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More evidence for capillary sampling in the determination of glycaemic index

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Introduction and objective. The glycaemic index (GI) can be determined using capillary blood or venous plasma sampling. The aim of this study was to compare the GIs of three South African oat products determined using both capillary blood and venous plasma.

Methods. Twenty healthy, non-smoking male students (mean age 21.0 years, standard deviation (SD) 1.28 years, mean body mass index (BMI) 24.55, SD 2.62) volunteered to participate. Each subject received a standard pre-test meal the evening before test days. The reference food used was glucose, and the test meals were three oat porridges, namely Jungle oats (66 g dry weight), Bokomo oats (72.8 g) and Oatso Easy (101 g) in the form they are normally consumed, namely with milk (150 ml) and sugar (20 g), or water only in the case of Oatso Easy, an instant porridge. Each meal contained 50 g glycaemic carbohydrate as determined by the Englyst method. Capillary blood glucose and venous blood samples were taken simultaneously at 0, 15, 30, 45, 60, 90 and 120 minutes and areas under the curve (AUC) and GI were calculated.

Results. The mean fasting glucose value obtained from venous plasma before all tests ($N = 80$) was significantly lower (4.5 mmol/l, SD 0.7, $p < 0.002$) than the mean capillary blood glucose concentration (4.8 mmol/l, SD 0.04). The mean incremental AUCs obtained from venous plasma after all test meals and the reference (oral glucose) differed significantly ($p < 0.01$) from the AUCs obtained from capillary blood. The SDs for GIs calculated from venous plasma samples were at least 3 times larger than the SDs for GIs calculated from capillary samples. No significant differences were found between the GIs of the three oat products.

Conclusion. The present data are consistent with previous results showing that glycaemic responses measured in venous plasma are lower and more variable than those in simultaneously obtained capillary blood. The most precise and accurate determination of GI may be achieved using capillary rather than venous blood sampling, supporting the recommendation of the working group on the standardisation of GI testing in South Africa.

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 portion of food containing 50 g glycaemic carbohydrate expressed as a percentage of the area under the curve elicited by 50 g glucose taken by the same subject.[1] Scientific evidence that low-GI foods produce beneficial effects on glucose control, hyperinsulinaemia, insulin resistance, blood lipids and satiety has recently been reviewed.[2] Reliable GI tables compiled from the scientific literature are necessary to improve the quality of research examining the relationship between GI and health and the practical application of GI. The revised international table of GI values[3] includes more than 750 types of foods. However, the GI value of many common foods is unknown and different GI values for similar foods are often reported by different investigators. Differences in GI values of similar foods could be due to inherent botanical differences from country to country, different testing methods, or the effects of random variation.[4] Differences in testing methods include use of different types of blood samples (capillary whole blood or venous plasma) and different portions of foods (50 g of total carbohydrate rather than of glycaemic carbohydrate). According to a joint US Food and Agriculture Organization/World Health Organization (FAO/WHO) expert consultation,[1] capillary blood gives similar GI values to venous plasma. However, 7 GI testing laboratories around the world recently participated in a study[5] to determine the degree of variation in GI values when the same centrally provided foods were tested, and results showed that the GI values of foods are more precisely determined using capillary than venous sampling. The within-subject variation from the 2 centres using venous plasma (including our laboratory)

was twice that from the 5 centres using capillary blood.[5]

The GI of breakfast food is of special importance because the metabolic responses after breakfast may influence glucose and insulin responses after the subsequent lunch meal ('second-meal effect').[6] The blood-glucose response after a standard lunch was significantly flatter when a 'slow-release' starchy breakfast was consumed compared with a 'rapid-release' starchy breakfast.[6,7] A major disadvantage in this regard is the shortage of low-GI breakfast foods. Many common starchy foods such as bread products and breakfast cereals have high GIs.[8]

The aim of this study was to determine glucose concentrations in capillary blood and venous plasma after consumption of three South African oat products and to relate the results to the GI. The oat products were consumed as eaten by the majority of consumers in South Africa (i.e. with milk and sugar). Since the GI is an index of the blood glucose-raising potential of the glycaemic carbohydrate in foods, carbohydrates that do not provide glucose for metabolism, such as non-starch polysaccharides and resistant starch,[8] should not be included in the calculation of glycaemic carbohydrates. The Englyst method[9] was used in this study to determine 50 g glycaemic carbohydrate portions of the test foods.

Methods

Twenty healthy, non-smoking male students, aged 21.0 years (standard deviation (SD) 1.28 years), with a mean body mass index (BMI) of 24.55 (SD 2.62), were recruited to take part in the study. Previous work in our laboratory[10] indicated that if a 10% range for a GI of a food is sought with 80% confidence, between 24 and 90 subjects should be included in a study using venous plasma samples. The subjects stayed overnight in the Metabolic Unit of the Potchefstroom Institute of Nutrition and were studied after a 10 - 12-hour fast on 4 mornings over a 4-week period. Subjects consumed a standard pre-evening test meal (60% carbohydrate, low in fibre) to optimise carbohydrate metabolic enzyme induction and to standardise potential 'second meal' effects.[6] On the test days fasting blood samples were obtained, after which subjects randomly consumed a test meal within 10 - 15 minutes; the meal included either 50 g glucose powder dissolved in 300 ml water, or 66 g Jungle oats cooked with 450 ml water, 72.8 g Bokomo oats cooked with 413 ml water or 101 g Oatso Easy instant porridge prepared with 480 ml water. The test meals of Jungle and Bokomo oats were each served with 150 ml milk and 20 g sugar. Each meal supplied 50 g glycaemic carbohydrates. The Englyst method[11] was used to determine 50 g glycaemic carbohydrate (Englyst Carbohydrates Research and Services Ltd, Southampton, UK). Carbohydrate fractions included in the 50 g portion were free sugar glucose and rapidly and slowly available starch (expressed as glucose). All resistant starch fractions were excluded.

Venous blood samples were obtained by a registered nursing sister using an indwelling catheter placed in a forearm vein and kept open by flushing with 1 - 2 ml saline and heparin. The saline and heparin were cleared before each blood sample by withdrawing and discarding 1 ml of blood. Whole capillary blood glucose was measured by three experienced specialised technicians trained and standardised in measuring capillary whole-blood glucose using SureStep test strips and SureStep glucometers (Lifescan; Johnson & Johnson, Milpitas, Calif.). This procedure was done in strict compliance with the protocol recommendations of the manufacturer and good laboratory practice guidelines. Venous whole-blood and finger-prick capillary blood samples were taken simultaneously at 0, 15, 30, 45, 60, 90 and 120 minutes.

Venous blood was collected in sodium-fluoride tubes and centrifuged within 15 minutes to remove the plasma. Thereafter it was frozen at –80°C until analysis. Plasma glucose was measured in duplicate using the enzymatic colorimetric method (Randox, Cat. No GL 2614 for 2 x 500 ml reagent, Randox Laboratories Ltd, Antrim, UK). Standard laboratory techniques, apparatus and standard reference ranges were used for analysis of the plasma samples.

The study protocol was approved by the Ethics Committee of Potchefstroom University, and the participants gave written informed consent.

Statistical analysis

Statistical analysis was done using the Statistica 6.1 software package. Results are expressed as incremental areas under the glucose curves, ignoring the area beneath the fasting level[1] and GI. For the three different test meals the means, 95% confidence intervals (CIs), SDs, medians, 25th and 75th percentiles were calculated for the GI, taking the capillary whole-blood and venous plasma glucose into account. Area under the curve (AUC) values were log transformed to normalise the data. *t*-tests for dependent samples were used to compare fasting baseline capillary whole-blood glucose and AUC with fasting venous plasma glucose and AUC. Because the calculated GIs were not normally distributed, Wilcoxon's matched pairs test was used to compare the GIs obtained from capillary and venous blood samples. The Friedman analysis of variance (ANOVA) was used to determine whether the differences in GI between the three different test meals were significant.

Results

Fasting values and postprandial glucose response (AUC during 120 minutes) were measured after both venous and capillary blood sampling. The results are shown in

Table I. The mean fasting glucose value obtained from venous plasma before all tests was significantly lower (4.5, SD 0.7 mmol/l, $p < 0.002$) than the mean capillary blood glucose concentration (4.8, SD 0.4 mmol/l). The mean AUCs obtained from venous plasma after all test meals as well as the reference, differed significantly ($p < 0.01$) from those obtained from capillary blood. The venous peak values at 30 minutes were about 1.1 (0.6 - 1.3) mmol/l lower than the corresponding capillary values ($p < 0.05$, data not shown).

The individual GI measurements found in the present study are given in Table II and Fig. 1. There were no significant differences in GI between the three oat meals as indicated by the means and SD, using both venous and capillary sampling. After excluding outlying values (chosen as values more or less than 2 SD from the mean) there were still no significant differences in GI between the three oat meals (Table II).

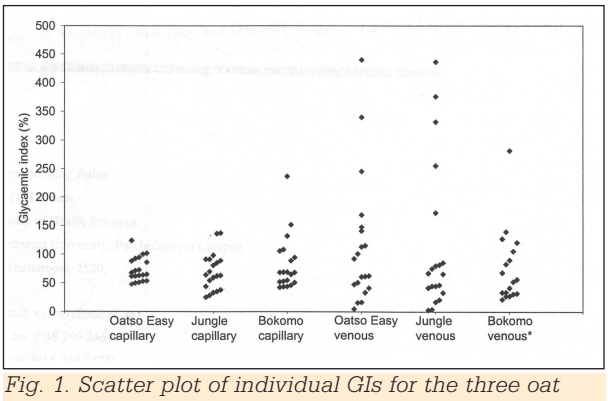

Fig. 1. Scatter plot of individual GIs for the three oat meals as measured when using capillary whole blood or venous plasma (two data points (675, 1 663) have been deleted from the GI values for Bokomo oats from venous plasma).*

The mean GIs of the three different oat meals as determined from capillary samples fell between the intermediate and high categories as defined by the draft Regulations Relating to Labelling and Advertising of Foodstuffs in the Foodstuffs, Cosmetics and Disinfectants Act of 1972 (Act No. 54 of 1972) which was published for comment on 8 August 2002. However, the median GIs of the three oat meals all fell in the intermediate category except for the GI of the Oatso Easy meal calculated from venous plasma, which fell in the high category. However after the exclusion of outliers, the median GIs of all three meals fell in the intermediate category.

The mean GIs of the oat meals, obtained from venous samples (115, 114 and 187 for Oatso Easy, Jungle and Bokomo oat meals respectively) were above 100, the value of oral glucose. The large SD from the mean venous plasma glucose values is reflected by individual GIs of 256 (subject No. 1, Jungle oats meal), 332 and 698 (subject No. 5 for Jungle and Bokomo oats meals, respectively), 340 and 376 (subject No. 7 for Oatso Easy and Jungle oats, respectively), 436 (subject No. 9 for Jungle oats) and 440 and 1 664 (subject No. 15 for Oatso Easy and Bokomo oats, respectively). These high GI values resulted from relatively low AUCs obtained from venous samples of these subjects after the reference test (results not shown).

Discussion

The main observation from the present work was the significantly greater AUC for the three oat meals and reference test when glucose values were obtained from capillary blood compared with venous samples. Although the GI values calculated from capillary blood samples were much lower than in the case of venous sampling, the difference was not significant owing to the large SD (Table II). The results correspond with those of Granfeldt *et al.*,[12] who reported significantly lower AUCs for venous samples (33 - 40%, $p < 0.05$) than the corresponding areas for capillary samples, but no differences in GI values.

Current recommendations are that capillary blood sampling is preferred for determining GI, but it is acceptable to use venous blood sampling.[1] However, more recently an interlaboratory study[5] found that GI values are more precisely determined in capillary blood because venous plasma values are lower and more variable than those of capillary blood. The SD of GI values with capillary blood was on average 25 (range 20.6 - 32.5 between the five centres), compared with an average SD of 42 for venous samples (40.3 - 44.6 for the two respective centres). The results of the study reported here confirm that capillary blood is the preferred measurement for determining GI.

In the literature, mean GIs are normally reported. Since the GIs of the three test meals were not normally distributed, statistically it would be more correct to report the median GIs and to base interpretation of results on medians rather than means. The discussion therefore focuses on median GIs after exclusion of outliers.

Venous glucose responses may be more variable than capillary responses for several reasons, as discussed by Wolever *et al.*[5] Blood glucose concentrations oscillate on a minute-by-minute basis,[13] partly as a result of pulses of insulin secretion.[14] The oscillations of blood glucose in different tissues in the body are presumably not in phase with each other, because it takes different lengths of time for the insulin pulses from the pancreas to reach them. The magnitude of the oscillations in the forearm venous blood may be greater than those in capillary blood because the vein drains a small volume of tissue with insulin oscillations in phase with each other while arterial blood is derived from all tissues in the body with insulin oscillations out of phase with each other, dampening the glucose oscillations in

Table I. Mean fasting and postprandial glucose characteristics (mean ± SD)

Glucose response during 120 minutes	Oatso Easy	Jungle oats	Bokomo oats	Oral glucose
Fasting concentration (mmol/l)				
Venous plasma	4.3 ± 0.5*	4.4 ± 0.6*	4.7 ± 0.7*	4.6 ± 0.9*
Capillary blood	4.8 ± 0.3*	4.7 ± 0.4*	4.8 ± 0.4*	4.8 ± 0.3*
Area under curve (mmol/l/min)				
Venous plasma	94.2 ± 68.7*	76.8 ± 53.9*	95.5 ± 83.3*	138.6 ± 118.2*
Capillary blood	126.1 ± 45.2*	122.8 ± 72.0*	130.1 ± 51.2*	176.3 ± 62.1*

*Values in capillary blood are significantly different from corresponding values in venous plasma ($p < 0.01$).

Table II. Individual GI values and descriptive statistics for the three meals as calculated using capillary blood and venous plasma

	GI from capillary whole blood			GI from venous plasma		
Subject No.	**Oatso Easy**	**Jungle oats**	**Bokomo oats**	**Oatso Easy**	**Jungle oats**	**Bokomo oats**
1	100.06	69.79	131.90	101.08	255.74	90.51
2	71.10	85.24	68.19	140.89	80.11	121.01
3	85.91	62.33	46.26	92.49	44.89	83.23
4	88.12	59.32	89.01	147.60	75.21	139.67
5	61.85	43.98	151.58	169.01	332.25	697.99
6	52.89	64.04	94.61	15.86	44.84	27.56
7	101.72	80.65	68.86	340.02*	376.17*	282.27
8	94.44	38.00	68.81	61.75	21.11	21.79
9	64.78	98.25	43.54	50.94	436.26*	127.54
10	50.73	54.45	64.81	33.56	17.20	27.76
11	72.51	89.12	52.16	47.57	46.76	41.70
12	47.57	136.94*	108.35	16.70	4.27	52.55
13	62.83	35.32	54.74	115.42	2.78	34.02
14	53.52	63.14	43.90	113.14	172.96	31.02
15	123.89*	25.25	236.89*	440.22*	82.11	1 663.71*
16	63.57	91.48	42.29	60.86	85.71	32.35
17	61.29	33.18	53.00	41.52	65.82	56.68
18	49.80	28.52	51.01	4.83	33.49	34.07
19	67.58	136.07*	105.75	245.65	41.77	105.97
20	92.42	91.4	68.25	62.63	67.28	68.31
N	20	20	20	20	20	20
Mean	73.3	69.3	82.2	115.1	114.3	187.0
SD	21.0	32.2	47.6	112.3	130.0	379.1
–95% CI	63.5	54.2	59.9	62.5	53.5	9.5
+95% CI	83.2	84.4	104.5	167.6	175.2	364.4
Median	66.2	63.6	68.2	77.6	66.5	62.5
Lower quartile	57.4	41.0	51.6	44.5	37.6	33.2
Upper quartile	90.3	90.3	100.2	144.2	129.3	124.3
N	19	18	19	18	18	19
Mean	70.7	61.9	74.1	84.5	81.9	109.3
SD	17.8	23.9	31.5	62.6	87.4	155.5
–95% CI	62.1	50.0	58.9	53.4	38.4	34.3
+95% CI	79.2	73.7	89.2	115.6	125.4	184.2
Median	64.8	62.7	68.2	62.2	56.3	56.7
Lower quartile	53.5	38.0	51.0	41.5	33.5	32.3
Upper quartile	88.1	85.2	94.6	115.4	82.1	121.0

* Outliers defined as > 2 SD of the mean.
N = number of subjects; SD = standard deviation; CI = confidence interval.

capillary blood.[5] There is also a small analytical error associated with measuring glucose and this has a larger proportional effect on the AUC when the rise in glucose is small. For example, a 0.1 mmol/l difference in the fasting glucose concentration results in a 12 mmol/l/min difference in the AUC over 2 hours, which is 20% of an AUC of 60, but only 6% of an AUC of 200.[5]

The median GI of the oat meals (65, 63 and 68 for Oatso Easy, Jungle oats and Bokomo oats as obtained from capillary sampling and after excluding the outliers) is in accordance with data from Jenkins *et al.*,[15] who reported a mean of 69 for muesli, and Granfeldt *et al.*,[12] who reported 65 for boiled rolled oat porridge, calculated with glucose as the reference food. The mean ± SD GI of oat porridge as determined in 8 studies included in the most recent international tables[3] is 58 ± 4 (range 42 - 75) and between 65 and 66 for instant oat porridge. The high metabolic response to oats is surprising, as rolled oats contain viscous beta-glucans. Granfeldt *et al*.[12] and Liljeberg *et al*.[16] did not find any significant effect of the naturally occurring beta-glucans in oat products on GI. However, the GI of oat bran breakfast cereal may be reduced by enrichment with additional beta-glucan and sweetening with fructose[17] or by making the porridge from boiled oat kernels (keeping the structure more intact).[12] We tested the three oat products in the form in which they are usually eaten by the majority of consumers, namely with 150 ml full-cream milk and 20 g sugar (sucrose), or water only in the case of Oatso Easy. The GI of milk (27 ± 4) and sucrose (68 ± 5)[3] might have had an effect on the overall GI of the meals. It has been shown previously that 15 g sugar added to dried cooked butter beans, a low-GI food, increased the GI from 29 to 54.[18] In the present study, sucrose (GI 68) did not change the GI of the Bokomo oats meal (68). For the Jungle oats meal, however, the GI tested as 63. Forty per cent (20 g) of the meal came from sucrose and 60% (30 g) from oats. Calculations show that the estimated GI of Jungle oats is 60, if the GI of sucrose is taken to be 68.

Blood glucose responses vary considerably from day to day within subjects.[1] The mean AUC of three trials of the reference food should therefore be used to calculate the GI because the mean of three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial.[1] In this study the reference food was tested only once. This was an important limitation of the study. While measuring the reference food more than once undoubtedly improves the accuracy of the GI the fact remains that the GI of a particular food will still have a large variation. In previous work in our laboratory using venous blood sampling, the reference food (oral glucose) was administered four times and the mean used to calculate the GI of white bread consumed on four occasions by the same 9 subjects.[10] The GI of bread still varied from 46% to 77%, after using the mean of four measurements for the reference food.

Conclusions and recommendations

The results reported here are consistent with previous results showing that glycaemic responses measured in venous plasma are lower and more variable than those in simultaneously obtained capillary blood[5] and support the recent recommendation of the working group on the standardisation of GI determination in South Africa, namely that capillary rather than venous blood sampling should be used for GI testing.

It is further recommended that in using GI to choose carbohydrate foods, patients and consumers should be made aware of the fact that physiological responses to a food may vary between individuals. Therefore, if a food is indicated as having a high GI it does not mean that it will have a high GI in everybody. It is quite normal for that food to have a high GI in some individuals and a medium or even a low GI in others.

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