Interindividual Variability and Intra-Individual Reproducibility of Glycemic Index Values for Commercial White Bread

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OBJECTIVE — We sought to assess the intra- and interindividual variability of glycemic index value determinations for white bread using glucose as the reference food.

RESEARCH DESIGN AND METHODS — A total of 23 healthy adults (aged 20–70 years) completed up to three sets of two visits per set. Each pair of visits assessed the glycemic response to 50 g available carbohydrates from commercial white bread and glucose, administered in random order. Glycemic index values were calculated by dividing the 2-h incremental area under the serum glucose response curve after each commercial white bread challenge by the mean area under the curve (AUC) for glucose.

RESULTS — The mean ± SE ratio of the AUC after white bread intake by the AUC after glucose intake for the first set of determinations was 78 ± 15 (n = 23; coefficient of variation [CV] 94%). When using glycemic index values calculated with the subset of participants who completed three sets of tests (n = 14), glycemic index values for each of the three sets of determinations were 78 ± 10, 60 ± 5, and 75 ± 10, respectively. CVs were 50, 28, and 50%, respectively. The mean glycemic index value of these three sets was 71 ± 6, with a CV of 30%. When an ANOVA approach was applied to these data, the interindividual CV was 17.8%, and the intra-individual variation was 42.8%.

CONCLUSIONS — These data suggest that in response to a challenge of white bread relative to glucose, within-individual variability is a greater contributor to overall variability than among-individual variability. Further understanding of all the sources of variability would be helpful in better defining the utility of glycemic index values.

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It has been recognized for the past 3 decades that carbohydrate-containing foods elicit widely different postprandial blood glucose responses (1,2). A system to classify foods on the basis of glycemic response, termed glycemic index, emerged to capture this information (3). The classification system has been endorsed for use as a tool to guide food choices to reduce chronic disease risk by some individuals and organizations (4–6) but not by others (7–9). Reluctance to universally recommend the system for use in formulating dietary guidance stems from a number of issues, some relating to uncertainties in reproducibility among people and variability in the composition and preparation of individual foods.

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Abbreviations: AUC, area under the curve; GRR, glycemic response ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The glycemic index value of a given food is determined by monitoring the incremental area under the curve (AUC) for blood glucose response over a 2-h period after feeding a 50-g carbohydrate portion of both a test food and standard food (50 g glucose or a 50-g carbohydrate portion of white bread) and expressing the data on a percentage of the test food relative to the standard food (6,10). By definition, any area below the fasting glucose concentration is not included in the AUC value. If white bread is used as the standard food, the glycemic index value for the test food is frequently corrected to glucose using a factor of 1.4 (6,10). An extensive compilation of glycemic index and glycemic load values derived from different laboratories worldwide is currently available (10,11).

Implicit in the recommendation to formalize glycemic index as a dietary guidance tool is the assumption that the glycemic response an individual has to a given food is similar among individuals regardless of metabolic and physiological factors. Guidelines for the determination of glycemic index values recommend that it be determined in six or more subjects and the values averaged (6). It is additionally recommended that the glycemic index value for more than one food ". . . be determined in one series of tests, for example, each subject might test four foods once each and the standard food three times for a total of seven tests in random order on separate days." It is further recommended that subjects should be studied on separate days in the morning after a 10–12 h overnight fast and a standard drink of water, tea, or coffee be given with each test meal.

There are limited data that have addressed the issue of reproducibility. Therefore, the current study was carried out to assess the interindividual variability (among individuals) and intra-individual reproducibility (within the same individual, when repeatedly measured) of glycemic index values for white bread, using glucose as the standard food, determined under controlled conditions.

RESEARCH DESIGN AND METHODS

— A total of 25 adults (aged 20–70 years) were recruited from

the greater Boston area. Exclusion criteria were as follows: *1*) known chronic diseases, including diabetes, untreated hypertension, irritable bowel syndrome or malabsorptive disorder, or established cardiovascular, kidney, or liver disease; *2*) smoking; *3*) BMI >35 kg/m^2; *4*) fasting glucose ≥7 mg/dl; *5*) abnormal blood chemistry or complete blood count; *6*) pregnancy or breastfeeding; *7*) alcohol consumption at >7 drinks/week; *8*) use of medications known to affect glucose metabolism (insulin, sulfonylureas, metformin, glucosidase inhibitors, thiazolidinedione insulin sensitizers); *9*) weight gain or loss ≥7 kg within 6 months before enrollment; and *10*) unwillingness to adhere to study protocol. Subject identification numbers were assigned sequentially in the order in which participants were enrolled. All study participants gave written informed consent. The study protocol was approved by the Human Investigation Review Committee of Tufts University-New England Medical Center.

All participants completed one set of two visits. A subset of participants completed three replicate sets (n = 14). Each set, or pair of visits, was used to assess the glycemic response to white bread and glucose, administered in random order. Visits within the same set were conducted no more than 7 days apart. One participant was withdrawn from the study because of poor venous access, whereas one participant was unable to complete a set of visits within 7 days because of unanticipated scheduling conflicts and was excluded from the analysis. During the testing period, participants were requested to maintain their habitual diet and physical activity patterns. Before each test day, participants were asked to fast and refrain from exercising 12 h before arrival at the Metabolic Research Unit at the Human Nutrition Research Center. At the beginning of the first test day, blood pressure, body height and weight, and waist and hip circumferences were measured using standardized procedures. Immediately thereafter, an intravenous indwelling catheter was placed in the forearm for blood drawing purposes, and a fasting blood sample was obtained. The test food (described below) was provided, and the participant was requested to consume the food within a period of 5 min, under observation, to mimic a bolus administration of the test food. Consistent with the recommended standardized protocol for nondiabetic individuals (3,6,12), additional blood samples were obtained 15, 30, 45, 60, 90, and 120 min thereafter. During the test time, participants remained in the Metabolic Research Unit under observation and were restricted to sedentary activities in their rooms.

For each set of visits, participants consumed 500 ml glucose solution (100 g/l; 50 g carbohydrate) and 96 g (50 g available carbohydrate) commercial white bread (Original White Bread; Pepperidge Farm, Norwalk, CT) with 500 ml water, in random order. Water, ad libitum, was available throughout the test period.

Biochemical measures

Blood was allowed to clot at room temperature for 20 min, and serum was subsequently separated by centrifugation at 1,100*g* at 4°C for 20 min. Glucose was measured using an enzymatic method (Olympus America, Melville, NY). The CV for the glucose determinations was 2%. Insulin was measured using a human insulin–specific radioimmunoassay kit (Linco Research, St. Louis, MO) (13). The CV for the insulin determinations was 5%. To characterize the study participants, baseline total, LDL, and HDL cholesterol and triglyceride concentrations were measured on a Hitachi 911 automated analyzer (Roche Diagnostics, Indianapolis, IN) using enzymatic reagents (14). The lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, GA.

Glycemic index calculations

The incremental blood glucose AUC was determined as previously described (12) by calculating the geometric sum of the areas of the triangles and trapezoids for the response over 2 h, excluding area that fell below initial fasting glucose concentrations. Glucose was used as the reference food to calculate the glycemic index of white bread. Glycemic index values were calculated by dividing the AUC obtained after the white bread challenge by that obtained after glucose intake, as previously described (3,6).

The evaluation of the variability of the glycemic response to white bread and glucose was assessed by comparing the data obtained from the first set of tests for all participants (n = 23). Glycemic response ratio (GRR) values were calculated by dividing the AUC value for the glycemic response to the first white bread challenge by the AUC value for the glycemic response to the first glucose challenge. GRR values differ from glycemic index values in that only one AUC for the reference food (glucose) was used instead of the mean of three values, used for the standard method (3,6).

The evaluation of the interindividual variability (among individuals) and intraindividual variability (within the same individual when repeatedly measured) of the glycemic index determination was assessed by comparing data from the subset of participants who were tested three times (n = 14). Glycemic index values were calculated by dividing the AUC value for the glycemic response to the first white bread challenge by the mean of the three AUC values after glucose intake. Glycemic index values for these comparisons were calculated by the standard procedures using separate individual AUC values for the glycemic response to white bread divided by the mean of the three AUC values after glucose intake, as previously described (3,6).

Statistical analyses

The data were entered into a spreadsheet and analyzed using SAS for Windows (version 9.1; SAS Institute, Cary, NC). Descriptive statistics and graphs (PROC UNIVARIATE and PROC MEANS) were used to summarize the overall effects of tests and distributions of the outcome measures. The overall intra- and interindividual variation was calculated through a variance component model (PROC VARCOMP). Data are presented in text and tables as means ± SD or SE, as indicated. Baseline characteristics of male and female participants were compared using independent samples t tests. Analyses were conducted at the 0.05 α level.

RESULTS — At baseline, blood pressure, anthropometric measures, and insulin and lipoprotein concentrations were similar between male and female participants (Table 1), whereas waist circumference, fasting glucose, and LDL cholesterol were greater in male than in female subjects ($P < 0.05$).

Figure 1 depicts the glycemic response ratios to white bread and glucose during the first set of tests for each participant (n = 23). Panels represent the response of each participant and are arranged in order of increasing GRR value from left to right and top to bottom. The mean GRR value was 78 ± 73 (SEM = 15; n = 23) with an interindividual CV of 94%. The mean serum glucose AUC for the white bread challenge was 2,135 ± 1,175 (SEM = 245; CV = 55%; n = 23), and the mean serum glucose AUC for the

Table 1—*Baseline characteristics of participants*

Variable	All participants	Male subjects	Female subjects	*P*
n	23	10	13	
Age (years)	42 ± 15 (23–70)	47 ± 14 (30–69)	38 ± 15 (23–70)	NS
Blood pressure (mmHg)	—	—	—	—
Systolic	116 ± 13 (89–142)	121 ± 12 (101–137)	112 ± 13 (89–142)	NS
Diastolic	71 ± 7 (54–81)	72 ± 8 (54–81)	71 ± 6 (62–80)	NS
BMI (kg/m^2)	25.8 ± 3.8 (20.3–32.5)	26.2 ± 3.2 (21.4–29.5)	25.8 ± 4.3 (20.3–32.5)	NS
Waist circumference (cm)	89 ± 13 (67–117)	96 ± 11 (80–117)	82 ± 11 (67–99)	0.02
Hip circumference (cm)	103 ± 9 (83–119)	102 ± 9 (91–119)	103 ± 10 (83–116)	NS
Fasting glucose (mmol/l)	4.8 ± 0.5 (3.8–6.0)	5.1 ± 0.4 (4.7–6.0)	4.5 ± 0.4 (3.8–5.2)	0.002
Fasting insulin (pmol/l)	67 ± 32 (10–171)	61 ± 22 (24–100)	72 ± 39 (10–171)	NS
HOMA*	2.06 ± 1.07 (0.27–5.58)	2.01 ± 0.79 (0.73–3.45)	2.09 ± 1.28 (0.27–5.58)	NS
Cholesterol (mmol/l)	—	—	—	—
Total	4.62 ± 0.83 (2.90–6.62)	4.76 ± 0.65 (3.85–5.79)	4.52 ± 0.97 (2.90–6.62)	NS
LDL	2.79 ± 0.67 (1.32–4.32)	3.06 ± 0.47 (2.30–3.85)	2.58 ± 0.74 (1.32–4.32)	0.01
HDL	1.45 ± 0.29 (1.07–2.15)	1.25 ± 0.13 (1.07–1.45)	1.60 ± 0.30 (1.11–2.15)	NS
Triglyceride (mmol/l)	0.86 ± 0.42 (0.45–2.09)	1.00 ± 0.40 (0.45–1.69)	0.76 ± 0.42 (0.49–2.09)	NS

Data are means ± SD (range) unless otherwise indicated. An unpaired *t* test was conducted for the comparison of male vs. female subjects. *Homeostasis model assessment (HOMA) = glucose (mmol/l) × [insulin (μU/ml)/22.5].

glucose challenge was 3,556 ± 1,686 (SEM = 351; CV = 47%; n = 23). The mean serum insulin AUC for the white bread challenge was 2,532 ± 1,591 (SEM = 332; CV = 63%; n = 23), and the mean serum insulin AUC for the glucose challenge was 2,945 ± 1,553 (SEM = 324; CV = 53%; n = 23) (see online appendix, located at http://dx.doi.org/10.2337/dc06-1598).

Figure 2 shows individual mean glycemic index values calculated for the subset of participants who were tested three times (n = 14). The mean glycemic index value for white bread calculated using AUC values from the first time in which white bread was consumed was 78 ± 39 (SEM = 10; CV = 50%; n = 14) (Table 2). The CV was smaller when the white bread was tested more than once (25% when tests one and two were included and 30% when the three tests were included). However, the range of glycemic index values obtained was broad (42–106 for the mean of tests one and two; 44–132 for the mean of the three tests) (Table 2, Fig. 2), suggesting that although the values may be valid for groups of people, there is some uncertainty when applying them to individuals, and it would be helpful to understand more about what contributes to this level of variability.

As shown in Table 2, there exists substantial variation in glycemic index values within each individual. For the mean of three tests, 50% (7 of 14) were at least 10 units away from the overall mean of 71, and 29% (4 of 14) at least 15. However, to calculate an estimate, we have averaged the individual CV from each subject (ranging between 7 and 75%).

To assess the components of variance within these tests, the overall interindividual variation was obtained from the PROC VARCOMP routine using the following model: $Y(ij) = u + S(i) + R(ij)$, where replicates (R) are nested within subjects (S). When the square root of the subject variance ($\sqrt{159.62}$) is divided by the mean glycemic index (70.69), the interindividual CV is 17.8%. The intra-individual variation was assessed using the same PROC VARCOMP routine, using the square root of the error term ($\sqrt{913.56}$) divided by the mean glycemic index to yield an intra-individual variability of 42.8%.

CONCLUSIONS — Despite the range of responses observed for individual subjects, the mean glycemic index value for white bread observed in the current study was 71 ± 22 (SEM = 6; CV = 30%, n = 14) (Table 2), which is virtually identical to the mean value used for reference purposes (10). However, the glycemic index values for white bread ranged from 44 to 132, with a CV of 30%. This value is consistent with the calculated CVs from other reports with healthy volunteers, which ranged from 12 to 53% (3,15–17). From a report evaluating the glycemic response to white bread (18), the mean among-subject CV for the AUC was calculated as 33% for subjects with type 2 diabetes and 39% for subjects with type 1 diabetes. Similarly, from a comparison of glycemic index values from different pasta foods in a sample of subjects with type 1 (57 ± 20, n = 6, CV = 34%) and type 2 (49 ± 23, n = 11, CV = 47%) diabetes, CVs were 34 and 47%, respectively (19). In another report (20), the mean among-subject CV of the AUC of the glycemic response was 25% for subjects with type 2 diabetes and 38% for subjects with type 1 diabetes.

The CV when the test was performed in triplicate ranged from 4 to 75%. Ramdath et al. (21) found significant differences ($P < 0.001$) in glucose AUC responses to white bread when the measure was repeated on three different occasions in subjects participating in a two-center study. In one of the few studies that reported repeated measurements (four times) within the same subject, the CV for white bread ranged between 4 and 46% (22). It has also been reported that the mean within-subject CV for the AUC of the glycemic response is 16% for patients with type 2 diabetes and 24 to 29% for patients with type 1 diabetes (20,23). The within-subject CV reported for the glycemic index value was of similar magnitude (16% for patients with type 2 and 25% for patients with type 1 diabetes).

In a study that assessed the variability in glycemic index value determinations among seven laboratories in normoglycemic volunteers (24), the CV of the mean glycemic index value for white bread ranged between 5 and 85%. Some sources of variability among laboratories in the

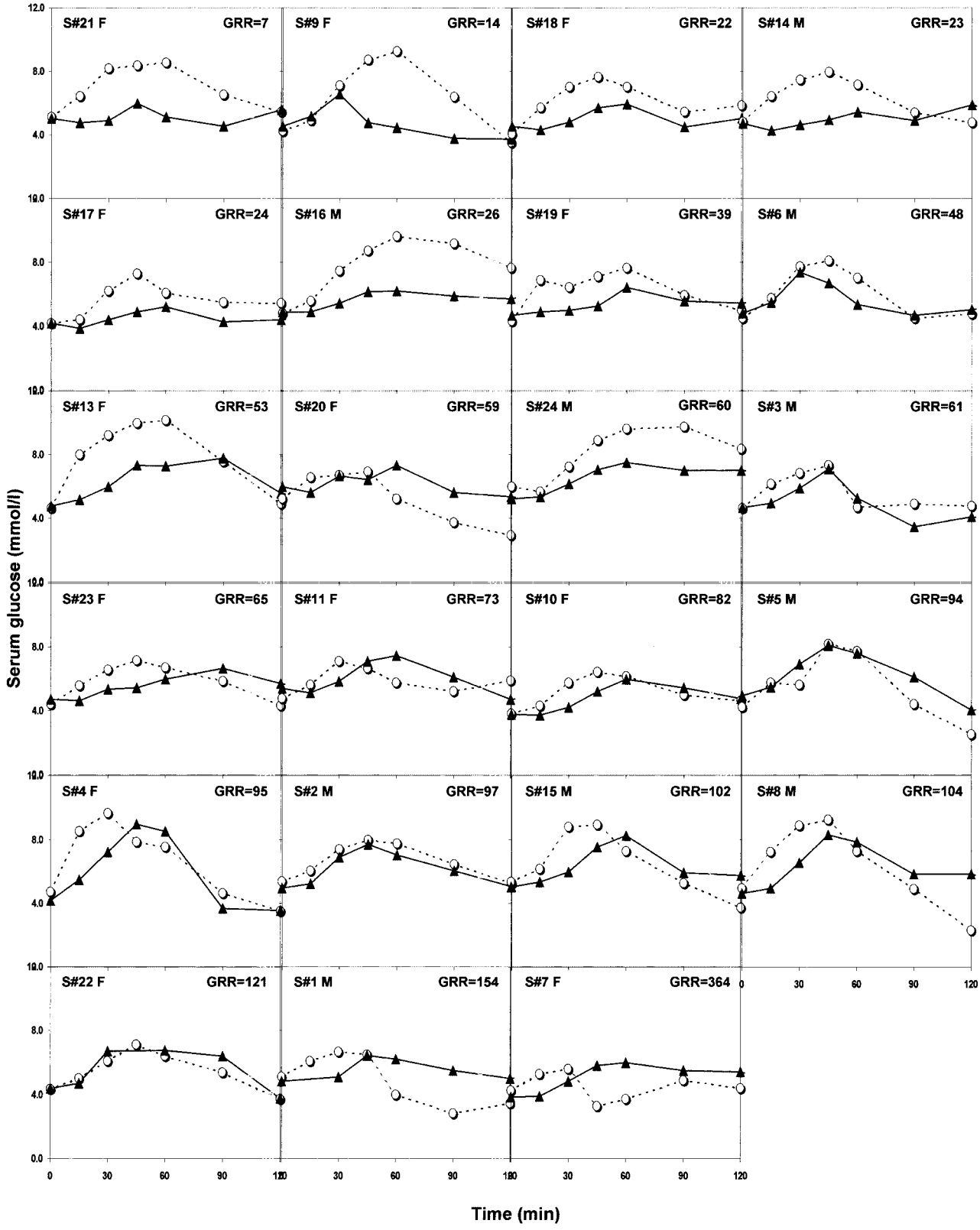

Figure 1—*Glycemic (postprandial glucose) response curves to white bread (▲) or glucose (○). Individual graphs arranged by increasing white bread GRR, denoted in upper right corner of each panel, from left to right and top to bottom. Participant number is denoted in upper-left corner of each panel. GRR = 78 ± 73 (SEM = 15; CV = 94%;* n *= 23). F, female; M, male.*

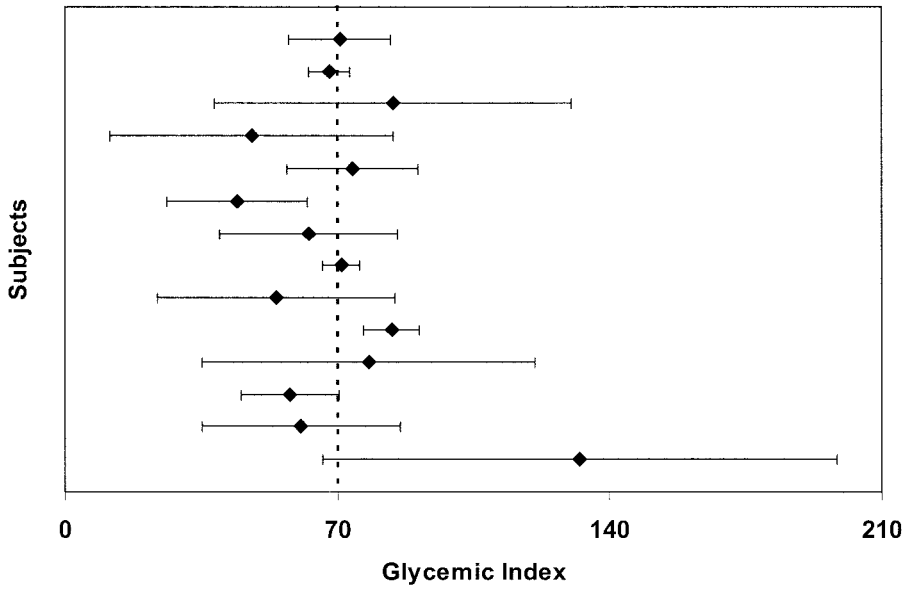

Figure 2—*Individual glycemic index values calculated for the subset of participants who were tested three times* (n = *14*). ◆ *represent the mean of three glycemic index determinations; horizontal lines represent ± SD.*

glycemic index value determinations noted were source of blood (venous vs. capillary), biological sample (whole blood vs. plasma), and method used for the glucose determinations (24). The former factor may have contributed to the CV observed in the current study.

The oral glucose tolerance test, widely used as an aid for diagnosis of diabetes, is also based on glycemic response to an oral glucose challenge. Due to concern about reproducibility (25,26), in the early 1990s, the American Diabetes Association shifted their recommendations and currently endorses the use of fasting plasma glucose concentrations for the diagnosis of insulin resistance and diabetes in the clinical setting (27). When variability of oral glucose tolerance test data were evaluated on the basis of age, sex, or obesity, no significant associations were identified (26).

The limitations of the current work are that the meals before the tests were not controlled, these initial studies did not assess a wider range of foods, and the habitual diet of the volunteers before the test days was not controlled. Variability may have inadvertently been introduced by using venous instead of capillary blood.

Table 2—*Glycemic index values for white bread and mean AUC for bread and glucose determined by measuring its glycemic response on three separate occasions*

	Test 1	Test 2	Test 3	Mean of tests 1 and 2	Mean ± SEM of 3 tests	CV(%) of 3 tests
Individual values	—	—	—	—	—	—
Participant 1 (M)	154	58	185	106	132 ± 38	51
Participant 2 (M)	89	53	40	71	61 ± 15	41
Participant 3 (M)	59	45	70	52	58 ± 7	22
Participant 4 (F)	126	44	64	85	78 ± 25	55
Participant 5 (M)	78	92	82	85	84 ± 4	8
Participant 8 (M)	86	52	25	69	54 ± 17	56
Participant 13 (F)	76	67	70	71	71 ± 3	7
Participant 15 (M)	81	37	70	59	63 ± 13	37
Participant 18 (F)	25	61	47	43	44 ± 11	41
Participant 19 (F)	56	77	89	67	74 ± 10	23
Participant 21 (F)	7	77	60	42	48 ± 21	75
Participant 22 (F)	121	33	99	77	84 ± 26	55
Participant 23 (F)	72	70	62	71	68 ± 3	7
Participant 24 (M)	56	75	81	65	71 ± 8	18
Mean ± SEM of GI	78 ± 10 (50)	60 ± 5 (28)	75 ± 10 (50)	69 ± 5 (25)	71 ± 6 (30)	
M subjects	86 ± 12 (38)	59 ± 7 (32)	79 ± 19 (65)	73 ± 7 (25)	75 ± 10 (36)	
F subjects	69 ± 17 (65)	61 ± 6 (27)	70 ± 7 (26)	65 ± 6 (25)	67 ± 6 (23)	

Data are means ± SEM or means ± SEM (CV) unless otherwise indicated; $n = 14$. Data obtained from participants who attended three sets of tests. CV values in the last column were calculated for the mean of the three tests. All values were calculated by dividing individual glycemic responses to white bread (AUC) by the mean glycemic response to three glucose challenges. GI, glycemic index; F, female; M, male.

Nonetheless, the conditions mimicked the standard protocol currently in use (3,6). The strengths of the current report are that the glycemic index value determinations were conducted using a single type and lot of white bread and followed a protocol that was as consistent as possible among volunteers. This included contracting the time during which the subjects consumed the 50 g carbohydrate; limiting the type of beverage that participants were allowed to consume during the test period (water) and the physical activities permitted during the 12 h before and during the test period; conditions of blood collection, processing, and analysis; and the general surrounds of environment of the test area (private room).

In conclusion, the level of interindividual variability and intra-individual reproducibility observed when the glycemic index for white bread was determined under controlled conditions was high despite efforts to standardize the study conditions and protocol as much as possible, yet the mean value was comparable with that previously published. When the intra- and interindividual variabilities were calculated using an ANOVA approach, it appears that the within-individual variability of 43% has a greater contribution to the overall observed variability than the among-individual variability of 18%. These data suggest that despite a reduction in the CV with replicate testing, glycemic responses to a single food, white bread, can be inconsistent, and a better understanding of the sources of this variability would be helpful in defining the utility of glycemic index values.

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