Intra- and inter-individual variation in blood glucose response to white bread and glucose in patients with type 2 diabetes mellitus

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Introduction and objective. Variability of glycaemic response can be due to several factors, including within- and between-subject variations. The aim of this study was to determine the within- and between-subject variation of fasting free fatty acids (FFAs), plasma glucose and serum insulin after the intake of white bread versus oral glucose in type 2 diabetic subjects.

Methods. Nine overnight-fasted subjects (4 men and 5 women, mean body mass index (BMI) 31.7, mean age 57.8 years) with type 2 diabetes mellitus were recruited. Each subject received a standard pre-test meal the evening before every test day. The reference foods used were 50 g available carbohydrate is in the form of white bread (101 g), and glucose. Each reference food was tested on four randomised occasions. Venous blood samples were taken before, and every 30 minutes for 3 hours after, test meals. Incremental areas under the curve (IAUC), areas from the lowest value (AUCL) and areas from zero (AUC0) were calculated for the glycaemic and insulin response curves.

Results. No significant difference ($p < 0.05$) for within-subject variation in plasma glucose response was found at any time point, while the coefficient of variation (CV) of the IAUC and AUCL were significantly higher for white bread. The only significant difference ($p > 0.05$) in plasma glucose response between the subjects was found at 180 minutes, while no significant differences were found between the variations according to the areas under the curve. No significant differences were found between glucose and white bread with regard to within and between-subject variation for serum insulin responses and fasting FFA concentrations.

Conclusion. According to the IAUC (used in glycaemic index (GI) calculations), within-subject blood glucose concentrations tend to be less variable after a glucose test meal than after a starchy test meal in type 2 diabetic subjects.

Research to date on carbohydrates has shown that dietary carbohydrates are a diverse group of substances with varied physiological properties of differing importance to health.[1,2] The physical structure of the cell wall of carbohydrates affects satiety[3,4] as well as the rate and extent of starch digestion, which is a major factor controlling blood glucose and insulin.[5-7]

Carbohydrates have been the main focus of diabetes nutrition management from as long ago as 1550 BC. Since the 1930s scientists have challenged the traditionally held view that the metabolic effects of carbohydrates can be predicted according to their classification as either 'simple' or 'complex'. In the 1970s researchers such as Otto *et al*.[8] and Crapo *et al*.[9] examined the glycaemic impact of a range of foods containing carbohydrates. To standardise the interpretation of glycaemic response data, they proposed the glycaemic index (GI). The GI is determined by comparing the acute glycaemic response of a test food with that of a standard food in individual subjects. Studies included subjects with type 1 diabetes mellitus (DM), type 2 DM and non-diabetics.[10] The GI concept disproved the assumption that equivalent amounts of carbohydrate from different foods elicit similar glycaemic responses, and researchers have concluded that the carbohydrate exchange lists that have regulated the diets of many diabetics may not reflect the physiological effect of foods.[11]

The GI of foods has important implications for the prevention and treatment of the major causes of morbidity and mortality, including type 2 DM, coronary heart disease and obesity.[12] There is clear evidence that low GI foods improve blood glucose control in diabetic subjects.[13-15] Furthermore it has been shown that a low GI diet improves hyperlipidaemia[16,17] and promotes weight control by both enhancing satiety and reducing insulinaemia.[18-20]

There seem to be particularly important reasons to promote low GI foods in the dietary choices of people with diabetes. In subjects with type 1 and type 2 DM, low GI diets, compared with high GI diets of similar nutrient composition, lead to improvement in glucose and blood lipid profiles.[14-17] Recently

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there has also been general agreement that sucrose in controlled amounts can be included in a diabetic diet because of its intermediate GI.[21-23]

Despite the evidence of the health benefits of low GI food choices, the GI approach has been dismissed on the basis of certain criticisms. Two of the major problems regarding the general implementation of the GI in diabetic diets is the reproducibility of the glucose response in the same subject, and the between-individual variation of glucose response to the same standard food.[24,25] Beebe[26] concluded that the application of the GI concept in diabetic diets needs further research because the application of the GI in different subjects is still seen as a barrier to its general practical application.

From the above it seems clear that more knowledge is necessary regarding the application of the GI in the diabetic diet. Further knowledge is essential regarding the most appropriate standard food with which to compare the glycaemic response to different foods. The aim of this study was therefore to examine the intra- (within) and inter- (between) subject variation in glucose and insulin response in type 2 diabetic subjects after consuming a standard amount (50 g) of carbohydrate as glucose and white bread.

Methods

Twenty-four type 2 diabetic subjects were recruited via the local media to take part in the study. Included were both male and female subjects with diagnosed type 2 DM treated with metformin (Glucophage) or diet alone, between the ages of 19 and 70 years, and with a body mass index (BMI) ranging between 20 and 35. A standard glucose tolerance test (GTT) according to the World Health Organisation (WHO) criteria,[27] was used to ensure the presence of type 2 DM (fasting plasma glucose > 7.8 mmol/l and/or > 11.1 mmol/l after 2 hours). For indication of blood glucose control, glycated haemoglobin was analysed, and subjects with an HbA_{1c} > 8% (normal 4.4 - 6.4%) were not included in the study. Exclusion criteria were impaired renal function according to serum creatinine and creatinine clearance tests (see Table I for normal ranges), smoking, and alcohol intake of more than 6% of the daily energy intake, according to an analysed food frequency questionnaire.[28] Subjects had to maintain their regular level of activity/exercise throughout the study period. All subjects gave informed consent and the study was approved by the Ethical Committee of the Faculty of Health Sciences, University of the Free State.

Ten type 2 diabetic subjects started the study and 9 successfully completed it (4 male, 5 female, aged 57 ± 5.9 years, BMI 31.7 ± 3.7, treated with metformin (Glucophage) or diet and fulfilling all the inclusion criteria). The study was conducted on eight separate mornings, with weekly intervals. All subjects consumed a standard pre-test meal (Table II) the evening before every test day, followed by an 8 - 12-hour overnight fast. The night before testing patients using oral drugs took their medication. On test days fasting blood samples were obtained for plasma glucose, serum insulin and free fatty acids (FFAs), after which subjects had to consume a test meal of either 50 g glucose powder dissolved in 250 ml water or 101 g white bread, each containing 50 g available carbohydrate, within 10 - 15 minutes. Subjects were allowed to consume either 450 ml water or black tea with the white bread test meal and their choice had to remain the same throughout the study. Each test meal was repeated four times by each subject in random order.

Venous blood samples were obtained using an indwelling catheter at 30, 60, 90, 120, 150 and 180 minutes after the subjects had started the meal. Blood samples were obtained by the staff at the Hoechst Research Clinic of the University of the Free State. Palatability and acceptability of the test meals were also assessed, using a questionnaire, with subjects indicating whether they experienced symptoms of headache, dizziness, stomach discomfort, bloating, belching, flatulence or diarrhoea after the test meals.

Blood samples for glucose and insulin were analysed at the Department of Chemical Pathology, University of the Free State, while FFAs were analysed at the Fibrinogen Unit at the Technikon Free State. Standard laboratory techniques, apparatus and standard reference ranges were used for the analyses of blood samples. These laboratories are part of the International Wellcome Control System, which specifies that

Table I. Subject characteristics

Parameter	Reference range	Mean	SD	Sample range
Age (yrs)	19 - 70	57	5.9	51 - 67
Body mass index (kg/m²)	24.0 - 35.9	31.7	3.7	26.5 - 35.7
HbA1c (%)	< 8	6.8	0.9	5.5 - 7.8
Serum-creatinine (µmol/l)	F: 53 - 97	F:73.8	F: 10.9	F: 65 - 93
	M: 80 - 115	M: 104.2	M: 9.5	M: 94 - 115
Creatinine clearance (ml/min)	F: 80 - 125	F: 111.8	F: 36.5	F: 71 - 164
	M: 90 - 130	M: 183.0	M: 30.7	M: 139 - 207

HbA_{1c} = glycated haemoglobin; SD = standard deviation; F = female; M = male.

every tenth sample should be a control sample of a known value, supplied by Wellcome Control. Serum immunoreactive insulin was measured using a Coat-A-Count Insulin radioimmunoassay (Diagnostic Products Corp., Los Angeles), plasma glucose was determined by a glucose oxidase method using the Technikon RA-100 system, and FFAs were analysed by NEFA-Quick 'BMY'.

The Department of Biostatistics at the University of the Free State did the statistical analyses. For each individual the mean and standard deviation (SD) of the four values obtained for the specific meal type were calculated and within-individual variation was then summarised for the specific meal type using the coefficient of variation (CV). For each meal type these within-individual CVs were summarised using the means, medians and SDs. To compare the within-individual variation for the two meal types, paired *t*-tests were performed, and 95% confidence intervals (CIs) were calculated for the mean difference. The mean of the four values for the specific meal type was calculated for each individual. These mean values were then summarised for the 9 subjects who completed the study using means, SDs and the CV, the latter indicating the between-individual variation.

To compare the results of between-subject variation for the two meal types, analysis of variance was performed separately for each meal type. Dividing the mean square error associated with subjects on the one meal type by the other, an F-statistic was calculated and its *p*-value reported. 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. In cases where a significant interaction was found, the responses to the meal types cannot be compared. To determine the GI of the white bread, each person's mean incremental area under the curve (IAUC) on the four occasions using white bread was divided by each subject's mean IAUC for glucose. This was summarised by the mean and SD. The following three areas under the blood glucose response curve were calculated: total area under the curve (AUC0),[29] IAUC,[11] and incremental area under the curve using the lowest blood glucose value as baseline (AUCL).[30] Power calculations were done to determine the number of subjects needed to determine the GI of foods.

Results

One subject dropped out because of poor compliance and 9 subjects successfully completed the study. Table II shows the characteristics of the 9 subjects at the beginning of the study. All the female subjects included in the study had had a hysterectomy. Weight changes were recorded during the study: a maximum weight loss of 1.2 kg and a maximum weight gain of 1.9 kg were observed. Seven of the subjects were treated with the oral hyploglycaemic agent metformin (Glucophage), while 2 subjects were treated by means of diet alone.

The mean energy intake of the study population was 8 655.7 kJ for the men and 6 540.8 kJ for the women, with mean intakes of 48.1% carbohydrate, 17.7% protein, 37.4% fat and 0.6% alcohol. All nutrient intakes except fat intake were within the recommended dietary allowances, and the mean alcohol intake was within the range of < 6% of total energy.

Table III summarises the mean, SD and median for the within-subject CV in plasma glucose response. After the white bread test meal, the mean CV in plasma glucose response varied from 11.6% at 90 minutes to 16.1% at 30 minutes, while the mean CV in plasma glucose response after the glucose test meal varied from 9.6% at 90 minutes to 14.5% at 0 minutes.

According to the areas under the blood glucose curve, the CV after the white bread was the highest for the IAUC and the AUCL at 27.9% and 21.8% respectively, while for the AUC0 the mean CV was only 10.6%. The mean CV for the area under the curves after the glucose test meal was also the highest for the IAUC and the AUCL, at 11.6% and 8.8% respectively, while the mean CV for the AUC0 was 10.0%.

There were no significant differences as indicated by the *p*-value and the 95% CI at any time point, but the CVs of the IAUC and AUCL were significantly higher on white bread than glucose.

The between-subject variations for plasma glucose responses are shown in Table IV and presented as the mean blood glucose response, SD and CVs calculated at every time point for white bread and glucose. The CV indicates the between-subject variation in glucose response. The mean blood glucose response between the subjects varied from 8.3 mmol/l at 0 minutes to 13.0 mmol/l at 90 minutes after the intake of the

Table II. Standard pre-test meal

Amount of food	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (kJ)
3 slices of white bread	45	-	6	870
60 g cheese	-	10	14	620
5 teaspoons of sugar or jam	20	-	-	340
3 teaspoons margarine	-	15	-	570
250 ml skimmed milk	12	-	8	345
2 x 70 g apple (without skin)	20	-	-	340
Total	97	25	28	3 085

Table III. Within-subject coefficient of variation for plasma glucose responses (*N* = 9)

	White bread (%)			Glucose (%)				
Time (min)	Mean	SD	Median	Mean	SD	Median	*p*-value	95% CI
0	14.7	5.3	14.5	14.5	6.4	14.8	0.919	–4.8; 5.3
30	16.1	5.1	14.2	12.2	5.5	13.3	0.105	–1.0; 8.8
60	13.4	5.5	13.7	11.2	5.0	9.3	0.508	–5.0; 9.3
90	11.6	8.7	8.0	9.6	2.9	9.8	0.570	–5.8; 9.8
120	13.7	6.3	16.2	12.2	2.8	13.7	0.527	–3.6; 6.6
150	14.4	10.3	10.0	13.3	4.9	13.7	0.766	–7.2; 9.5
180	14.5	8.2	14.5	14.4	7.2	10.3	0.991	–9.7; 9.8
IAUC	27.9	15.5	23.5	11.6	5.0	11.1	0.012	4.7; 7.7
AUCL	21.8	9.5	23.5	8.8	3.6	8.7	0.007	4.7; 21.4
AUC0	10.6	5.1	10.4	10.0	3.4	9.8	0.762	–4.7; 27.7

IAUC = incremental areas under the curve; AUCL = incremental area under the curve using the lowest blood glucose value as baseline; AUC0 = total area under the curve.

Table IV. Between-subject coefficient of variation for plasma glucose response (*N* = 9)

	White bread			Glucose			*p*-value for comparison of mean response	*p*-value for comparison of between-subject variation
Time (min)	Mean (mmol/l)	SD	CV (%)	Mean (mmol/l)	SD	CV (%)		
0	8.3	1.7	20.6	7.9	1.6	19.7	0.255	0.401
30	11.1	2.5	22.5	12.9	2.3	17.9	*	0.410
60	12.9	2.4	19.0	14.9	2.3	15.6	*	0.443
90	13.0	2.2	16.6	13.5	2.0	14.8	0.268	0.170
120	12.2	1.8	15.2	11.7	1.8	15.1	0.205	0.453
150	10.9	2.1	19.5	10.0	1.8	17.5	0.019	0.302
180	10.0	2.1	21.0	8.7	2.0	23.0	0.001	0.448
IAUC	596.0	137.6	23.1	718.0	142.6	19.9	0.001	0.463
AUCL	632.7	135.5	21.4	755.3	128.6	17.0	0.313	0.448
AUC0	2 078.7	342.6	16.5	2 139.3	327.0	15.3	0.001	0.443

* Significant interaction between subject and meal type.
IAUC = incremental areas under the curve; AUCL = incremental area under the curve using the lowest blood glucose value as baseline; AUC0 = total area under the curve.

white bread test meal. According to the CV, the least variation after testing white bread was found at 120 minutes (15.2%), while the variation at 30 minutes (22.5%) was the greatest.

The mean blood glucose response after the glucose test meal varied from 7.9 mmol/l at 0 minutes to 14.9 mmol/l at 60 minutes. According to the CV the least variation after the glucose test meal was found at 90 minutes (14.8%), while the highest variation was found at 180 minutes (23%). The only significant difference ($p < 0.05$) in the mean blood glucose response values between the two meals was found at 180 minutes, while a significant interaction between the subject and meal type was found at 30 minutes and 60 minutes.

The area under the blood glucose curve was found to be significantly higher ($p < 0.05$) after the glucose test meal than after the white bread test meal for the IAUC and AUC0: IAUC 718.0 (glucose) versus 596.0 (white bread), AUC0 2 139.3 (glucose) versus 2 078.7 (white bread). However, the AUCL was not significantly higher after the glucose test meal than after the white bread test meal: AUCL 755.3 (glucose) versus 632.7 (white bread).

The CVs for the IAUC, AUC0 and the AUCL after the white bread test were 23.1%, 16.5% and 21.4% respectively, while for the glucose test meal similar results were found with CVs of 19.9%, 15.3% and 17.0%. No significant difference ($p < 0.05$) was found between the variations in the areas under the curve between the two test meals.

The within-subject variations for serum insulin response are summarised in Table V, using the mean, SD and median for the CVs. The mean CV after the intake of the white bread test meal varied from 17.4% at 20 minutes to 29.4% at 180 minutes, while the mean CV for the glucose test meal varied from 18.9% at 0 minutes to 24.0% at 60 minutes. According to the areas under the blood glucose curve, the CV after the white bread test meal was the highest for the IAUC and the AUCL, at 37.1% and 27.8% respectively, while for the AUC0 the mean CV was 15.4%. Similar results were found for the CV after the intake of the glucose test meal, with a mean CV of 35.3%, 29.0% and 15.4% for the IAUC, AUCL and AUC0 respectively. No significant differences were found between glucose and white bread with regard to within-subject variations.

The between-subject variations for serum insulin response are summarised in Table VI. The CV indicates the between-subject-insulin response variation. The mean serum insulin response between the subjects varied from 33.9 IU/ml at 0 minutes to 68.8 IU/ml at 90 minutes after the intake of 101 g white bread, while the CV calculated at every time point varied from 37.8% at 30 minutes to 55.1% at 90 minutes.

The mean serum insulin response with 50 g glucose varied from 28.9 IU/ml at 0 minutes to 64.3 IU/ml at 60 minutes. According to the CV, the least variation after the glucose test meal was found at 0 minutes (44.5%) and the highest variation at 60 minutes (69.5%). A significant difference ($p < 0.05$) in mean serum insulin concentrations at the different time points

Table V. Within-subject coefficient of variation for serum insulin responses (*N* = 9)

	White bread (%)			Glucose (%)				
Time (min)	Mean	SD	Median	Mean	SD	Median	*p*-value	95% CI
0	28.0	28.3	17.6	18.9	8.2	18.6	0.293	–9.5, 27.6
30	24.7	6.4	24.4	21.4	7.9	19.7	0.271	–3.1, 9.7
60	20.9	4.7	18.7	24.0	10.7	21.8	0.378	–11.1, 4.7
90	19.1	9.1	23.0	23.3	9.2	21.6	0.418	–15.6, 7.2
120	17.4	5.4	18.6	20.5	10.9	18.7	0.399	–11.2, 5.0
150	20.1	10.6	18.5	19.6	7.2	21.8	0.898	–6.8, 7.6
180	29.4	29.0	18.4	20.3	8.1	19.2	0.329	–11.1, 29.4
IAUC	37.1	22.4	32.1	35.3	22.6	32.8	0.877	–24.6, 28.2
AUCL	27.8	13.4	29.4	29.0	16.6	28.9	0.883	–19.4, 17.0
AUC0	15.4	5.2	17.4	15.4	7.3	14.1	0.971	–5.5, 5.7

IAUC = incremental areas under the curve; AUCL = incremental area under the curve using the lowest blood glucose value as baseline; AUC0 = total area under the curve.

Table VI. Between-subject coefficient of variation for serum insulin response (*N* = 9)

	White bread			Glucose			*p*-value for comparison of mean	*p*-value for comparison of between-subject
Time (min)	Mean (µU/ml)	SD	CV (%)	Mean (µU/ml)	SD	CV (%)	response	variation
0	33.9	16.9	49.9	28.9	12.9	44.5	0.149	0.228
30	52.5	19.8	37.8	51.9	24.2	46.7	*	0.293
60	62.7	26.8	42.8	64.3	44.7	69.5	*	0.086
90	68.8	37.9	55.1	52.4	28.8	54.9	0.001	0.228
120	57.6	29.0	50.3	46.5	21.1	45.5	0.001	0.195
150	52.1	23.9	46.0	44.5	23.1	51.9	0.004	0.458
180	50.1	20.9	41.6	40.9	18.5	45.1	0.010	0.368
IAUC	4 335.0	2 105.7	48.6	3 658.5	2 679.4	73.2	0.060	0.255
AUCL	4 532.6	2 054.1	45.3	3 740.3	2 606.0	69.7	0.015	0.258
AUC0	10 068.6	4 386.2	43.6	8 834.3	4 535.7	51.3	0.002	0.463

* Significant interaction between subject and meal type.
IAUC = incremental areas under the curve; AUCL = incremental areas under the curve using the lowest blood glucose value as baseline; AUC0 = total area under the curve.

between the different test meals was found at 90, 120, 150 and 180 minutes.

The area under the serum insulin response curves was significantly higher ($p < 0.05$) after the white bread test meal than after the glucose test meal according to the AUCL and the AUC0. The CVs for the IAUC, AUCL and AUC0 after the white bread test meal were 48.6%, 45.3% and 43.6% respectively, while for the glucose test meal the CVs were 73.2%, 69.7% and 51.3% respectively.

The within- and between-subject variations for fasting FFAs were as follows: the mean within-subject CV for fasting FFA before the white bread meals was 20.1% (SD 6.5), while the mean CV for fasting FFA before the glucose meals was 23.6% (SD 13.3). No significant difference ($p = 0.471$) was found between the mean CV for the two test meals. The mean fasting FFA between the subjects before the white bread meals was 0.6 mmol/l (SD 0.2), while the mean fasting FFA before the glucose meals was 0.7 mmol/l (SD 0.2). The CVs for the white bread and glucose meals were 27.2% and 25.9% respectively. No significant difference ($p < 0.05$) was found between the subjects for the mean FFA concentration or for the variation between the two test meals.

According to correlations between fasting FFA and fasting glucose, fasting FFA and fasting insulin and fasting glucose concentrations, the following were found: a signficant ($p = 0.012$) negative correlation ($r = –0.78$) was found between the CV for fasting FFA and fasting glucose before the glucose test meal, while no correlation ($r = –0.15$) was found before the white bread test meal. No correlation could be found between the mean values for fasting FFA and fasting glucose before the glucose test meal ($r = 0.01$) or before the white bread test meal ($r = –0.04$).

No correlation was found between fasting FFA and fasting insulin for CV and mean values before both test meals (Table VII). Significant correlations ($p < 0.05$) were found between the CV and mean values for fasting insulin and fasting glucose before the white bread test meal. The correlations before the glucose test meal were somewhat lower.

Table VII. Correlations between fasting values for plasma glucose, serum insulin and fasting free fatty acids (FFAs)

	White bread		Glucose	
CV	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
FFA with glucose	–0.15	0.700	–0.78	0.012
FFA with insulin	–0.20	0.606	–0.36	0.331
Insulin with glucose	0.83	0.005	0.56	0.111
Mean values				
FFA with glucose	–0.04	0.915	0.01	0.965
FFA with insulin	–0.21	0.574	–0.23	0.542
Insulin with glucose	0.68	0.042	0.66	0.050

CV = coefficient of variation.

Using results from consecutive visits to calculate GIs, the mean GIs were 88.2 (SD 18.1), 81.4 (SD 17.0), 66.5 (SD 33.0), and 101.2 (SD 36.6). Using the mean values of the IAUCs for the four occasions on a specific test occasion, the mean GI was estimated as 83. The power calculations based on the smallest SD found indicated that a minimum of 24 subjects are necessary to determine the GI of bread with 80% accuracy.

The symptoms experienced after test meals were as follows: none of the subjects had any symptoms of nausea, bloating, belching, flatulence, diarrhoea or other symptoms not described in the questionnaire. With regard to headaches and dizziness, the subjects had similar experiences on white bread and glucose. Subjects experienced hunger more frequently on glucose than white bread. One subject experienced hunger on one occasion after testing with white bread, while 1 subject felt hunger on one occasion, 4 subjects on two occasions and 2 subjects on three occasions after testing with glucose.

Discussion

Within-subject variation in blood glucose responses

Within-subject variation in plasma glucose responses in the type 2 diabetic subjects was similar at every point for both white bread and glucose. These results correspond with those found by Wolever and co-workers,[31] who also compared within-subject variability of plasma glucose measured 2 hours after a GTT with 75 g glucose versus a standardised test meal (50 g available carbohydrate). They also determined the relationship between the two sets of plasma glucose measurements.

Wolever and co-workers[32] concluded that the variability in 2-hour plasma glucose concentrations is two to three times greater after oral glucose than after starchy foods in normal subjects. They suggest that standardised starchy test meals may provide a more precise way of assessing carbohydrate tolerance than oral glucose because of the greater reproducibility of 2-hour blood glucose. However, because these results pertained to normal subjects, Wolever and co-workers[32] suggested that before being considered valid, these results would have to be reproduced in subjects with DM. Therefore, the present study looked at the variation in type 2 diabetics, but did not find a significant difference in blood glucose or white bread. It can therefore be concluded that a starchy meal may not provide a more precise way of assessing carbohydrate tolerance than oral glucose in type 2 diabetic subjects.

The present study together with other studies[33,34] shows a fairly consistent picture of a fasting plasma glucose variability of 14 - 20%. According to Oleerton and co-workers[33] the daily biological variability accounts for 14% of the 15% total variability in fasting plasma glucose content. While diet or

lifestyle changes were unlikely to have had a significant effect on the results because of the relatively short period between the tests, the variability is probably due to other unexplained within-subject biological factors that influenced fasting plasma glucose, including the dawn phenomenon.

Another possible influence on fasting plasma glucose concentrations could be the different diabetic treatments of the subjects. Although Wolever and co-workers[35] have found that the within-individual variation in glycaemic response in type 2 diabetic subjects on insulin is virtually identical to that in type 2 diabetics treated by diet alone or diet plus oral hypoglycaemic agents, Gannon and Nuttal[36] concluded that the effect of the medication (insulin or oral hypoglycaemic agents) might vary from day to day. Gannon and Nuttal[36] suggested that this could influence glycaemic and insulin responses. However, the number of patients in the present study was too small to evaluate the effect of treatment.

Wolever *et al*.[31] suggested that the physiological mix of nutrients in a starchy meal might stimulate gastric motor activity, resulting in more consistent gastric emptying and hence more consistent postprandial plasma glucose concentrations than an oral glucose tolerance test. However, this was not found in the present study.

The mean within-subject CV of 27% (IAUC) after the white bread meal was significantly higher than the CVs found by Wolever *et al*.[35] (CV = 16%), Wolever *et al*.[37] (CV = 16%) and Rasmussen *et al*.[38] (CV = 19%), all tested on diabetic subjects. However, the mean within-subject CV of 11.6% after the glucose meal found in the present study corresponds with the results of Wolever and co-workers[31] (CV = 10.3%).

According to Wolever and co-workers[35] type 2 diabetics appear to show less variation in blood glucose response from day to day than either non-diabetic or type 1 diabetic subjects. After testing, Wolever and co-workers[35] found the highest variation among type 1 diabetic subjects when compared with type 2 diabetics and non-diabetics. The present results for type 2 diabetics (CV = 27%) correspond with those found by Wolever and co-workers[35] for type 1 diabetics (CV = 29%, suggesting that type 2 diabetics may be just as variable as type 1 diabetic subjects after testing white bread.

Although both test meals in the present study had identical carbohydrate content, the differences in rate of digestion and absorption of the test foods could account for the different plasma-glucose responses. The fact that glucose was taken as a liquid could possibly contribute to higher plasma-glucose response peaks, thus influencing the areas under the blood glucose response curve, because foods taken as liquids are reported to result in higher glucose and insulin responses than the same foods taken as solids.[39] Furthermore, oral glucose is more rapidly absorbed than most other foods due to the fact that it is a monosaccharide and does not need to be broken down by means of digestive processes before it can be absorbed.[40]

On the other hand white bread contains protein and fat in addition to the carbohydrate, compared with glucose which contains 0 g fat and 0 g protein. Fat is known to decrease the glycaemic response because of the slow rate of carbohydrate absorption induced by the reduced rate of gastric emptying.[41] Furthermore, protein ingestion stimulates more insulin secretion in type 2 diabetic subjects and blood glucose responses appear to be increased rather than decreased by the addition of protein to a meal.[42] However, these effects are not seen unless relatively large amounts (25 g fat and protein per 50 g carbohydrate) are added.[41] Therefore, the presence of fat and protein in the white bread could not have affected the blood glucose response in such a way that it influenced the variations seen in the subjects.

Within-subject variation in serum insulin responses

The within-subject variations in serum insulin concentrations were two to three times greater than those of plasma glucose. No significant difference ($p > 0.05$) in variation was found at any time point between the two test meals. These results correspond with the results of Wolever and co-workers[31] who found no significant difference in the within-subject CV for plasma insulin after a starchy meal (50 g available carbohydrate) versus 75 g oral glucose. Wolever and co-workers[31] concluded that true within-subject variation may be due to the fact that insulin is secreted in pulses, so that plasma concentrations for both glucose and insulin fluctuate minute to minute.

According to the areas under the serum insulin response curve, no significant difference ($p > 0.05$) was found between the two test meals for all three methods calculated. The results of Rasmussen and co-workers[38] for white bread (IAUC) (CV = 45%) were similar to those in the present study. These researchers concluded that the variance could be a result of a large day-to-day variation in insulin resistance in type 2 diabetic subjects because the blood glucose excursions were fairly constant at varying plasma insulin levels in the subjects.

Although insulin release in response to a carbohydrate load is augmented by fat, possibly due to the increase in gastric inhibitory polypeptide (GIP) levels after a fatty meal,[43] protein-stimulated insulin secretion is increased in type 2 diabetics.[42,44] However, the presence of small amounts of fat and protein in the white bread meal in the present study could not have influenced glucose or insulin responses such that these could contribute to the variations seen within the subject or between the two test meals.

Within-subject variation in fasting FFA concentrations

No significant differences in the within-subject variation for fasting FFA concentrations were found. Wolever and co-workers[45] concluded that fasting FFA concentrations tend to be

quite variable, because only a small increase in plasma insulin is required to inhibit hormone-sensitive lipase and to reduce FFA release from adipose tissue.

Although the within-subject CV for fasting serum insulin in the present study could have contributed to the within-subject variations seen in fasting FFA concentrations, no correlation was found between the fasting CV for FFA and the fasting CV for serum insulin.

Between-subject variation in plasma glucose responses

The mean fasting plasma glucose concentrations were within the fasting reference ranges for diabetics after the 10 - 12-hour fasting that preceded testing.

The only significant differences in blood glucose responses between the two test meals were found at 150 minutes and 180 minutes, while the highest variation after the glucose meal was found at 60 minutes and after the white bread meal, at 90 minutes. Wolever and co-workers[37] and Jenkins and co-workers[46] also found the highest glucose variation after 90 minutes in type 2 diabetic subjects after a white bread meal (50 g carbohydrate). Similar results for the highest variation at 60 minutes after a glucose meal (50 g carbohydrate) were also found by Krezowski and co-workers[47] and Indar-Brown and co-workers.[48] The differences in rate of digestion and absorption of the test meals could probably account for the different peak plasma glucose responses.

According to the CV calculated for every time point, no significant differences ($p > 0.05$) were found in variation at any time point between the two test meals. The areas under the blood glucose response curves were significantly higher ($p < 0.05$) after the glucose meal than after the white bread meal for the IAUC and the AUCO. Similar results were found by Indar-Brown and co-workers.[48] The mean between-subject CV for blood glucose responses according to the areas under the blood glucose curve for both test meals showed no significant difference in variation in the three areas that were calculated.

The mean variation for the IAUC calculated for both meals in the present study corresponds with those found by Wolever and co-workers in two separate studies.[35,37] However, higher between-subject CVs were reported in similar studies by Wolever and co-workers[35] and Rasmussen and co-workers[38] on type 2 diabetic subjects.

According to different researchers[49-51] the various factors that could influence the between-subject glycaemic responses to the same food are the following: presence of diabetes, type and treatment of diabetes, age, sex and race. The participants in the present study were all classified as type 2 diabetics according to a standard oral GTT, and were either treated by means of diet or with metformin. Gannon and Nuttal[36] concluded that the effect of the medication may vary from day to day, and might influence glycaemic and insulin responses. The treatment could therefore have influenced the between-subject results in the present study.

Although the reference range for age was wide in the present study, the sample range was quite narrow, representing small differences in age between the subjects. Rasmussen and co-workers[52] failed to show a significant influence of gender on glycaemic and insulinaemic responses in middle-aged type 2 diabetic subjects. The sample population represented one race group — it has been demonstrated that there is no significant difference in blood glucose response between different races.[53]

The GI values of white bread are higher than the GI values of glucose by a factor of 100/73 = 1.37, where 100 is the GI value of glucose and 73 is the mean GI value of white bread.[10] In the present study the mean GI calculated for the white bread was 83%, using the mean response to glucose as standard. The power calculations[54] for this study showed that to have 80% confidence that the bread will be in a 10% range, 24 - 128 subjects would be needed to determine the mean GI.

Between-subject variation in serum insulin responses

The mean fasting serum insulin of the subjects in the present study was elevated. According to Kahn and Weir[55] a high fasting insulin concentration indicates the presence of insulin resistance. The most common and important cause of insulin resistance is obesity. According to the sample range and mean BMI of the present study population, the subjects were either overweight or obese, which could therefore explain the high fasting serum insulin concentrations. A possible explanation for the insulin resistance in obese subjects is the reduced binding of insulin to target tissues and also that the release of FFA by omental fat into the portal circulation enhances gluconeogenesis and therefore interferes with insulin action on the liver.[55]

The variation seen in the fasting serum insulin was high for both test meals, with no significant differences between the two test meals. When compared with the fasting CV for plasma glucose, the CV for the fasting insulin was two to three times greater than for plasma glucose. Wolever and co-workers[31] concluded that the magnitude of fasting insulin fluctuations, expressed as percentages of the mean, are two to three times greater than those for plasma glucose and may explain why the CV of plasma insulin was two to three times that of fasting plasma glucose.

The peak insulin response was found 90 minutes after the white bread meal and 60 minutes after the glucose meal. According to Kahn and Weir[55] early insulin responses at 30 minutes in type 2 diabetics are often lower than those in non-diabetic subjects. This failure of early insulin release, coupled with poor suppression of glucagon secretion within the first 30 minutes, probably leads to enhanced hepatic glucose

production and resultant hyperglycaemia, which feeds back to stimulate insulin secretion.

In the present study the serum insulin response was significantly higher after the white bread meal compared with the glucose meal at 90 minutes, 120 minutes and 180 minutes. After 3 hours, the serum insulin was still elevated above the fasting value after both test meals. The elevated concentrations of both the plasma glucose and the serum insulin were expected, because 4 - 5 hours is required for plasma glucose to return to the fasting concentration, while the insulin concentration may still be moderately elevated 5 hours after ingestion of 50 g glucose in type 2 diabetics.[56]

According to the areas under the curve, the mean between-subject serum insulin response was significantly higher after white bread than glucose for all three areas calculated. The higher peak rise in serum insulin response at 90 minutes after the white bread meal could possibly have contributed to the greater areas under the curve.

When comparing the variation calculated for the different areas under the curve, small differences in variation were found after the white bread meal. However, the IAUC and AUCL were higher than the AUCO after the glucose meal. As pointed out by Wolever and Jenkins,[57] any differences in responses to foods will appear smaller if the absolute area (AUCO) is determined. In addition, differences in fasting glucose or insulin values may strongly influence absolute areas under the curve.[36] The variation between fasting glucose and insulin concentrations could therefore have influenced the variations seen for the areas under the curves.

The variations in serum insulin response found between the subjects in the present study could also be the result of the rate of starch digestion, the amount of rapidly available glucose and resistant starch, the degree of osmolality, the viscosity of the gut's contents[58] as well as variations in fasting insulin concentrations, BMI and drug treatment.[48] Another factor that may influence the postprandial insulin response is the postprandial blood glucose response, because Holt and co-workers[58] reported that glycaemic responses to different foods are predictors of the insulin responses, but account for only 23% of the variability in insulinaemia. Macronutrient (protein or fat, water, sugar and starch) content of foods is also a significant predictor of insulin response, but together these account for only another 10% of the variability found. Therefore according to Holt and co-workers[58] only 33% of the variation of insulin response between subjects can be explained.

Between-subject variation in fasting FFA concentrations

The mean fasting FFA concentrations between the subjects for white bread (0.6 mmol/l) and glucose (0.7 mmol/l) correspond with the results of Axelsen and co-workers.[59] Plasma FFA concentrations are raised in type 2 diabetics and may contribute to insulin resistance by reducing glucose oxidation and increasing hepatic glucose and triglyceride production.[45] Raised plasma concentrations reduce glucose uptake primarily by reducing glycogen synthesis, initially by reducing glucose transport or phosphorylation and later by reducing muscle glycogen synthase activity. Therefore, the mean fasting FFA concentrations in the present study could have contributed to the increased plasma glucose responses by inducing insulin resistance at the peripheral and hepatic level.[45] However, no significant correlation was found between the mean fasting FFA concentration and both the mean fasting serum insulin and plasma glucose concentrations.

No significant difference in between-subject CV for fasting FFA was found between the two test meals.

Occurrence of symptoms

According to Wolever and co-workers[31] starchy test meals are more palatable and acceptable than a glucose test meal. However, the present study did not show a significant difference in symptoms between the two meals. The hunger and dizziness occurred slightly more frequently after the glucose meal. However, in contrast to the study by Wolever and co-workers,[31] no other symptoms of stomach discomfort were recorded after the glucose meal in the present study.

Conclusions and recommendations

Information gained from this study with regard to the variation found within and between subjects highlights further questions and suggestions for future research. According to Wolever,[43] if the response to white bread is unusual in one subject, this will affect the GI of every other food taken by that subject, and increase the variability of the mean GI value for each food. Therefore, to obtain a representative value for the glycaemic response to white bread, Wolever[43] suggests that each subject be tested on white bread for an average of three times and that the mean value in GI calculations be used. According to the IAUC (used in GI calculations) in the present study, the blood glucose concentrations tend to be less variable after a glucose test meal than after a starchy test meal in type 2 diabetic subjects. This suggests that a glucose meal may be a more reliable standard food to use in GI calculations for type 2 diabetics. Since variations were found between the results for the different methods that were used to calculate the areas under the curve, it is clear that standardisation of the methods used to determine variation within and between subjects is of paramount importance if results of various researchers are to be compared. Therefore, according to these conclusions, further research regarding a standard test meal with least variation, which can be applied as a reference food in GI calculations for type 1, type 2 and non-diabetic subjects is recommended. To

accomplish this the results of the present study need to be compared with similar studies on type 1 diabetics and non-diabetic subjects.

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