within 5 minutes. Subjects repeated the glucose meal and white bread test four times. The white bread (Blue Ribbon) (nutritional values taken from the South African Food Composition Tables) was bought in bulk to ensure that the same batch was given to all subjects. Crusts were removed before the bread was portioned (101 g to provide 50 g of carbohydrate) and frozen in plastic bags.

Furthermore, the subjects were instructed not to change their physical activity patterns during the period of this study. It has been suggested by Fukagawa et al. that change in activity might influence peripheral sensitivity to insulin and thus might have an effect on the glycaemic and insulinaemic indices.

Venous blood samples were collected in fluoro-oxalate tubes and plain tubes before and at t15, 30, 45, 60, 90, and 120 after the test meals were consumed. Serum and plasma were then frozen at ~8°C until analysis of glucose and insulin concentrations. Plasma glucose concentrations were determined in duplicate using the enzymatic colorimetric method of Randox (Cat. no. GL 2614 for 2 × 500 ml reagent, Randox Laboratories Ltd., Antrim, UK). Serum insulin was analysed in duplicate using a radio-immunoassay (RIA) kit for human insulin supplied by IBL in Hamburg, Germany (12SI-Insulin-RIA-100, Cat. no. IC 13021). The coefficients of variance (CVs) for the experimental procedures were 2% for glucose analysis and 25% for insulin.

Incremental areas under the blood glucose response curves (AUC) were calculated using two known methods. The first method ignores the area below the baseline, and the other method uses the lowest value obtained during the test as baseline. The GI of bread (four repeats) was calculated using glucose as the standard and the AUCmin.

**Side-effects**

Any side-effect experienced by the subject was noted.

**Statistical analysis**

The statistical analysis was done by the Statistical Consultation Service of the Potchefstroom University for Christian Higher Education (PU for CHE) using the SAS System for Windows Release 6.12. The means and standard deviations (SDs) for the four repeats of glucose and white bread were calculated for each subject for both blood glucose and insulin concentrations. The CVs were also calculated by expressing the SD as a percentage of the mean. Analysis of variance was performed with subject and test meal as the variables, and the Neuman-Keuls procedure used to adjust for multiple comparisons. Pearson’s correlation coefficients were calculated. Power calculations were done to determine how many subjects would be needed to determine the GI of bread with an 80% chance that the range of the GI would not be more than 10%, using the method of Berger. Analysis of variance with repeated measures over treatments using a mixed model with a person-by-treatment interaction was performed. Analysis of variance with subject, meal type and subject-meal type interaction was performed to determine the significance of differences between meal types in responses. Where significant interaction was found, the response of the meal types could not be compared.

**Ethical considerations**

The Ethics Committee of the PU for CHE approved all procedures (ethics number: VGE 3M6-96) and the subjects gave written consent. The subjects were not students of any of the involved researchers.
Results

Glucose responses
The mean plasma glucose responses for glucose and white bread are illustrated in Fig. 1. The mean blood glucose concentration tended to be higher after glucose than after bread (statistically significant at t15, \( p < 0.05 \)). A sharper increase in plasma glucose levels after the intake of oral glucose is seen with a higher peak (t30) as well as a hypoglycaemic effect at the end (t120) compared with bread. One subject had a maximum glucose concentration of 14.7 mmol/l at t30 after one of the four repeats of oral glucose. For the other three repeats, the values for this subject were 8.6, 8.3 and 8.1 mmol/l.

The mean incremental areas under the blood glucose curve for the two methods of calculation are given in Table I. The mean incremental area under the blood glucose curve (AUC0) after glucose was given (106.6 ± 61.0 min.mmol/l) was significantly greater (\( p < 0.05 \)) than that after bread (62.5 ± 44.4 min.mmol/l). When using the AUCmin method glucose also showed a significantly greater (171.6 ± 63.9 min.mmol/l) area compared with bread (102.2 ± 44.9 min.mmol/l). When the mean plasma glucose responses to the four oral glucose and white bread meals were compared it was evident that the oral glucose yielded a greater area under the blood glucose curve.

Table II lists the coefficients of variation (CVs) for plasma glucose within and between subjects at the different time intervals. The CVs of plasma glucose for the four repeats of oral glucose tended to be larger than for the bread tests. The mean within-individual CV of 2-hour glucose was 19.2 ± 7.7% and 16.2 ± 7.6% after oral glucose and bread, respectively. The within-individual CVs in the incremental area were 21.4% and 45.0% for oral glucose (AUCmin and AUC0 respectively) and 36.2% and 63.8% for white bread (AUCmin and AUC0 respectively), with no significant differences observed between the CVs for the two standards. The within-individual CV in the AUC (both methods) tended to be larger for white bread than for oral glucose. However, the AUCmin method had smaller within-individual variation for oral glucose and white bread compared with the AUC0.

The 2-hour CV between individuals was 25.1% for oral
glucose and 16.5% for white bread. The mean between CVs for all the time intervals were 16.3 ± 1.5% and 22.0 ± 5.5% for bread and oral glucose, respectively. The between-individual variation tended to be smaller with bread than oral glucose. The use of the AUC\textsubscript{min} method showed a tendency towards a smaller overall variation. No statistically significant differences (p > 0.05) were found. Furthermore, no correlation was observed between the within-individual variation in plasma glucose concentration with oral glucose and white bread, as well as for the two methods used to calculate AUC.

**Side-effects**

The only side-effect experienced by subjects (N = 3, once each) was nausea after the intake of the oral glucose solution.

**Insulin responses**

The mean serum insulin curves after oral glucose and white bread are illustrated in Fig. 2. The oral glucose resulted in the fastest rise (t30) and highest peak (t45) compared with white bread. Also, the insulin concentrations were below the fasting level at t120 for both white bread and oral glucose. Significant differences between the insulin responses to oral glucose and white bread were observed at time interval t120 (p < 0.05), while highly significant differences were found at t0, 45, 60, and 90 (p < 0.01).

Table III illustrates the mean areas under the serum insulin curves before and after four oral glucose and white bread tests. Both methods used for calculating the AUCs showed highly significant differences (p < 0.01) between oral glucose and white bread. The oral glucose resulted in the highest serum insulin concentration compared with the white bread. It seems, therefore, as if glucose stimulated insulin more effectively than the starchy meal.

There were no significant differences observed between areas calculated with the two methods. No significant differences were found between CVs after oral glucose and white bread at the different time intervals (Table IV). No relationship was found between the within- or between-individual CV in serum insulin after the oral glucose and white bread tests. The 2-hour within-subject variation after white bread was 46.1 ± 22.0% and 50.8 ± 25.4% after oral glucose.

![Fig. 2. Mean serum insulin responses to four oral glucose and bread tests at the different time intervals. Values are mean, minimum and maximum.](image)

### Table III. Mean incremental areas under the serum insulin curves above fasting and minimum values after oral glucose and white bread

<table>
<thead>
<tr>
<th></th>
<th>Glucose Mean ± SD</th>
<th>White bread Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC\textsubscript{0}</td>
<td>117.5 ± 21 412.9*</td>
<td>11 131.8 ± 8 970.3*</td>
</tr>
<tr>
<td>AUC\textsubscript{min}</td>
<td>33 436.0 ± 19 345.2*</td>
<td>22 436 ± 10 845.9*</td>
</tr>
</tbody>
</table>

*Means in the same row not containing the same letter are significantly different (p < 0.01).

### Table IV. Coefficients of variation within and between subjects for serum insulin after oral glucose and white bread at the different time intervals*

<table>
<thead>
<tr>
<th>Time intervals (min)</th>
<th>Within subjects</th>
<th>Between subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>White bread</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>T0</td>
<td>48.8 ± 21.5</td>
<td>38.0 ± 20.5</td>
</tr>
<tr>
<td>T15</td>
<td>65.2 ± 21.1</td>
<td>66.5 ± 23.5</td>
</tr>
<tr>
<td>T30</td>
<td>35.3 ± 21.1</td>
<td>38.6 ± 17.7</td>
</tr>
<tr>
<td>T45</td>
<td>67.9 ± 29.1</td>
<td>43.0 ± 18.8</td>
</tr>
<tr>
<td>T60</td>
<td>65.3 ± 37.0</td>
<td>35.8 ± 18.3</td>
</tr>
<tr>
<td>T90</td>
<td>54.8 ± 27.6</td>
<td>37.6 ± 17.9</td>
</tr>
<tr>
<td>T120</td>
<td>50.8 ± 25.4</td>
<td>46.1 ± 22.0</td>
</tr>
<tr>
<td>AUC\textsubscript{0}</td>
<td>101.6 ± 58.1</td>
<td>73.5 ± 35.1</td>
</tr>
<tr>
<td>AUC\textsubscript{min}</td>
<td>50.1 ± 34.2</td>
<td>42.6 ± 24.8</td>
</tr>
</tbody>
</table>

*SD = standard deviation; AUC\textsubscript{0} = area under the serum insulin curve using fasting level as baseline; AUC\textsubscript{min} = area under the serum insulin curve using the minimum level measured as baseline.

*No significant differences.
within-individual CVs in the incremental area of serum insulin responses were 50.1% and 101.6% (AUCmin and AUC0 respectively) for oral glucose and 42.7% and 73.3% (AUCmin and AUC0 respectively) for white bread. Again, the AUC0 method showed less variation compared with the AUCmin method.

The between-individual CVs in 2-hour serum insulin responses were 37.7% for oral glucose and 24.5% for white bread. The mean CVs between subjects for all the time intervals (not shown in Table IV) were 27.2 ± 5.4% and 30.9 ± 6.0% for bread and oral glucose, respectively. The between-individual CVs for the AUC0 method were 62.8% (oral glucose) and 81.7% (bread), and for the AUCmin method 43.2% and 56.4%.

The between-individual variation tended to be less for white bread compared with oral glucose (p > 0.05).

The GIs of the four bread meals were calculated with glucose as the standard. The mean GI of bread was 62.59. The variations in the GI of the bread and 95% CI are given in Table V.

To determine whether the power of statistics contributed to the variance, Berger’s method was used to calculate an 80% power. Using the GI’s largest standard deviation (SD) obtained for bread and a 10% range of a GI value the following formula was used. For 80% statistical power: 0.1/0.326 = 0.307. This number was looked up in the Student’s t-distribution table and yielded a total of N = 90 subjects (0.1 = 10% range; 0.326 = largest SD of the mean GI of bread found in this study; N = number of subjects).

When the smallest SD obtained in this study for the GI is used (0.170), using Berger’s method, the number of subjects necessary to determine the GI with 80% power for a 10% spread will be 24 subjects.

**Discussion**

The variability of glycaemic as well as insulinaemic responses can be ascribed to several factors such as methodology, test meal-related factors, physiological factors, as well as the standard used. Originally Jenkins et al. used oral glucose as standard food but it later became evident that white bread was a more physiologically acceptable standard. We studied the within- and between-individual variation in glucose and insulin responses to the two standards, oral glucose and white bread.

The glucose responses to oral glucose and white bread were looked at first. It was found that the mean glucose response after oral glucose was higher, with a hypoglycaemic event after 2 hours (value lower than baseline). Three of the subjects reported nausea once after the oral glucose was taken. It is well known that glucose solutions can cause an osmotic effect which in turn may lead to delayed gastric emptying. Also, an increase in blood glucose after an oral glucose load can be seen as a stressful event which in turn releases cortisol. This hormone increases blood glucose in stress reactions.

Significant differences were observed in the blood glucose responses (AUC) between oral glucose and white bread. The response to white bread was lower compared with that after oral glucose. The CV within individuals 2 hours after oral glucose was 19.2%. We found a variation of 16.2% within subjects after bread was taken. Wolever et al. found a mean within-individual CV of 12.9 ± 2.8% (oral glucose) and 5.2 ± 0.8% (white bread) after a 2-hour glucose tolerance test. The large difference in results might be due to the capillary versus venous blood sampling. It has been suggested that results for capillary blood are less variable than those for venous blood. Wolever et al. suggested that within-subject variation might be due to the fact that insulin is secreted in pulses and that this might cause periodic fluctuations in glucose and insulin concentrations. Bansal et al. found a large between-subject variation (45.6%) in the GI of bread with glucose as the standard in 55 adults who were newly diagnosed as having type 2 diabetes mellitus. Glucose was measured in venous blood samples in this study.

It is known that bread contains not only carbohydrate, but that fat and protein are also present. Protein is known to stimulate insulin secretion. Wolever et al. reported a negative correlation between protein content and the GI of foods. Nutall et al. found that after 30 g and 50 g protein was added to a 50 g carbohydrate load the glycaemic response was reduced only after the 50 g protein load. The protein content of the bread meals used in our study was 9 g and it would, therefore, have had little or no effect on the insulin response. The oral glucose resulted in a higher insulin response compared with white bread. Again, it is speculative whether stress hormones might have played a role here.

The two methods used to calculate the AUC were also examined as possible sources of variation. Highly significant differences (p < 0.01) were noted between the two methods for

**Table V. Variations in the GI of bread (using AUCmin)**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread 1</td>
<td>59.63 ± 32.55</td>
<td>34.31</td>
<td>84.66</td>
<td>54.60</td>
</tr>
<tr>
<td>Bread 2</td>
<td>46.21 ± 16.97</td>
<td>33.16</td>
<td>59.25</td>
<td>36.73</td>
</tr>
<tr>
<td>Bread 3</td>
<td>76.64 ± 24.50</td>
<td>57.81</td>
<td>95.46</td>
<td>31.96</td>
</tr>
<tr>
<td>Bread 4</td>
<td>67.87 ± 26.37</td>
<td>47.60</td>
<td>88.14</td>
<td>38.85</td>
</tr>
</tbody>
</table>
The AUCmin method is a more relevant physiological calculation variation than the fasting level as baseline method. We feel that minimum level as baseline method appeared to have less would lead to less variation in the GI calculation. The glycaemic response as well as insulinaemic response. This glucose as standard would ensure the lowest variation in the compared with white bread. It would appear that using oral foods. Variation in serum insulin responses between individuals (AUC) tended to be smaller with oral glucose compared with white bread. It would appear that using oral glucose as standard would ensure the lowest variation in the glycaemic response as well as insulinaemic response. This would lead to less variation in the GI calculation. The minimum level as baseline method appeared to have less variation than the fasting level as baseline method. We feel that the AUCmin method is a more relevant physiological calculation method. However, more research regarding the standardisation of methods determining the GI is needed. The development and implementation of a standardised method will ensure that variation within and between subjects is minimised. Consensus should be reached on the method of calculation of the incremental area under the blood glucose and insulin response curves.

Another source of variation should be considered. The pre-test meal that subjects took the evening before fasting commenced was standardised to optimise carbohydrate metabolism enzyme induction and to prevent differences in possible ‘second-meal’ effects.\(^2\) It is possible that these subjects could have experienced this standardised meal as an additional stress, influencing the glucose response the following morning. This aspect needs more research.

The larger variations observed for the insulin response compared with the glucose response (not statistically significant) may be partly because of methodology. The RIA method for insulin determination had a CV of 25\%, while the enzymatic glucose method had a CV of only 2\%.

The fact that the within-individual variations were not significantly smaller than the between-individual variations (especially with bread) has an important practical application. It suggests that in GI determinations (either for research or for example labelling purposes in the food industry) it would not be necessary to use the same subjects repeatedly. Larger groups of subjects, provided that they are at least as homogeneous as the group participating in this study, could be used.

The question that should be addressed is whether these relatively large variations in glucose response to the two foods usually used in GI determinations as standards, would influence the credibility and usefulness of the GI. The GI of bread on the four occasions, using the mean response to glucose as standard (Table V), showed mean values varying from 46\% to 77\%. The 95\% CI varied from 33\% to 95\%. This means that in this group of subjects, the GI of bread could actually be any value between 33\% and 95\%. The power calculations showed that to have 80\% confidence that the GI of bread will be in a 10\% range, 90 subjects would be needed to determine the mean GI.

If foods on a scale from 0 - 100 are classified as having a low, medium or high GI, the ranges would probably be 0 - 55 (low), 56 - 70 (medium) and 70 and over (high).\(^6\) The ideal would be to classify the same food in the same category consistently. It seems that to do this with confidence, the mean GI of a particular food should be determined in larger groups (based on our results, from 24 to 90 subjects). If a larger range than 10\% is deemed sufficient, fewer subjects may be used.

Conclusions

From the results of this study it can be concluded that:

1. The CV of glucose response was not statistically significantly different between and within subjects after ingestion of 50 g carbohydrate as glucose or bread.

2. Variations in insulin response were generally larger than variations in glucose responses.

3. Variations in plasma glucose were smaller after bread intake compared with glucose intake at 2 hours, but the variations in the AUC were generally larger for bread than for glucose. However, these differences were not statistically significant in this relatively small group of nine subjects with four repetitions of each test (oral glucose and bread).

4. The power calculations based on the variations of the GI of bread, using a mean glucose response of four glucose tolerance tests, indicated that if a 10\% range for a GI of a food is sought with 80\% confidence, between 24 and 90 subjects should be used to determine the mean GI of the food.

5. The use of oral glucose as standard in this study showed less variation than bread.
Recommendations

The first recommendation is that more research is needed to standardise methodology in order to minimise the variations in the GI. Notably, the pre-evening meal, venous versus capillary blood, the way in which the AUC is calculated, as well as the number of subjects used should receive attention. Another recommendation would be to use oral glucose as the standard food instead of bread, as it was shown in this study that oral glucose had less variation.

The second recommendation is that in using the GI of foods to choose carbohydrate-containing foods, patients and clients (consumers) should be made aware of the fact that the physiological responses to a food vary between individuals and also in the same individual from time to time. Therefore, when advising on the GI it should be mentioned that the GI of a particular food is usually low, medium or high, but that exceptions can be expected and that these exceptions are normal.

References