A COMPARISON OF THE GLYCEMIC INDEX (GI) RESULTS OBTAINED FROM TWO TECHNIQUES ON A GROUP OF HEALTHY AND A GROUP OF MIXED SUBJECTS

by

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Abstract

This comparative study was conducted in two parts.

Part 1

Objectives

- To compare the mean GI-values of two foods, i.e. Muesli (M) and Apple juice (A) from a mixed group of subjects (healthy, type 1 and type 2 diabetic), using the Medisense Precision QID Glucometre (MPQIDG) extra-laboratory (EL) to the mean GI-values of the same two foods from a group of healthy subjects intra-laboratory (IL), using laboratory equipment (YSI Analyser or YSI) and the MPQIDG, and determine whether the former method is an acceptable alternative for the latter.
- To compare the Area under the curve (AUC), their means and GI values of each healthy subject, using the YSI and MPQIDG (IL).

Subjects and Methodology

A group of 12 trained subjects, aged 29-54 years (41 ± 7), BMI 18-30 kg/m^2 (24 ± 4), were tested IL (Group 1) under well-controlled conditions, as recommended by the FAO/WHO (1998).[3] M and A were each tested once and the reference food (glucose) was tested on 3 occasions, using the MPQIDG and the YSI. Capillary blood glucose was measured fasting and every 15min for 2h, after the glucose/test food was consumed (diabetic subjects measured blood glucose concentrations over 3h).

Mean GI-values of M and A, obtained IL by Group 1 were compared to the mean GI-values of M, obtained EL by a mixed group of subjects (Group 3), and of A, obtained EL by another mixed group of subjects (Group 4), and the mean GI-values of M and A, obtained EL by the group of 12 healthy subjects (Group 2), using ANOVA. The AUC and GI values of each healthy subject of Group 1 were compared using Pearson's correlation coefficient (r). Lin's concordance correlation coefficient (r_c) was used for testing agreement (4). Statistical significance was set at $p=0.05$.

Results

The mean GI-values of M and A, as determined by Groups 1, 2, 3 and 4 (using ANOVA) did not differ significantly for M ($p=0.2897$) and A ($p=0.8454$).

Pearson's **correlation coefficient** (r) was acceptable and significant for the AUC-values of Glucose 1 ($r=0.7$; $p=0.0081$), good and significant for the GI-value of A ($r=0.8$; $p=0.0043$) and very good and highly significant for the AUC-values of Glucose 2 ($r=0.9$; $p<0.0001$), Glucose 3 ($r=0.9$; $p<0.0001$), M ($r=0.9$; $p<0.0001$) and A ($r=0.9$; $p<0.0001$) and the GI-value of M ($r=0.9$; $p=0.0003$), respectively, after removal of outliers. The mean AUC$_{MPQIDG}$ of all the foods tended to be higher than the mean AUC$_{YSI}$, after removal of outliers, but this was not significant ($p=0.69301$; $p=0.20838$; $p=0.43311$; $p=0.32926$; $p=0.49199$ for Glucose 1, 2 , 3 M and A, respectively).

Lin's concordance correlation coefficient [which tests reproducibility/agreement (r_c)] was acceptable for the AUC-value of Glucose 1 ($r_c=0.7$) and the GI-value of A ($r_c=0.7$), good for the AUC-value of Glucose 2 ($r_c=0.8$) and very good for the AUC-value of Glucose 3 ($r_c=0.9$), M ($r_c=0.9$) and A ($r_c=0.9$) and the GI-value of M ($r_c=0.9$), respectively, after removal of outliers.

Part 2

Objective

To compare the GI-values from a mixed group of subjects using the MPQIDG, EL with GI-values from a group of healthy subjects IL, using laboratory equipment laboratories A-E who took part in an inter-laboratory study[39] and determine whether the former method is an acceptable alternative for the latter.

Subjects and methodology

A mixed group of 10 trained subjects (5 male: 2 diabetic and 3 healthy; 5 female: 3 diabetic and 2 healthy) were tested EL under well-controlled conditions, as recommended by the FAO/WHO (1998)[3] The test foods were White bread, Barley, Rice, Instant potato and Spaghetti (reference food: glucose). Capillary blood glucose concentrations were measured fasting and every 15min after the glucose/test food was consumed for 2h (healthy subjects) and 3h (diabetics), using the MPQIDG. Mean GI-values were compared to the weighted mean GI-values of the 5 laboratories (laboratories A–E), using the analysis of variance (paired t-test). The standard error (SE), confidence interval (CI) width and mean GI-values of each of the laboratories for each of the five foods were compared to the weighted means of the rest of the laboratories, for these parameters. The mean GI-values were also compared using ANOVA.

Results

The GI-values of all five foods for each laboratory were compared to the weighted means of the rest of the laboratories (including EL). There was no significant difference for laboratories A and B. There were significant differences ($p \leq 0.05$) for one of the foods for laboratories D, E and EL and three of the foods for laboratory C, respectively. The results of the ANOVA test for variance confirmed these findings.

Conclusion

Using a mixed group of subjects and the MPQIDG to conduct GI-tests EL seems to be an acceptable alternative to using a conventional group of subjects and laboratory equipment, IL.

Key words

GI methodology, Glycemic Index, GI-values.

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LIST OF ABBREVIATIONS

ADA	- American Diabetes Association
AOAC	- Association of Official Analytical Chemists
AUC	- Area under the curve
AUC_{min}	- Area under the curve (minimum)
AUC_0	- Total area under the curve
BMI	- Body Mass Index
CHD	- Coronary Heart Disease
CHO	- Carbohydrate(s)
CV	- Coefficient(s) of variation
CVD	- Cardiovascular disease
e.g.	- for example
EL	- Extra-Laboratory
FBG	- Fasting blood glucose
FCT	- Food composition tables
FFA	- Free Fatty acid(s)
g	- grams
GI	- Glycemic Index
GL	- Glycemic Load
GTT	- Glucose Tolerance Test
HbA1c	- Glycated hemoglobin
HDL	- High-density lipoprotein
h	- Hour(s)
HRT	- Hormone Replacement Therapy
IAUC	- Area under the curve (above fasting baseline) or Incremental Area under the curve
i.e.	- that is
ID	- insulin response diet
IGT	- Impaired glucose tolerance
IL	- Intra-laboratory
ILSI	- International Life Sciences Institute
kJ	- kilojoules
LDL	- Low-density lipoprotein
LPL	- Lipoprotein lipase
min	- minutes
mm	- millimetres
mL	- millilitres
MPQIDG	- Medisense Precision QID glucometre
MUFA	- mono-unsaturated fatty acids
N/A	- Not applicable
ND	- isoenergetic, balanced diet
NEFA	- Non-esterified fatty acids
NSP	- Non-starch polysaccharides
PUFA	- Poly-unsaturated fatty acids
RAG	- Rapidly available glucose
RER	- Respiratory Exchange Ratios
RS	- Resistant starch

SA   - South Africa(n)
SAG   - Slowly available glucose
SCFA   - Short Chain Fatty Acids
SD   - Standard Deviation
SE   - Standard Error
SMBG   - Self-monitoring of blood glucose
TG   - Triglyceride(s)
VLDL   - Very low- density lipoprotein
vs.   - versus
y   - year(s)
YSI   - Yellow Springs Instruments

1. BACKGROUND TO THE STUDY

1.1 The Glycemic Index and its use

The Glycemic Index (GI) is a classification of the blood glucose raising ability of carbohydrate (CHO) rich foods. It is defined as the area under the blood glucose response curve (AUC) elicited by a 50 grams (g) available/glycemic CHO portion of a food in a specific subject, expressed as a percentage of the mean AUC after consumption of 50g CHO from a standard/reference food, tested by the same subject on three different occasions.[1,2,3]

During the 70's it was shown by researchers that not all CHO foods have the same effect on blood glucose levels [4] and after the introduction of the GI as a method of classifying CHO according to their glycemic response [1], it was noted that the observed glycemic response after consumption of a food becomes lower as the GI of that food product lowers.[5]

The positive effects of low GI diets have been reported in 15 studies from all over the world, such as the UK, Sweden, France, Canada and Australia [6] and cannot be ignored. Evidence from prospective or epidemiological studies, based on prospective cohorts, have shown that low GI diets are associated with reduced diabetes risk in men and women [7,8], reduced cardiovascular disease (CVD) risk [9] and reduced cancer risk.[10] In short-term intervention studies or clinical trials in humans, low GI diets have been shown to cause a statistically significant ($p \leq 0.05$) improvement in glycemic control over 12 weeks in type 2 diabetic subjects [11] and an improvement in lipid and glucose metabolism over 3–6 week periods in type 1 diabetic subjects [reported by Fontvielle et al (1988) and Collier et al (1988)], as measured by glycated albumin or hemoglobin (HbA1c) [2,11]; a significant reduction in total and LDL (low-density lipoprotein)-cholesterol and triglyceride (TG) levels over one month in hyperlipidemic subjects [12]; a significantly ($p<0.001$) lower HOMA index (a measure of insulin sensitivity) in middle-aged men with one or more cardiac risk factors over 24 weeks (when compared to a high GI diet) [13]; and reduced body weight and food intake, as well as a greater fall in insulin resistance, which could only be attributed to weight loss.[14] A short-term (9 weeks) intervention study in animals has also shown better glycemic and insulinemic control, higher plasma adiponectin (a newly identified risk factor for type 2 diabetes) and lower TG concentrations, less disruption of beta-cell architecture and significantly less body fat ($p=0.015$) and more lean body mass ($p=0.12$) in young growing rats on a low GI rather than a high GI diet.[15] This was confirmed in a long-term (32 weeks) intervention study by Pawlak et al (2000) on young adult rats, in which those placed on a low GI diet had a 16% lower weight and significantly lower fat mass ($p \leq 0.05$) than those placed on a high GI diet.[16] The GI also has relevance for sports performance, where lower GI CHO foods are the preferred choice before prolonged exercise, whereas higher GI foods and drinks are considered beneficial during prolonged exercise [17]; as well as after exercise [18]; appetite control through higher satiety [19,20] and memory, as found by Benton et al (2003).[21] Liu et al (2000) found that classifying CHO according to GI was a better predictor of CVD risk than classifying them as either simple or complex.[9]

These findings prompted the Joint FAO/WHO Expert Consultation Group on carbohydrates in Human Nutrition (1998) to recommend the consumption of a high CHO diet [that is (i.e.) $\geq$55% of total energy], most of which should come from low GI foods and that foods can be ranked as low, medium or high GI [3], confirming the recommendation made by Jenkins and co-workers in 1981.[1] The European Association for the Study of Diabetes, the Canadian Diabetes Association and the Dieticians Association of Australia have all recommended high fibre, low GI foods for individuals with diabetes as a means of improving postprandial glycemic and weight control[22] and in countries like France, Sweden, Canada, Australia and South Africa (SA), the GI concept has been incorporated into the dietary guidelines given by health professionals.[21]

1.2   Substantiation of the research

In the past it was assumed that complex CHO (containing mainly polysaccharides or starches) had a smaller effect on blood glucose levels than simple CHO (containing mainly mono-, di- and oligo-saccharides), which was assumed to have a major effect on blood glucose levels. It was also believed that the former contained significant amounts of other nutrients, including fibre, whereas the latter were generally not nutrient-rich, but mainly sources of "empty calories". However, this was a major oversimplification of CHO, and their effect on blood glucose levels, was inaccurate [23] and was mainly based upon an experiment done by Frederick N. Allen (1910), when starch or sucrose was given to totally pancreatectomized diabetic dogs. The dogs' blood glucose levels only rose after the ingestion of sucrose, because the animals were lacking the exocrine pancreas and were therefore unable to absorb significant amounts of the CHO from starch.[24]

CHO sources that elicit a low glycemic response generally have a low GI and are not the same as foods that are high in complex CHO and fibre, and those that elicit a high glycemic response are generally high GI and not necessarily high in simple sugars. The foods that produce the highest glycemic responses include many of the starchy foods consumed in the typical western diet, including refined bread, highly processed breakfast cereals and potatoes, whether these foods are high or low in fibre.[4,25] The human diet contains many different types of CHO, which are all digested and absorbed at different rates and to different extents [4,26] and therefore it is not merely the amount of CHO that determines glycemic response, but more so the rate of CHO digestion and absorption.[5] Crapo et al (1977) went one step further, claiming that it was mainly the absorption, and not so much intraluminal digestion, that was the rate-limiting step in over-all assimilation, especially of starch.[4] There are many factors that influence the effect that different CHO foods have on blood glucose levels and not merely the presence of sugar (refer to 2.4.3). It was found that the GI values of foods containing naturally occurring sugars were not significantly different from those of foods containing added sugars and that there was no significant difference between blood glucose responses to sugar containing vs. sugar free confectionery.[27] The fasting blood glucose (FBG) and insulin levels of healthy men and women were also not affected by the consumption of 50–107g sucrose per day, for a period of one to two weeks.[28]

Jenkins et al (1987) showed that there was no significant difference in the glycemic response curves and GI values of maltodextrin, a cornstarch hydrolysate consisting of 22 glucose units (GI=109±11) and corn syrup, which contains polymers of six glucose units (GI=113±7). Classification of foods as simple or complex CHO does, therefore, not appear to be physiologically useful and should no longer be used to indicate differences between the rate of digestion, absorption and glycemic responses of sugars and starches. Although many factors play a role in determining the glycemic response of CHO foods, the degree of complexity in terms of polymeric chain length did not appear to be one of them.[29]

As gastrointestinal digestive and absorptive processes do not seem to treat all starches identically, grouping all complex CHO together may cause confusion in the evaluation of epidemiologic and other study findings and may also contribute to less effective and less precise diet therapy.[4] Seeing that it has also become clear during the 20th century that different foods that contain the same amount of CHO have different effects on blood glucose and insulin responses [1,4], the CHO exchange lists, that have been used to plan diabetic diets for more than 30 years (y), may not be sufficient anymore for this purpose, as they do not reflect the physiological effect that the same amount of different CHO sources have on blood glucose levels.[1]

Another system is therefore needed to classify CHO rich foods instead of using the terms simple and complex CHO, which has become obsolete and should not be used anymore.[3,23] Jenkins et al (1981) proposed a classification of CHO rich foods based on the effect of specific foods on blood glucose levels,

in comparison to a standard food. This is called the glycemic index (GI)[1] and was also recommended as a useful indicator of the impact that different foods have on blood glucose levels and to be used to compare foods of similar composition within food groups.[3]

GI values are now available for a substantial number of CHO foods.[22,30] Including the GI in food labeling may eliminate problems with understanding of the terms "complex and simple CHO". However, to implement a well-balanced low GI diet, a much wider range of low GI products will have to be available, particularly for breakfast cereals and breads [23], which constitutes 50% of the CHO of the typical western diet [25] and currently few low GI breads, muffins, scones and breakfast cereals are available on the market.[23] A challenge to the food industries, therefore, exists to develop new and palatable low GI foods that also have to be tested for their GI [23,25], as many people see low GI foods as less acceptable, e.g. legumes and heavy breads. This will give food companies a new marketing edge with long-term benefits to public health.[25]

Only foods and beverages that are rich in CHO should be labeled, as labeling low CHO foods for GI is not meaningful. It was suggested that only foods containing 10g CHO per 100g of food or those that supply 40–50% of energy from CHO, should be labeled.[23] However, according to the draft SA regulations, only food products with a CHO content of 40% or more of total kilojoules (kJ) and that do not comprise more than 42% of total kJ as protein and/or 30% as fat, will be allowed to be labelled in categories of low, intermediate or high GI. Said regulations will only allow labelling of a product with the words "legal/suitable for diabetics", if the product is indeed low GI and has a reduced fat content as well. The claim "sugar free" or "contains no added sugar/sucrose" will not be allowed if the product contains any high GI sweeteners, e.g. maltodextrin, and there will be a compulsory listing of the GI range on food products that bear these claims.[31]

However, the GI values of many SA foods have not been determined yet and conducting GI testing in a laboratory set-up, when the GI values of many foods have to be determined, is very expensive and time consuming, which could be regarded as impractical for the food industries and the clinical world, as well as inconvenient for test subjects. For many years both healthy and diabetic subjects have been used as test subjects in GI tests [22,30] The glycemic response to different CHO foods are similar in healthy and diabetic subjects [5] and there seems to be no significant difference between GI values obtained using healthy versus (vs.) diabetic subjects.[1,19,32,33] However, diabetic and healthy subjects are not usually used in the same GI test teams, but rather either healthy or diabetic subjects are used.[22,30]

Glucose metres (glucometres) have begun to be used to measure glycemic responses and GI values of foods, as they are inexpensive, convenient to use, require little training and yield quick results. The One Touch Ultra (OTU) glucometre was recently evaluated against a reference technique or gold standard [Yellow Springs Instruments Analyzer (YSI)], by comparing the AUC and GI values of seven potato meals in healthy subjects. The OTU showed more variation and did not agree well with the values obtained using the YSI. The OTU was therefore not recommended for determining AUC or GI values in healthy subjects. The researchers, however, concluded that this did not necessarily apply to other glucometres and recommended that the performance of these meters should be evaluated.[34]

The Medisense Precision QID glucometre (MPQIDG) has been found in an independent head to head comparison conducted by the International Diabetes Institute (Melbourne, Australia) on many glucometres, to show the smallest difference from the reference method (YSI) across a whole range of mean glucose levels.[35] The coefficient of variation (CV) of the new test strip of the MPQIDG ranged from 2.1-5.6% over a range of blood glucose readings from 2.2-26mmol/L for 80 replicate tests across four lot numbers, and fell mostly within the recommended level of 3-5%, with the only value above 5%

being that for very low blood glucose readings.[36] Ideally, methods with a CV<3% should be used for scientific purposes [21], such as the YSI.[37]

In the light of the new SA draft regulations [31], many food companies need to have the GI values of their foods determined and the number is likely to increase once the draft legislation is accepted and legislated. This task will be simplified if the MPQIDG can be used Extra-laboratory (EL) to conduct reliable GI tests, instead of having to conduct all GI tests IL (Intra-laboratory), using expensive equipment, since it will yield GI values at a more affordable price and at a faster pace. It could even mean more accurate results, due to the fact that subjects eat the test food and take their blood glucose readings in their natural surroundings, without added stress that may affect GI results [38], which could be experienced when GI tests are conducted IL.

1.3   Investigation

The need has therefore been identified to compare the outcome of GI tests done by a mixed group of subjects (i.e. healthy and diabetic subjects) by using the MPQIDG (EL), with the results of GI tests done IL by a specific group of subjects (e.g. healthy or diabetic subjects), using a laboratory instrument (e.g. the YSI), to see whether the former method is as an acceptable alternative for the latter method in determining reliable GI values of foods. This will be much more cost effective, less invasive and time consuming for test subjects and will cause the GI values of more foods to be able to be determined more quickly and labeled as such, which will benefit the public in the end.

This study also wished to make a contribution in finding ways to reduce within-subject variation in glycemic responses, as an inter-laboratory study [39] found that between-laboratory variation in GI could be attributed mainly to day-to-day variation of glycemic responses within subjects. In the standardization of the protocol, the following testing activities would be controlled:

- standardized techniques, i.e. training of subjects in using the MPQIDG, lancet and test strips,
- subject involvement by having them choose a standard meal [21] the night before all GI tests were conducted, consuming it before 20h00 on the night before testing and
- lifestyle-confounding factors. Subjects would be asked to standardize their consumption of caffeine (by consuming either decaffeinated drinks at all times or a standard amount of caffeine containing drinks), alcohol (by consuming either no alcohol or a standard volume of alcohol on the evening before all GI tests) and medication (by either consuming medication every day or not at all, and refraining from conducting a GI test if they took other medication. They would also have to standardize their exercise habits (by keeping to the same exercise program, especially on the day before all GI tests were conducted, with the last exercise to be done by 12h00), sleeping habits (by trying to go to bed at the same time every evening) and smoking habits (by either smoking the same number of cigarettes the day before a test or not smoking at all). Female subjects would be advised to not conduct a GI test during their menstrual period and all subjects would be advised to not conduct a GI test if they had an infection in their bodies, e.g. a cold, toothache, etc. in order to reduce the effect of day-to-day within-subject variation in glycemia. This is due to the fact that caffeine [40,41], alcohol [42,43], exercise [44,45], medication [23], lack of sleep [46], smoking [21], hormonal fluctuations [47], stress [38] and infections [5,48] have all been found to affect blood glucose response. An effort would be made, however, to standardize these lifestyle-confounding factors in such a way, so as to not place additional stress [38] on the subjects, by involving them in these choices and keeping their lifestyle as normal as possible.

2. LITERATURE REVIEW

2.1 Introduction

The GI is a ranking of foods on a scale from 0–100 and gives an indication of the blood glucose raising ability of CHO foods, relative to a standard (glucose or white bread). The AUC elicited by a 50 grams (g) glycemic or available CHO portion of a food is expressed as a percentage of the mean AUC elicited by a 50g CHO portion of a reference food, taken by the same subject on three different occasions.[1,2,3] The GI in a specific person is therefore calculated as follows:

$$\frac{AUC_f \text{ (above fasting baseline)}}{\text{Mean } AUC_s \text{ (above fasting baseline)}} \quad x \quad \frac{100}{1}$$

where:
AUC_f (above fasting baseline) = Area under the curve above fasting baseline of a food
Mean AUC_s (above fasting baseline) = Mean area under the curve above fasting baseline of three determinations of the standard food.[2]

The AUC (above fasting baseline) is also called the incremental area under the blood glucose response curve (IAUC) and includes only the area above the fasting level, which is calculated geometrically. Any area beneath the fasting value is ignored in the calculation.[2,3,21] The GI value of a food is the mean of 8–12 volunteers of the percentage expression in each volunteer.[3]

Aim and approach

The aim of the literature study was to investigate whether any work had been done regarding GI testing (EL), using a mixed group of subjects, i.e. healthy and diabetic (type 1 and/or type 2) subjects. A literature search and review was therefore done on the methodology of GI testing regarding number and type of test subjects, venous vs. capillary blood, laboratory instruments vs. glucometres, EL testing, etc. To contextualize the use of the GI values, the literature review also covered a historical perspective, the application and clinical use of GI values, arguments against the GI and factors that influence the GI and the variability of the GI values of foods.

2.2 Historical perspective

2.2.1 Pioneers

Wagner & Warkany (1927) and Conn & Newburgh (1936) were some of the first researchers who showed that similar amounts of different CHO foods produced different glycemic responses. Otto and co-workers (1973 and 1980) were the first scientists who classified CHO foods on a systematic basis, according to their glycemic responses, and incorporated them into the diabetic diet in amounts inversely proportional to their glycemic responses, thereby keeping the glycemic impact of the diet constant.[2,49] In the 1970s, Crapo et al examined the glycemic and insulinemic effect of a range of CHO rich foods, each containing a 50g glucose load, on 16 healthy volunteers and found that "not all CHO were created equal". Dextrose and potato caused similar, higher glycemic responses, whereas corn caused lower glycemic responses, rice and white enriched wheat bread. In addition, dextrose and potato elicited similar and larger insulinemic responses than white bread, which showed an intermediate response, and especially larger than rice and corn, which elicited low insulinemic responses.[4] Crapo et al (1981) were also able to demonstrate similar glycemic and insulinemic responses to these foods in diabetic individuals.[49] In 1980, Jenkins et al also found different glycemic responses to 35 CHO rich foods, each containing 50g CHO,

using ten healthy volunteers. The mean glucose AUC and peak blood glucose values after the consumption of cooked beans were respectively 52% (p<0.001) and 41% (p<0.001) of the values for grains; 51% (p<0.01) and 45% (p<0.001) of the values for bread and spaghetti; 49% (p<0.001) and 43% (p<0.001) of the value for biscuits; 51% (p<0.001) and 48% (p<0.001) of the value for breakfast cereals and 55% (p<0.01) and 45% (p<0.001) of the value for tubers.[50] This disproved the assumption that equivalent amounts of CHO from different CHO rich foods and drinks had similar physiological effects on the body and confirmed the findings of Crapo et al (1977), namely that all CHO, if consumed in amounts that all yield the same amount of glucose (usually 50g) to the body, did not have the same effect on blood glucose and insulin levels.

2.2.2. Coining of the concept

However, as the methods of presentation of data on the glycemic effect of different CHO foods were not standardized, the results of different studies could not always be compared directly.[49] Therefore, in order to standardize the interpretation of these different glycemic responses in reaction to equal amounts of different CHO foods, Jenkins and colleagues of the Department of Nutritional Sciences of the University of Toronto (Ontario, Canada], proposed (in 1981) the GI. The GI was proposed as a classification of CHO foods according to their effect on blood glucose levels [1] or their blood-glucose raising potential so to speak, to supplement the information found in food composition tables, as well as the CHO exchange lists used by dieticians, which is merely based on the amount of CHO present in foods/drinks. This was deemed necessary, as the CHO exchange lists, which have been used by dieticians for about 30y to regulate the diets of diabetic subjects, do not reflect the physiological effect of foods on blood glucose levels and are therefore no longer sufficient to control blood glucose levels. Scientists proved with research done over the past 25y that it is not so much the amount of CHO, but rather its rate of digestion and absorption that determined the glycemic response of humans to CHO rich foods.[5]

2.2.3 Measurement and interpretation

The very first GI tests were conducted in 1981 on 5–10 healthy volunteers. Fifty grams CHO portions (as calculated from food composition tables) from 62 different CHO rich foods were consumed with tea made with one tea bag and 50mL milk, after an overnight fast. The area under the 2 hour (h) blood glucose response curve (above fasting baseline) was calculated using a specific formula and was expressed as a percentage of the mean 2h IAUC when an equivalent amount of CHO was taken as glucose, together with 550mL tea with 50mL milk, which was done on more than one occasion. The GI value of a food is the mean of 5–10 volunteers of the percentage expression in each volunteer.[1]

In 1983 Jenkins et al also determined the GI on groups of 5–7 diabetic volunteers from a pool of 12 subjects, of which one was type 1, two type 2 on oral medication and the rest type 2 diabetic subjects on insulin. Fifty grams CHO portions (as calculated from food composition tables) from 15 different CHO rich foods or CHO and protein meals were consumed after an overnight fast. The area under the 3h IAUC was calculated using the same formula already mentioned and expressed as a percentage of the mean IAUC of three tests of an equivalent amount of CHO taken as white bread. The GI values of the foods were also calculated as the mean of the percentage expression in each volunteer and were found to be significantly related (r=0.756; p<0.01) to the GI values obtained on the same foods, when the GI values of 62 foods were determined in healthy individuals over 2h, with glucose as the reference food, by Jenkins et al (1981). The IAUC of the 3h tests on 15 foods in diabetic subjects was also significantly related to the IAUC of the 2h tests on the same foods in healthy volunteers (r=0.753; p<0.01).[32] This data showed that diabetic subjects could also be used for GI testing, in addition to healthy subjects, and also indicated that white bread could be used successfully as standard/reference food.

However, GI values using white bread as standard food are higher than GI values using glucose as standard food, but can be adjusted to yield GI values using glucose as standard by multiplying by the factor of 70/100, where 100 is the GI value of glucose and 70 is the mean GI value of white bread from several studies. Therefore, if a GI value with white bread as reference food needs to be converted to a GI value with glucose as reference food, the value must simply be multiplied by 70/100 [2,27] or divided by 1.4. The relative difference between the GI of foods is the same, regardless of whether glucose or white bread is used as reference food.[2]

GI conversion factors for different reference foods

Glucose to white bread: x 1.4

White bread to glucose: x 0.7

Rating foods according to their GI values made it possible to compare CHO sources tested by different investigators from around the world in different groups of subjects more readily and a large number of foods have been classified thus.[22,30] However, there are different methods to calculate the AUC and GI (refer to 2.4.3.2).

2.2.4 Arguments against the use of GI values

Some scientists are concerned about the possible problems that may be encountered in incorporating GI advice into therapeutic nutrition, as well as the potential adverse effects it could have on food choices and fat intake. For this reason the American Diabetes Association (ADA) does not recommend the use of GI values for dietary counseling [22], although the ADA does not question the fact that consumption of the same amount of different starches can lead to different glycemic responses.[51] [Some critics of the GI even acknowledge that different, single CHO containing foods cause different glycemic responses.[52]] Omitting the incorporation of the GI in dietary recommendations for diabetic individuals and advising them to rather place priority on the amount of CHO, ignores the two- to threefold difference in glycemic responses for the same amount of CHO in different foods. In fact, two slices of low GI bread produced a similar glycemic response to only one slice of regular bread. Although the amount of CHO consumed is important, as seen in the glycemic load (GL); one usually decides [in the words of Brand Miller et al (1999)] "what to eat" before deciding "how much to eat".[25]

2.2.4.1 Lack of agreement in GI values obtained between different centers (refer to 2.4.3.2)

Although the GI values of many CHO foods tested in different centers around the world are similar for the same type of foods, different GI values for the same foods have been observed from one center to another, especially for potato and rice.[22,30] However, sometimes slightly different weights of the same food were tested, like in the case of potato, due to the use of different food composition tables (FCT) in different countries of the world, which partly explains the differences in glycemic responses. There could also be real differences between the more powdery russet potato that was tested by Crapo et al (1977) and the variety of potatoes that were tested by Jenkins et al (1981) and Jenkins et al (1983). In addition, Crapo et al (1977) probably found a lower response to rice as they used parboiled rice, whereas the studies that found higher responses, i.e. Jenkins et al (1981) and Jenkins et al (1983), used regular rice. Studies that were conducted since then have shown that parboiled cereal grains like rice [53] and wheat [19] usually result in relatively flat blood glucose responses. These differences are not due to lack of agreement between centers, but due to true differences in the physiological effects of these foods that were previously erroneously considered to be the same. Goddard et al (1984) found that long grain rice may be

higher in amylose starch and therefore elicited a flatter blood glucose response than short-grain varieties, which are higher in amylopectin [49], and which are more easily digested.

An inter-laboratory study revealed an average inter-laboratory SD of 9, indicating that if published GI values of the "same" food, which was determined by different GI testing laboratories in the world, differ by more than 18, it is possibly a true difference in GI due to real differences in the food.[39]

2.2.4.2   Individual variation in glycemic responses (refer to 2.4.3.2)

As mentioned before, Crapo and co-workers (1977 and 1981) demonstrated similar glycemic and insulinemic responses to four starches (bread, potato, rice and corn) in healthy [4] and diabetic individuals [49], in two separate studies. Several other studies have also shown similar responses to different CHO foods in healthy [1], type 2 diabetic [32] and type 1 and type 2 diabetic subjects.[19,53] However, it has been said that using average glycemic responses is not accurate enough, as they may not reveal large differences in response in different individuals.[54] Jenkins and colleagues (1988) therefore examined the individual data that were used to determine the GI values of a range of low GI foods, as determined in two sets of type 1 and type 2 diabetic subjects in the two studies mentioned above. They established that the overall response to the whole range of low GI foods that were tested was consistent for each individual, i.e. the mean value for all the low GI foods in each subject was significantly lower than that of the high GI food, white bread and was similar to or below the predicted mean GI value for these foods, in spite of the wide variation in individual responses to a specific food. Therefore they are of the opinion that the GI can be applied to individual diets that contain many different CHO sources.[49]

2.2.4.3   Glycemic response of mixed meals

Jenkins et al (1984) found that if two CHO sources with different GI values were incorporated into a meal, the overall glycemic response of the meal was intermediate in relation to the GI values of the two CHO sources. They concluded that the GI value of foods could be used to predict the glycemic response to a mixed meal that contains different sources of CHO, as well as protein and fat.[55] The GI can therefore be used to predict the glycemic response of mixed meals by calculating the weighted GI value of the meal.[3]

The observed GI values of mixed meals were within 2% of the predicted GI value, when the latter was calculated prior to testing.[56] At least 12 studies showed that the GI values of individual foods predicted the glycemic response to mixed meals containing these foods.[2] In a study conducted by Chew et al (1988), the correlation coefficient for the observed glycemic response vs. the predicted response was as high as $r=0.88$ ($p<0.01$). The lack of effect in mixed meals found in one study could possibly be attributed to faulty methodology.[25]

In the first of three studies conducted by Coulston and Hollenbeck et al (which took place in 1984, as reported by Hollenbeck et al in a review article on these studies), a noon meal was fed to type 2 diabetic subjects, in which 66% of the total CHO came from a single CHO, i.e. either potato, rice, spaghetti or lentils (foods with a wide range of GI values). They did not find any significant differences in glycemic or insulinemic responses to these mixed meals, except for the meal containing potato, which showed significantly higher blood glucose and insulin responses than the other meals. However, these responses were not in line with the predicted responses, based on the GI values of these foods.[52]

In the second study on six healthy individuals and nine individuals with type 2 diabetes, the observed differences in glycemic and insulinemic responses to mixed meals that were given at lunch, in which the overall CHO from starch, fruit and vegetables were predominantly high (GI 71), intermediate (GI 48) or

low GI (GI 34), were much less marked than the predicted responses, and not in line with the calculated GI values of the meals, as determined by using the published GI values of the CHO in the meal. Although plasma glucose response areas seemed to follow the predicted order of glycemic response in the type 2 diabetic subjects, these differences were not significant and the response areas were almost identical for the different meals, especially in the healthy subjects. The higher the overall GI of the meal was, however, the higher the blood insulin levels were, although not statistically significant.[57]

In a third study, breakfast, lunch and dinner were fed to type 2 diabetic subjects. The three test days contained mixed meals with an overall high (GI 71), intermediate (GI 54) and low GI (GI 38) respectively, as predicted by using published GI lists. The glucose response after breakfast, lunch and supper did not vary substantially between the low, intermediate and high GI meals, except for after lunch, in which the total plasma glucose response was 9% lower after consumption of the low GI meal, when compared to the high GI meal.[52]

This lack of difference in the GI values of mixed meals could possibly be attributed to faulty methodology pertaining to the following:

- **second meal effect:** Staub (1921) and Traugott (1922) found that the response to a second meal is smaller than to the first in healthy individuals, when blood glucose response is determined after the ingestion of oral glucose loads given about 4h apart. This is known as the Staub-Traugott or second meal effect.[58] Jenkins et al (1982) found a significantly lower blood glucose response to lunch after consumption of a low GI breakfast than after consumption of a high GI breakfast.[59] The lack of difference between the glycemic responses to the low, intermediate and high GI meals taken at lunch in the above mentioned three studies [Coulston et al (1984); Coulston et al (1987); Hollenbeck et al (1988)], could possibly be due to the Staub-Traugott effect, especially in the studies conducted by Coulston et al (1984) and Coulston et al (1987). The GI of the breakfast meal in the former study could have been low, but the article does state what was given for breakfast [50] and the GI of the breakfast meal in the latter study could have been low or intermediate or high, depending on the type of fruit juice that was given with the white bread.[57] If the GI values of the breakfasts were low GI, this could have affected the results of the GI tests that were conducted at lunch, as Jenkins et al (1982) found a significantly lower blood glucose response to lunch after consumption of a low GI breakfast than after consumption of a high GI breakfast.[59] Wolever and Bolognesi (1996) confirmed this, as they found that the difference in glycemic response between two breakfast cereals that were studied in eight healthy individuals in the morning after a 10–12h overnight fast, as well as at lunchtime (12h00) after a standard breakfast had been eaten, were significantly larger in the morning than at lunchtime, in spite of the fact that physical activity was restricted.[60] For this reason, it has been recommended by the international committee for the standardization of global GI methodology, that GI tests be conducted in the morning, after an overnight fast [21];

- **venous vs. capillary blood:** Venous blood was used by Coulston et al (1984) and Coulston et al (1987), and probably by Hollenbeck et al (1988) as well [52], whereas capillary blood is the preferred method of blood sampling to observe differences in glycemic responses to different foods [21,39];

- **frequency of blood sampling:** Blood glucose measurements were only taken every 30min (min) for the first hour in the study conducted by Coulston et al (1987) and only every 60min for the next 2h, instead of every 15min for the first hour and thereafter every 30min, as is the standard protocol for GI tests.[2,21] In the study conducted by Coulston et al (1984), blood glucose measurements were only taken every 60min. The highest postprandial value in healthy individuals is mostly at 45min, and Krezowski et al (1986) showed that blood glucose concentrations were usually back to baseline after 90min. The highest postprandial value in diabetic subjects could be anything from 60-90min. Researchers, such as Gannon et al (1986)

and Krezowski et al (1987), showed that it can take up to 4–5h for plasma glucose to return to baseline in these subjects.[61] The highest values and lowest values in both healthy and diabetic subjects could therefore have been missed, due to this faulty method of blood sampling;

- **lack of sufficient difference in GI between the three meals**: It does not come as a surprise that there were no significant differences in the glycemic and insulinemic responses to rice, spaghetti and lentils, three of the four CHO meals given to subjects in the study conducted by Coulston et al (1984), as lentils, high amylose rice and durum wheat spaghetti all have low GI values ranging from 29 to about 50. The overall GI of the high GI meal in the study conducted by Coulston et al (1987) could also have been lower than anticipated, if high amylose rice was used, which has a much lower GI than high amylopectin rice. In addition, banana was used in the high GI meal, whereas it has an intermediate GI and, if green bananas were used, the GI of this fruit would have been low.[22] The GI of overripe bananas (yellow skin with 1–2mm brown spots), as tested in ten well-controlled type 2 diabetic subjects, was 52, whereas the GI of under ripe bananas in the same ten subjects came to only 30, which was mainly attributed to the fact that 100g under-ripe bananas contained 15.8g starch (91%) and 1.6g sugars, in contrast to 2.3g starch (13%) and 14.8g free sugars in 100g over-ripe bananas. The starch in bananas has been found by other researchers to be hydrolyzed slowly by alpha amylase in humans.[62] The intermediate GI meal was also not truly intermediate, as it contained only one intermediate GI food, i.e. beetroot, and two low GI foods, namely orange and spaghetti, and had an overall low GI, i.e. 48, whereas intermediate GI meals and foods, strictly speaking have GI values between 56 and 69;

- **macro-nutrient distribution**: The meals in the study conducted by Coulston et al (1984) were relatively low in CHO (only 45% of total energy) and high in fat (i.e. 40% of total kJ) and fat is known to slow gastric emptying, especially if used in large quantities.[58] The glycemic response to CHO in the diet can be lowered substantially by increasing the protein and fat content of the meal.[57] However, Wolever et al (1985) found that, if protein and fat were added to CHO in mixed meals, the observed blood glucose responses still related significantly to the meal GI values, as calculated from the individual foods, and therefore concluded that the type of CHO largely determined glucose responses, even when a meal contained as much as 40% of total energy in the form of fat, as tested in type 2 diabetic volunteers.[33,53] The variation in findings could probably partly be due to the fact that this phenomenon is dose responsive. The addition of 10g protein to a 50g glucose load in persons with type 2 diabetes did not affect the glucose response curve significantly. With the addition of 30g protein the mean was reduced modestly and only with the addition of 50g protein was there a reduction that was statistically significant ($p \leq 0.05$). It was also found that there was a first order relationship between the insulin response and dose of protein added to 50g glucose, with a significant increase in insulin response at 30g protein or more.[58] The varying findings could also possibly be due to the fact that the amount of macronutrients was not always kept constant and only the type of CHO changed, which should be done if one wants to establish whether meal GI can be predicted from tested GI values;

- **lack of free days between testing**: In study one, which was conducted by Coulston et al (1984), it is mentioned in the article that "each subject received all three test meals in random order on three separate days". If this happened on consecutive days, it could have added to the lack of difference between the meals, as a washout period of two days between GI tests has been recommended to prevent the food tested in the first session to influence the glycemic responses of the food tested in a second session.[21]

Nuttall et al (1983) showed that when CHO foods were taken as part of mixed meals, the differences in glycemic responses were the same. However, in this study [Nuttall et al (1983)], AUC_0, rather than IAUC was used to compare postprandial glycemic responses. As GI classifications are based on IAUC, this

method of calculation should also be used for mixed-meal responses, as large variations in starting values could mask differences between meal responses when expressed as absolute postprandial levels, especially if AUC_0 is used.[49] Wolever et al (1985) found the observed GI of mixed meals to be within 2% of the expected value [56], which probably indicates how important it is that the GI values of individual CHO components of a mixed meal are determined before the glycemic effect of these foods in mixed meals are predicted or examined; a factor which was not always kept in mind when the observed GI of mixed meals were compared to the expected value. [49]

2.2.4.4 The use of 50g CHO portion sizes in GI testing

This has been criticized, as it does not constitute a normal serving size. However, Holt et al (1996) found that the glycemic response to a reasonable serving size of a 1000 kJ portion of a whole range of foods correlated highly with the published GI values of those foods.[25] (refer to 2.4.3.2)

2.2.4.5 Lack of demonstration of long-term benefits of low GI foods (refer to 2.3.1)

2.2.4.6 The GI and its complexity

The GI is not complex, as some critics of the GI profess. In Australia, where it has been used for over 10 years, it has been found to be simple and useful for both lay persons and professionals, by using simplified educational material that had been developed by dieticians.[25] Incorporating the GI into dietary advice given to diabetic subjects, is simply a matter of a few substitutions, which are summarized in Table 1.

Table 1: Substituting low GI foods for high GI foods [Adapted from Brand Miller et al (1999)[25]]

Higher GI	Lower GI
Bread, white, brown or whole meal	Bread containing a high proportion of whole grains e.g. Seed loaf and Pumpernickel bread
Processed breakfast cereal	Unrefined and/or very high fibre breakfast cereals e.g. High Fibre Bran (Kellogg's) or Fibre Plus (Bokomo)
Refined biscuits and crackers	Biscuits that contain fruit, dairy, legumes and/or lower GI cereal grains
Cakes and muffins (lower fat)	Cakes and muffins (lower fat) that contain fruit, dairy, legumes and/or lower GI cereal grains
Tropical fruits such as melons	Deciduous and citrus fruits
Potato (whole, mashed or french fries)	Sweet potato or baby potatoes with skin
Rice, especially "sticky" rice	Lower GI, high amylose and/or parboiled rice e.g. Tastic rice, Old Mill Stream Brown rice and Basmati rice
Pasta: homemade or made from wheat flour or soft wheat semolina	Pasta made from Durum wheat or Durum wheat semolina

Choosing low GI breads and cereals, which supply 50% of the total starch in the typical western diet, reduces the total GI by about 13 units. Alternatively, choosing a lower GI starch at the main meal and as one snack choice will have the same GI lowering effect. Those who eat more fruit can reduce the GI of their diet significantly by substituting lower GI fruit for higher GI fruit. Low GI CHO foods can be eaten more freely without the risk of hyperglycemia, as a bigger portion of a low GI CHO generally has the same glycemic effect as a smaller portion of its high GI equivalent.[25]

2.2.4.7   Insulin response versus glycemic response (refer to 2.4.2.1)

2.2.4.8   Sucrose versus starch: different effects on body weight, lipidemia and glycemia? (refer to 2.4.3.1)

Chantelau et al (1983) and other scientists have shown that regular use of up to 40g sucrose per day, by intensively treated type 1 diabetic subjects, had no negative effect on metabolic control.[24]

Sugar in certain confectionery items produced a blood glucose response similar to that of bread, with no evidence of rebound hypoglycemia. In fact, plasma glucose and insulin levels had returned to fasting levels within 2h in healthy individuals after the consumption of 17 foods containing only naturally-occurring sugars and 21 foods containing added sugars, except in the case of bread. It was found that most foods containing sugars have GI values that are lower than most common starchy foods.[27]

It is now common knowledge that not all sugars have a high GI value. Restrictions on sucrose intake for diabetic individuals can therefore be relaxed and should be similar to the recommendation on sugar intake for healthy persons.[63] Anderson (1997) found that the consumption of a moderate amount of refined sugars (10-12% of total energy) was not associated with obesity, micronutrient deficiency or undesirable effects on blood lipids or insulin sensitivity. In fact, several researchers have shown that a low sugar intake is rather associated with a higher fat, especially saturated fat, diet, higher body weight and a higher GI diet. Sugar improves dietary compliance by improving the palatability of bland cereal grains, such as oat porridge.[25]

Liu et al (2000) found that classifying CHO according to GI was a better predictor of CVD risk than classifying them as either simple or complex.[9]

2.2.5   Driving forces

Since an article was published on GI methodology and clinical implications [2], the FAO/WHO has compiled an internationally recommended method for GI testing.[3] An inter-laboratory study was conducted to determine the extent and sources of variation of GI values of the same foods, as determined by experienced investigators in different GI testing centres all over the world, using the method recommended by the FAO/WHO (1998).[3] This study showed that venous blood sampling was the only variable that was significantly associated with within-subject variability and therefore capillary blood sampling was recommended for GI testing. It was also recommended that, for international standardization, the GI values of all foods be expressed relative to glucose. As already mentioned, this study revealed that the average between-laboratory SD of GI values was 9.0, which implied that when published GI values of the "same" food, as determined by different laboratories, differ by more than 18, the difference in GI is probably real, rather than accidental.[39]

In the early 2000's, an international committee for the standardization of GI testing methodology was appointed by the International Life Sciences Institute (ILSI), the outcome of which was recently published.[21] In 2003, ILSI also commissioned a few experts from around the world to discuss "the role of diet in blood glucose response and related health outcomes", the results of which were recently published. The attendants of this workshop searched the literature, in order to establish whether there was sufficient evidence that a low GI/GL diet reduced the risk of developing lifestyle diseases like diabetes, CVD, obesity/overweight, cancer and dental caries, so that such a diet could be recommended to the general, healthy public. This exercise revealed that there are plenty of prospective studies, as well as randomized controlled animal trials and short-term clinical trials in humans to support such a

recommendation. However, is was recommended that more long-term clinical trials in healthy humans be conducted, before such a recommendation could be made.[64]

2.3 Current scenario

2.3.1 Application and clinical use of GI values

2.3.1.1 Dietary GI in relation to metabolic risk factors and prevalence of lifestyle diseases

In 1998 the United Nations FAO/WHO Expert Consultation Group on carbohydrates in human nutrition recommended the use of the GI to classify CHO as low, intermediate or high GI and in making food choices. They also recommended a high CHO diet (i.e. ≥55% of energy should come from CHO), most of which should come from foods that are rich in non-starch polysaccharides (NSP) with a low GI and which intake should be encouraged gradually from about 2y onward. This is due to the fact that diets that are rich in slowly digested CHO assist in disease prevention, especially the prevention of obesity and other chronic diseases of lifestyle. However, foods should not only be chosen on account of their GI, as some foods might have a low GI, but a high fat content [3] or low fibre and micronutrient or high sodium content, etc. The total amount of CHO, the amount and type of fat and fibre, micronutrient and sodium contents of foods should also be considered in deciding whether a food is a healthy choice or not. Preferring a low GI high fat food to a high GI low fat food does not hold any health benefits, as although high fat foods reduce glycemic response by slowing down gastric emptying, glucose tolerance to the following meal is impaired.[25]

These recommendations are in line with the findings of the Zuthpen Elderly study, which was conducted on 389 men, who were free of recently diagnosed diabetes. This study found that raised insulin levels during an oral glucose tolerance test (GTT) were associated with a reduced intake of total CHO. In addition, fasting C-peptide and body mass index (BMI) were inversely associated with the intake of total CHO.[65] However, Franz et al (1994) reported that short-term studies, such as that conducted by Garg et al (1988) have shown that high CHO (60% of energy), low fat (20–25% of energy) diets might aggravate high TG levels, reduce high-density lipoprotein (HDL)-cholesterol and increase postprandial blood glucose and insulin levels in type 2 diabetic subjects, whereas LDL-cholesterol levels did not change. They were more in favour of replacing saturated fats with mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA), as they reported that in the study conducted by Garg et al (1988), in which a high CHO low fat diet was compared with a moderate-CHO (45% of energy), high fat (40% of energy; saturated fats ≤10% of energy) diet, the adverse effects of a high CHO diet were eliminated.[18]

However, these varying findings could possibly be attributed to the fact that no distinction was made between the types of CHO. A long-term (32 weeks) intervention study conducted by Pawlak et al (2000) on young adult rats showed that those that were placed on a low GI diet had a 16% lower weight and significantly lower fat mass (p≤0.05) than those placed on a high GI diet.[16] A crossover study conducted on 17 middle-aged men with one or more cardiac risk factors (e.g. BMI>25kg/m^2, raised total cholesterol:HDL>5 and/or increased visceral adiposity), who were placed on one of four diets, i.e. a high fat diet (50% fat), a high CHO low GI diet, a high CHO high sucrose diet (90g/day) or a high CHO high GI diet, for a medium-term period of 24 days, confirmed this. The high fat diet led to lower postprandial insulin and glucose levels over time, but higher TG and non-esterified fatty acids (NEFA), which has been proposed as an independent risk factor for CHD risk, and would therefore counteract against any other metabolic benefits. There was also a significant increase in the 6h TG concentration on day one (p<0.01) on the sucrose diet, although this was 10% lower than that seen on the high fat diet. Postprandial median changes in HOMA index from day 1-24 were negative for the high fat, low GI and sucrose diets, but positive for the high GI diet and the percentage change in the latter was significantly different from

the other three diets (p<0.0001). There was a tendency to reduced energy intake on the low GI diet, causing significant weight loss on the low GI compared to the sucrose diet (p<0.02), in spite of identical energy intake. However, there was a spontaneous increase in energy intake on the high fat diet and increased weight on the high fat and high GI diets. The high GI diet also increased postprandial insulin resistance over the study period.[13] This was furthermore confirmed in a short-term (9 weeks) intervention study in animals, in which young, growing rats that were placed on a low GI diet showed better glycemic and insulinemic control, higher plasma adiponectin, lower TG concentrations, less disruption of beta-cell architecture and significantly less body fat (p=0.015) and more lean body mass (p=0.12) than those who were placed on a high GI diet.[15] It can therefore be seen that not all CHO sources have the same effect on blood profiles and that low GI CHO are the preferred choice (refer to Figure 1).

Low GI diet

↓

Increased starch malabsorption

↓

Increased colonic fermentation

↓

Increased short chain fatty acid (SCFA) production

↓

Decreased free fatty acids (FFA)

↓

Improved insulin tolerance

Reduced blood pressure   Improved lipid levels   Improved glycemic control
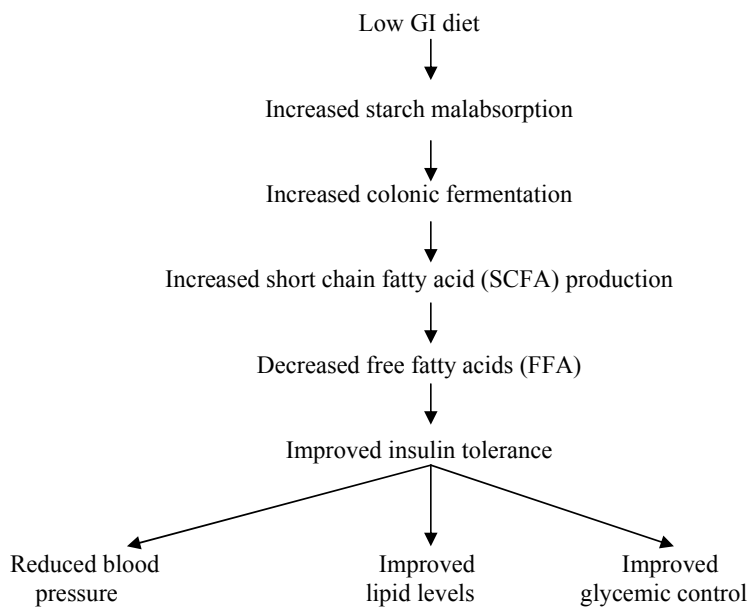
Figure 1: Advantages of a low GI Diet [59]

2.3.1.1.1 Insulin Resistance Syndrome and insulin sensitivity

Clinical predictors of hyperinsulinemia and insulin resistance are first and foremost central adiposity and others are high blood TG levels [59,66], low levels of HDL-cholesterol [59], impaired glucose tolerance (IGT), hypertension, arteriosclerosis, polycystic ovarian syndrome and ethnicity.[67] A high fat [65], high protein [67], high GI [4,11,15,20], high alcohol [42,65] diet as well as higher LDL-, very low-density lipoprotein (VLDL)-cholesterol [59] levels and BMI also seem to play a role. In addition, inactivity, overeating, aging, high blood glucose levels, increased plasma FFA and the effects of some medication are all acquired causes of insulin resistance.[66] Table 2 summarizes a number of studies done on the effect of a high GI/GL diet on insulin resistance/sensitivity.

Table 2: Summary of studies done on the effect of a high GI/GL diet on insulin resistance/sensitivity

Researchers	Study Population	Results
Brynes et al, 2003 [13]	17 Middle-aged men with one or more cardiac risk factors	Postprandial HOMA (an indicator of insulin sensitivity) median changes from day 1-24 were negative for high fat, low GI and high sucrose diets respectively, but positive for a high GI diet and the percentage change in the latter was significantly different from the other three diets ($p<0.0001$). The high GI diet also appeared to increase postprandial insulin resistance over a study period of 24 weeks.
Brand-Miller et al, 2003 [68]	30 Lean, healthy subjects	Stepwise increases in dietary glycemic load (GL) led to significant and predictable increases in both blood glucose ($p<0.001$) and insulin ($p<0.001$) levels.
Pawlak et al, 2004 [15]	30 Male partially pancreatectomized 6-week old rats	This short-term (9 weeks) intervention study in animals showed greater increases over time in the AUC for blood glucose and insulin levels when challenged with a GTT, lower plasma adiponectin concentration and a much higher proportion of abnormal islet cells, significantly more body fat ($p=0.015$), which was predominantly visceral fat, and less lean body mass ($p=0.12$) on a high GI vs. a low GI diet.
Brand-Miller et al, 2002 [16]	Adult rats	In a long-term (32 weeks) intervention study in animals, the high GI group gradually gained weight and were 16% heavier at the end of the study in comparison to the low GI group, although both groups were fed diets that were similar in energy distribution to modern western diets (comprising 45% of total kJ as CHO, 20% as protein and 35% as fat); the only difference being that the CHO of the one group came mainly from high GI starch, whereas that of the other group came from low GI starch. Total fat mass was significantly higher in the high GI group ($p\leq0.05$).
Brand, 1991 [11]	16 Well controlled, overweight type 2 diabetic subjects	Similar insulin levels in the presence of a lower blood glucose profile on a low diet vs. a high GI diet suggested improved insulin secretion on a low GI diet relative to prevailing blood glucose levels.
Slabber et al, 1994 [14]	30 Obese, hyperinsulinemic women	Reduced body weight and food intake, as well as a greater fall in insulin resistance, which could only be attributed to weight loss, were noted on a low vs. a high GI diet, probably due to the fact that the fasting insulin concentrations dropped significantly more after the low than after the high GI diet in both arms of the study ($p=0.01$ and $p=0.00$, respectively).

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Chronic high insulin levels, as well as high TG, are believed to play an important role in the development of type 2 diabetes. Unlike blood glucose responses, insulin responses to foods are affected by the degree of insulin resistance in the individual, which is determined by age, obesity and genetic inheritance.[69] Insulin sensitivity can be defined as the ability of insulin to lower blood glucose levels, by suppressing glucose production by the liver and stimulating glucose uptake by the cells of the muscles and adipose tissue. Insulin resistance is basically a condition of reduced insulin sensitivity.[66] Factors that improve insulin sensitivity are the following: physical activity [44,65,70], a low fat diet and substitution of PUFA for saturated fats [65], a high fibre diet [65,71], a moderate protein diet [67] and a low GI diet [11,14], as well as weight loss due to a low GI diet.[14] Table 2 summarizes a number of studies done on the effect of a low GI/GL diet on insulin resistance/sensitivity.

2.3.1.1.2 Obesity

Obesity and insulin resistance are risk factors for diabetes and CVD. Obesity and sedentary behaviour are also associated strongly with increase insulin requirement, as inactivity causes insulin insensitivity in muscle tissue.[64] Obesity, especially central intra-abdominal obesity, is associated with hyperinsulinemia, insulin resistance and type 2 diabetes. Hypertension, hyperuricemia and dyslipidemia are also more prevalent in persons with central obesity and type 2 diabetes.[18]

Lowering the fat content of the diet has been the main strategy of dietary prevention and treatment of overweight and obesity for over 20y. However, many low fat, high CHO diets may be counterproductive to weight management, as they increase postprandial blood glucose and insulin levels substantially, promoting CHO oxidation at the expense of fat oxidation, which is conducive to body fat gain instead of loss.[16]

Low GI foods may benefit weight control in the following ways:
- **by promoting fat oxidation at the expense of CHO oxidation** [16] High GI meals yielded lower rates of fat oxidation than low GI meals of similar composition[17] and some evidence supports the hypothesis that the chronic hyperglycemia and hyperinsulinemia induced by high GI diets, can reduce the body's ability to oxidize fat, leading eventually to a significant increase in body fat storage.[16] When Pawlak et al (2000) fed two groups of young adult rats iso-energetic, nutrient balanced diets that either contained high GI starch (amylopectin) or low GI starch (amylose), the high GI group gradually gained weight, whereas the low GI group remained weight stable. Total fat mass was significantly higher (40%) in the high GI group (p≤0.05) and the average weight of visceral fat in the high GI group was twice that of the low GI group. There was also a marked decrease in whole body fat oxidation in the high GI group, as well as higher rates of hepatic lipogenesis and higher liver and red oxidative muscle glycogen stores. These findings challenged the assumption that "a calorie is a calorie".[16] A short-term (9 weeks) intervention study in animals showed significantly less body fat (p=0.015) and more lean body mass (p=0.12) in young growing rats on a low rather than a high GI diet [15];
- **by reducing insulinemia** [14] A 12-week parallel study was conducted on 30 hyperinsulimemic, obese female subjects, in which 15 persons consumed an energy-restricted, low insulin response diet (ID) and the other 15 an iso-energetic, balanced diet (ND), that were identical in macronutrients. The only difference was that the ID contained mainly low GI CHO and that the starch was not fed at the same times as the protein in the diet, whereas the ND contained mainly high GI CHO, which was fed with protein. This was followed by a 12-week washout period and a crossover study on 16 of the subjects. It was found that body weight decreased more on the low compared to the high GI diet (-9.3 vs. –7.4 kg, p=0.14 in the parallel study and –7.4 vs. –4.5 kg, p=0.04 in the crossover study). This could probably be attributed to the fact that fasting insulin levels dropped significantly more after the low than after the high GI diet in both arms of

the study (p=0.01 and p=0.00 respectively).[14] It is well known that insulin leads to more fat storage, as insulin resistant individuals usually synthesize more VLDL-cholesterol, which leads to increased serum TG levels and reduced clearance of lipids due to reduced activity of the enzyme, lipoprotein lipase (LPL) [59];

- **by enhancing satiety** [16] Low GI foods are also generally more satiating than high GI foods [20] and Lawton et al (1993) have shown that CHO foods generally have a greater satiety value than fat.[63] According to Lavin et al (1999), the higher satiety induced by the consumption of low GI foods, can be attributed to the fact that nutrient receptors in the small intestine of the gastrointestinal tract are stimulated for a longer period of time, as these foods are digested and absorbed at a slower rate, which leads to prolonged feedback to the satiety center in the brain, through signals such as cholecystokinin and Glucogon-like Peptide-1.[16]

2.3.1.1.3 Diabetes

- **Factors that play a role in diabetes**

The high blood glucose levels observed in diabetes can be due to higher rates of glucose appearance in the blood stream or lower rates of glucose disappearance [72] and hypertension, hyperuricemia and dyslipidemia is more prevalent in persons with central obesity and type 2 diabetes.[18] In addition, blood glucose is an independent risk factor that contributes to CVD risk [64] and coronary heart disease (CHD) is the major cause of morbidity and mortality in patients with diabetes.[73] A high GI/GL diet has also been implicated as a risk factor in the development of diabetes. Table 3 summarizes two studies done on the effect of a high GI/GL diet on the risk of diabetes.

Table 3: Summary of studies done on the relation of GI/GL to the risk of diabetes

Researchers	Study population	Results
Salmeron et al, 1997 [7]	A cohort of 42 759 healthy men	This large prospective study (1986–1992) revealed that the GI of the diet (and not the fat, type of fat or total CHO) was positively associated with and the best predictor of the risk of developing type 2 diabetes, after adjustment for factors such as age, BMI, physical activity, family history, smoking, alcohol consumption and total energy intake. It also showed that a high GL and low cereal fibre (especially whole grains) diet increased the risk of type 2 diabetes in men, especially when both were present, as a high GI diet increased insulin demand and hyperinsulinemia in type 2 diabetic subjects.
Salmeron et al, 1997 [8]	A cohort of 65173 healthy women	In this prospective study (1986–1992), it was also found that the GI of the diet (and not the fat, type of fat or total CHO) was positively associated with and the best predictor of the risk of developing type 2 diabetes, after adjustment for age, BMI, smoking, physical activity, family history of diabetes, alcohol consumption, cereal fibre intake and total energy intake. It also confirmed that a high GL, low cereal fibre intake increased the risk of diabetes, compared to a low GL, high cereal fibre intake.

- **Treatment and recommendations**

Sustained and effective glycemic control can prevent or delay the onset of many of the complications of diabetes mellitus.[74] The most comprehensive and decisive effort in showing the link between hyperglycemia and structural damage that could lead to micro-angiopathy was the North American DCCT study, one of the largest, most well-planned and –performed, as well as most costly scientific studies. This study revealed that the foremost goal in the treatment of type 1 diabetes is the prevention of micro-angiopathic complications, by maintaining blood glucose levels in diabetic patients that are as close to normal as possible. The prognosis of type 2 diabetic subjects, however, was largely determined by macro-angiopathy and its resulting excessive rates of morbidity and mortality.[24]

Table 4 summarizes the outcome of a few studies done on the effect of a low vs. a high GI diet on diabetic control, as measure by 24h urinary C-peptide output, FBG levels, HbA1c or fructosamine.

Table 4: Summary of studies done on the effect of a low vs. a high GI diet on the measures of diabetic control

Researchers	Study population	Results
Burke et al, 1982 [12]	Type 2 diabetic subjects	Short-term studies using a low GI, high legume diet, demonstrated ↓ 24h urinary C-peptide outputs (an indication of reduced 24h insulin secretion). Subjects had ↓ mean daily blood glucose levels, but no significant change in FBG levels.
Brand et al, 1991 [11]	16 normolipidemic, well-controlled, overweight type 2 diabetic subjects	This 12-week study showed a statistically significant ($p \leq 0.05$) improvement in glycemic control, as the 8h plasma glucose profile was ↓ and the mean HbA1c was 11% ↓ at the end of the study on a low compared to a high GI diet. This was in spite of the fact that it was relatively low in total fibre (26g) and soluble fibre (11g). Other studies that showed improved diabetes control contained much more fibre (40–65g/day), much of it soluble fibre in legumes. It was concluded that a low GI diet per se, that was not high in soluble fibre, gave a modest improvement in long-term blood glucose control, but not necessarily in plasma lipids.
Opperman et al, 2004 [75]	A meta-analysis of seven human, clinical trials (one study on type 1 and four on type 2 diabetic subjects and two studies on healthy subjects)	There was a significant improvement in glycemic control as measured by fructosamine or HbA1c. There was a ↓ in fructosamine in subjects on a low compared to a high GI diet ($p=0.05$), but when the studies were considered separately, there was a non-significant improvement in each group (diabetic: $p=0.12$ and healthy subjects: $p=0.25$).
Opperman et al, 2004 [75]	A meta-analysis of eight clinical trials on diabetic subjects	There was a significant ↓ in mean HbA1c concentrations on a low ($p=0.03$) compared to a high GI diet. All the studies, except that of Lafrance et al (1998), found an improvement in HbA1c concentrations.
UK Prospective Diabetes Study [76]	Diabetic subjects	A 10% ↓ in HbA1c (a measure of glycemic control) would predict an approximate 10% ↓ in the risk of the complications of diabetes.

Jenkins et al (1985) showed good improvement in the blood lipid profile of 12 hyperlipidemic patients with the use of low GI diets over a period of one month, but legumes played an important role in the diet.[12] At least two studies in type 1 diabetic subjects showed improvements in both glucose and lipid metabolism after 3-6 weeks of consuming low GI foods, rather than high GI foods.[11] Table 5 summarizes the outcome of a meta-analysis of a few studies conducted on the effect of a low vs. a high GI diet on the blood lipid values of diabetic subjects.

Table 5: Summary of a meta-analysis of studies conducted on the effect of a low vs. a high GI diet on the blood lipid values of diabetic subjects

Researchers	Study population	Results
Opperman et al, 2004 [75]	Meta-analysis of eight studies on diabetic subjects	Neither high- nor low GI-diets seemed to have an effect on mean HDL-levels.
Opperman et al, 2004 [75]	Meta-analysis of seven studies on diabetic subjects	There was a non-significant ↓ in LDL-cholesterol on a low vs. a high GI diet (p=0.06), larger ↓ in LDL-cholesterol in type 2 diabetic subjects than in subjects with CHD or healthy subjects and larger in ↓ LDL-cholesterol for two longer studies conducted on well-controlled type 2 diabetic subjects, except for a non-significant ↓ in mean LDL-cholesterol concentration after six months, as reported by Tsihlias et al (2000).
Opperman et al, 2004 [75]	Meta-analysis of nine studies on diabetic subjects	Low GI diets vs. high GI diets caused a significant ↓ in total cholesterol (p=0.0003).
Opperman et al, 2004 [75]	Meta-analysis of ten studies on diabetic subjects	Low GI diets vs. high GI diets caused a non-significant ↓ in TG (p=0.35).

The following has been recommended in the dietary treatment of diabetic subjects:
- that legumes be re-introduced, as a high fibre, high CHO diet that contains substantial portions of beans (i.e. more than 6% of total kJ) led to the discontinuation of insulin in most diabetic subjects who were taking less than 30 units per day [1,32];
- that using the classification of foods according to their effect on blood glucose levels, i.e. the GI, is useful to obtain better blood glucose control, due to the differences in response from one CHO food to another.[1,32] Even if only half of the CHO in the diet is replaced with low GI choices, the overall GI of the diet is lowered by about 15 units, which is sufficient to bring about clinical improvements in glucose metabolism in diabetic subjects.[11] The FAO/WHO Expert Consultation Group on carbohydrates in human nutrition has recommended that 50% of the diet of all humans should come from CHO, the majority of which should come from low GI choices.[3] The European association for the Study of Diabetes (EASD 2000), the Canadian Diabetes Association (2000) and Diabetes UK (2003) all encouraged the use of the GI in choosing CHO rich foods [75];
- that more whole grains e.g. parboiled cracked wheat (bulgur) and Pumpernickel bread be used, as they produce significantly lower glycemic responses than whole meal breads made from these milled whole grains and quite a few studies showed beneficial effects of traditional methods of processing cereals [19];
- that there seems to be no scientific evidence to restrict sucrose in the diet of diabetic subjects, due to concern about its adverse effects on blood glucose levels, as the results of ten studies that were conducted on diabetic subjects indicated that caloric-caloric replacement of starch with sucrose had no adverse effects on glycemia [18];

- that supplements of the soluble fibre (guar) be consumed, as studies conducted by Maskkola-Vuoinen et al (1992) and Groop et al (1993) showed that these reduced blood lipids, HbA1c and serum fructosamine in type 1 and type 2 diabetic subjects [77];
- that overweight diabetic subjects should lose weight, as Henry et al (1986) found that weight loss in obese type 2 diabetic subjects seemed to increase insulin sensitivity and normalize glucose production by the liver.[18] This was confirmed by Coulston et al (1997), who are of the opinion that it is very important that more effective approaches should be developed, in order to achieve and maintain weight loss in overweight patients with type 2 diabetes.[73]

2.3.1.1.4 Coronary Heart Disease (CHD)

- **Risk of CHD in different population groups**

Both Depres et al (1996) and Salonen et al (1998) found fasting hyperinsulinemia to be an independent risk factor for CHD.[25] Blood glucose is also an independent risk factor that contributes to CVD risk, as was found in a prospective study on a cohort of 75521 healthy nurses, in which a high GL diet was directly associated with risk of CHD after adjustment for age, smoking status, total energy intake and other CHD risk factors and was most evident among women with BMI$\geq$23 kg/m^2.[9] However, the prospective Zuthpen Elderly Study [Van Dam et al (2000)] showed no supporting evidence that a high GI diet affects metabolic risk factors, such as total cholesterol, HDL-cholesterol, TG or fasting or postprandial insulin or glucose unfavourably or increases the risk of CHD]. However, this could possibly be attributed mainly to the fact that a small number of subjects participated in this study (<1500), as well as to the fact that most of them were fairly old at the commencement of the study (65–84), with the result that many of them had either already died at follow up, or were excluded due to diabetes or CHD.[78] Obesity and insulin resistance are also both risk factors for CVD.[64]

De Vegt et al (1999) found a significant association between the 2h postprandial blood glucose concentrations and the 8y risk of cardiovascular death in the Hoorn study in subjects with normal FBG readings, even after adjusting for known risk factors.[22] Temelkova-Kurktschiev et al (2000) found blood glucose spikes and postprandial blood glucose to be better than FBG or HbA1c in predicting CVD risk in healthy individuals. Chiasson et al (2003) found that if postprandial glycemia is targeted using the alpha-glycosidase inhibitor (Acarbose) in persons with IGT, the risk of developing heart disease could be delayed or reduced.[64]

The Adult treatment Panel III (2001) has stated that low HDL-cholesterol levels is a strong independent predictor of CHD and can be due to several factors, such as insulin resistance, raised TG levels, overweight and obesity, physical inactivity and type 2 diabetes.[75] CHD is the major cause of morbidity and mortality in diabetic patients.[73] Toeller et al (2001) showed that higher HDL-cholesterol levels were seen in type 1 diabetic subjects from the northern, eastern and western European centers who participated in the EURODIAB study and who consumed low GI diets.[22]

- **Treatment and recommendations**

Anderson (1979) showed that diets that caused flatter glycemic and insulinemic responses lowered the fasting TG concentration in subjects with high TG levels.[50] They also advised that using the classification of foods according to their effect on blood glucose levels, i.e. the GI, is useful for patients with CHO induced hyperlipidemia, due to the differences in response from one CHO food to another.[32] In spite of the fact that total CHO intake, total dietary GI, GL, total energy intake and BMI were all significantly related to TG concentrations in a cross-sectional study on a group of postmenopausal healthy women, dietary GL showed the strongest association, which includes both the quantity of CHO, the GI

(quality of CHO) and their interaction with each other.[9] When data of Jeppesen et al (1997) was used to look at the association between GL and fasting TG concentrations among women with BMI <25 and $\geq$25 kg/m^2, the slope for increasing GL was nearly four times greater among women with higher BMI.[76]

Table 6 summarizes the outcome of a few studies conducted on the effect of a low vs. a high GI diet on blood lipid values.

Table 6: Summary of a few studies done on the effect of a low vs. a high GI diet on blood lipids

Researchers	Study population	Results
Jenkins et al, 1981 [1]	Hypertriglyceridemic men	A high fibre high CHO diet that contained substantial daily portions of beans (more than 6% of total energy), ↓ serum TG levels successfully, in spite of the fact that it has been reported that ↑ CHO diets caused high serum TG.
Jenkins et al, 1985 [12]	12 Hyperlipidemic patients	This 3-month study showed that a significant ↓ in the mean GI of diets of from 82$\pm$1 to 69$\pm$2 units or 20% (p<0.001) over the middle month in a 3-month study, led to a significant ↓ in total cholesterol (9$\pm$2%; p<0.005) and serum TG (16$\pm$3%; p<0.001) and a small mean ↓ in LDL-cholesterol (10$\pm$4%; p$\leq$0.05), in comparison with the mean lipid values for the first and third control months. No significant changes in HDL-cholesterol levels were found. This was mainly achieved by substituting lower GI cereal products for higher GI ones and not by a significant increase in the fibre content (4.1$\pm$13; p<0.01) of the diet as in previous studies done by Anderson et al (1980 and 1984), although the percentage of total fibre in this study from oats and barley (good sources of viscous fibre such as beta-glucan), ↑ significantly during the low GI period (9.8$\pm$1.9%; p<0.01). Subjects who ↓ the GI of their diets by more than 13% by taking more than 50% of their CHO in the form of low GI foods, showed the greatest ↓ in serum TG. The ↓ in GI showed a significant correlation to the falls in both total cholesterol (r=0.634: p$\leq$0.05) and LDL-cholesterol (r=0.794; p<0.001). The low GI diet did not contain significantly more fibre than the control diet and the macronutrient content of the diet was also kept constant.
Opperman et al, 2004 [75]	Meta-analysis of eleven studies conducted on diabetic (eight studies), healthy (two studies) and CHD subjects (one study)	There was no significant change in overall mean HDL-cholesterol concentrations (p=0.23) on a low vs. a high GI diet and no significant change in HDL-cholesterol levels in CHD subjects (p=1.0). This could be attributed to the length of the studies.

Table 6/......

Table 6 (continued)

Researchers	Study population	Results
Opperman et al, 2004 [75]	Meta-analysis of ten studies conducted on healthy (two studies), diabetic (eight studies) and CHD (one study) subjects	There was a non-significant ↓ in LDL-cholesterol concentrations on a low vs. a high GI diet (p=0.06) and no significant difference in the ↓ LDL-cholesterol concentration on a low vs. a high GI diet in CHD subjects (p=0.37).
Opperman et al, 2004 [75]	Meta-analysis of 13 studies conducted on healthy (two studies), diabetic (nine studies) and CHD (two studies) subjects	There was an overall statistically significant ↓ in total cholesterol levels on a low vs. a high GI diet (p<0.001) and a larger ↓ in total cholesterol concentrations in subjects with ↑ total cholesterol levels at baseline. However, the two studies on CHD subjects conducted by Frost et al (1996 and 1998) found no change in subjects with CHD.
Opperman et al, 2004 [75]	Meta-analysis of 14 studies conducted on healthy (two studies), diabetic (ten studies) and CHD (two studies) subjects	There was an overall non-significant improvement in TG concentrations on a low GI diet (p=0.73) and no change was observed when the studies were subdivided. This could possibly be due to the fact that not all factors that influence serum TG levels were controlled for in all the studies.

The following has been recommended in the dietary treatment of hyperlipidemic subjects:

- legumes, which form a major part of the diet in rural Africa, India, Asia and Latin America and are so absent from the modern industrial Western diet, should be reintroduced into the diet [1];
- the dietary stimulus to insulin secretion should be reduced, as several studies with high fibre diets have shown that serum TG levels could thus be reduced [12];
- a possible role for whole grains e.g. parboiled cracked wheat (bulgur) and Pumpernickel bread, has been suggested, as quite a few studies have shown beneficial effects of traditional methods of processing cereals [19];
- Taking 5-15g of soluble fibre like guar, pectin, psyllium or beta-glucan (in oats and barley) has been suggested, as researchers like Anderson et al (1990) and Jenkins et al (1993) have demonstrated that taking soluble, viscous fibre in these amounts on a regular basis led to a reduction in serum cholesterol levels by 5% or more, whether the fibre intake was increased by means of foods or supplements. Many high-fibre foods that lower LDL-cholesterol also have low GI values, e.g. barley, beans, etc.[77]

2.3.1.1.5 Hypoglycemia

Jenkins et al (1983) advised that using the classification of foods according to their effect on blood glucose levels, i.e. the GI, is useful for post-gastric surgery patients, who suffer from hypoglycemia after large rises in blood glucose and insulin levels after meals, due to the differences in blood glucose and insulin responses from one CHO food to another [32], whereas these patients previously had to avoid CHO rich sources for quite a while. Kaufman et al (1996) showed that episodes of nocturnal hypoglycemia were reduced when lower GI foods were incorporated into the diet.[63]

2.3.1.1.6 Cancer

A modest but positive association between GI or GL and breast cancer risk was shown in a study, which supported the role of hyperinsulinemia in breast carcinogenesis, as insulin might raise levels of insulin-like growth factors, which might influence breast cancer risk.[10] An Italian case-control study by Franceschi et al (2001) showed that the higher the GI and GL of the diet, the greater the risk of colorectal cancer.[10,78]

2.3.1.2 Mood and cognitive performance

Benton et al (2003) found that the GI also had relevance for memory.[21]

2.3.1.3 Sports Performance

The GI also has relevance for sports performance. Table 7 summarizes a number of studies and their findings on the relevance (or not) of the GI for sports performance.

Table 7: Summary of studies done on the relevance (or not) of GI for sports performance

Researchers	Results/Findings		
	Pre-exercise	During exercise	Post-exercise
Thomas et al, 1991[17]	Foster et al (1979) found that, when high GI glucose was taken 15-60min before exercise, a rapid ↑ in blood glucose and insulin concentrations ↓ endurance. The ↑ use of glycogen during exercise could possibly be attributed to an inhibition of FFA mobilization, caused by the insulin surge in response to the consumption of high GI CHO pre-exercise.	Coyle et al (1986) found that high GI CHO (e.g. glucose polymers) taken during exercise maintained blood glucose levels and ↓ onset of fatigue, by providing exercising muscles with CHO, when muscle glycogen levels became low. Gollnick et al (1981) showed that glucose uptake by skeletal muscle was ↑ during exercise.	
Thomas et al, 1991[17]	Low GI lentils led to a significantly ↑ endurance (20min), when eight trained cyclists pedaled to exhaustion at 67%VO$_{2max}$, 1h after equal CHO portions of low GI lentils, high GI potato, high GI glucose or water. The low GI meal caused ↓ hyperglycemia and hyperinsulinemia before exercise, but healthy blood glucose levels and higher blood FFA during exercise. The lentils and water also caused significantly ↓ levels of plasma lactate (before and during exercise) than the higher GI meals (p≤0.05). The average respiratory exchange ratios (RER) and CHO oxidation during the first 90min of exercise were significantly ↓ in the low than in the high GI group, suggesting ↓ glucose oxidation. This could imply that glycogen stores were being depleted at a ↓ rate after low than after high GI food, confirming the findings of Bergstrom et al (1967) and Jansson et al (1980).		

Table 7/…

Table 7 (continued)

Researchers	Results/Findings		
Franz et al, 1994 [18]			Costill et al (1981) and Burstein et al (1985) reported that the immediate consumption of easily digested (or high GI) CHO ensured a higher degree of repletion of muscle glycogen stores. For the athlete with diabetes, this may also help to prevent post-exercise hypoglycemia.
Febbraio et al, 1996 [79]	Similar results to Thomas et al (1991) were found regarding blood glucose, insulin and FFA levels, in a study conducted on 6 endurance trained men who cycled 120min at $70\%VO_{2max}$ and then did a 15min performance test, 45min after consumption of a low GI meal (lentils), a high GI meal (mashed potato) or a control meal (diet jelly). No differences were found between RER and CHO oxidation of the low and high GI meals or in serum lactate levels during rest or sub-maximal exercise when comparing the 3 trials, although lactate concentrations were higher in all 3 trials after commencement of exercise. There was also no difference in muscle glycogen concentration at rest or post-exercise, when the three trials were compared, although exercise resulted in a lower glycogen concentration at the completion of exercise.		

2.3.2   Need for expanding food lists

GI values are now available for a substantial number of CHO sources [22,30] and an increasing number of food companies all over the world are marketing low GI foods.[21] Including the GI in food labeling may eliminate problems with understanding of the terms "complex and simple CHO". In order to implement a well-balanced low GI diet, however, a much wider range of low GI products will have to be available, particularly for breakfast cereals and breads in SA [23], which constitutes 50% of the CHO of the typical western diet.[25] Currently few low GI breads, muffins, scones and breakfast cereals are available in SA.[23] The challenge to the food industries therefore exists to develop new and palatable low GI foods that also have to be tested for their GI [23,25], as many people see low GI foods as less acceptable, e.g. legumes and heavy breads. This will give food companies a new marketing edge, with long-term benefits to public health.[25] According to Golay et al (1992) the technological means exists to lower the GI of starches significantly by choice of raw materials and minimizing processing, and that this is especially important, as metabolic control improves significantly in type 2 diabetic subjects, when as little as the conventional high GI breakfast is replaced by a low GI one.[23] Expanded foods lists, that have been tested and classified according to their GI values, are also needed.[49]

2.4   Evolution of measurement

2.4.1   Reflection on methodology and interpretation

2.4.1.1   Techniques

The GI was originally based on measuring glucose responses in whole capillary fingerprick blood, as this method of blood sampling is fairly simple and non-invasive, which allows for extensive GI testing on different foods.[1] However, many laboratories use venous blood to conduct GI tests [39,80,81,82], as venous blood was regarded as an acceptable method of blood sampling for GI tests by the FAO/WHO (1998). However, as glycemic responses in capillary blood are greater than those in venous whole blood or plasma, allowing detection of smaller differences in glycemic responses to different foods, capillary blood sampling was preferred for determining GI values.[3]

According to Pileggi et al (1974), whole capillary blood can be used, as is with enzyme-impregnated strips, for the self-monitoring of blood glucose (SMBG). Venous blood, however, is usually centrifuged and the serum or plasma thereof is used for enzymatic glucose determination. Therefore the glucose concentration that is measured may differ. Glucose is freely diffusible between plasma and red blood cells and the glucose concentration therein is the same. However, the water content of red blood cells is 72%, whereas that of plasma is 94%. Gannon et al (1987) therefore concluded that the true glucose concentration in whole blood is a function of the hematocrit. Whole blood glucose would be 2.3mmol/L for a plasma glucose reading of 2.59mmol/L based on a hematocrit of 45, indicating that the plasma glucose reading is 11.2% higher than the reading in whole blood glucose. The glucose reading in venous blood drawn from a peripheral or antecubital vein may be lower than that in an artery or capillary blood from a finger, as the removal of glucose by the body tissues like the skin and muscle of the forearm can be substantial [61], especially after glucose has been ingested, due to the stimulation of glucose uptake by insulin.[21] Figure 2 shows the relationship of venous and capillary whole blood and plasma to each other. It can be seen that whole blood usually yields lower readings than plasma, and venous blood yields lower readings than capillary blood.[83]

```
Capillary plasma e.g. 11.88mmol/L  ←───  Venous plasma e.g.10.84mmol/L
        ↑                                          ↑
      +11.2%              -3.2%                   +11.2%
Capillary whole blood e.g. 10.5mmol/ ───→
        ↑
      +9.6%              Venous whole blood e.g. 9.58mmol/L
```
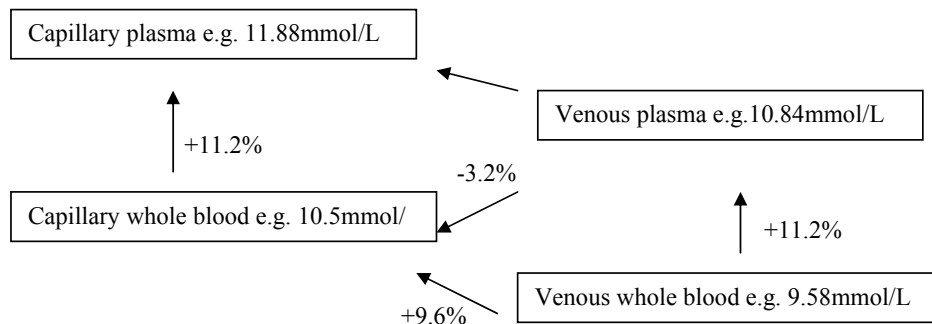
Figure 2: Relationship of specimen type [83]

2.4.1.1.1 Laboratory testing

- **Venous Blood**

Venous whole blood or plasma can be used for blood glucose determinations. A method like the HK-G6PD (which uses a hexokinase method) and an instrument like the Hitachi 7170 automated chemical analyzer can be used to measure blood glucose in both whole venous blood or plasma.[83]

- **Capillary blood**

Capillary whole blood glucose or plasma glucose can be determined by various laboratory instruments, e.g. the YSI and the Analox glucose Analyzerr, which both use a glucose oxidase method and can measure whole blood or plasma; the Nova technique, which also uses a glucose oxidase method, but accepts whole blood without dilution; the APEC glucose analyzer, which can measure whole blood or plasma [84] and the Beckman Dri-stat glucose HK-endpoint, which uses a hexokinase glucose-gamma-phosphate dehydrogenase method and which measures capillary plasma.[85]

2.4.1.1.2 Self-Monitoring of Blood Glucose (SMBG)

Reports on enzyme-impregnated strips (precursors of the dextrostix) for rapid assessment of glucose levels date back to the late 1950's. The very first volume of Diabetologia (1965) contained a detailed report on the quality and "clinical application" of this new technology in diabetes. Several potential clinical applications were discussed, but nothing was mentioned regarding the most important issue, which is to encourage the patient to determine his/her own blood glucose levels. In actual fact, it took another 15y until Sönksen and Tattersall finally started to turn over this long-available technology to the patient for SMBG – an element without which modern diabetes care is difficult to imagine.[24]

Glucometres for SMBG are available from several different manufacturers and usually work with a glucose oxidase method, although some are based on the hexokinase method.[84] The analytical performance of glucometres decreased between 1990 and 1996, which could possibly be attributed to the aging of meters. However, this was restored between 1997 and 1999, although the technical accuracy of glucometres does not seem to have improved further since then and up to 2003.[37] The test strips of most glucometres are calibrated to a reference method, such as the YSI [84], as is the case with the MPQIDG.[36,37] Although most glucometres use whole blood, they actually analyze plasma glucose.[84]

During the last decade most glucose metres were calibrated with a capillary whole-glucose technique, such as the YSI as reference. However, some manufacturers, like Abbott/Medisense, changed their calibration method after 1998 from capillary whole blood to capillary plasma, which can explain the relative improvement in the performance of glucose metres from 1996 onwards.[37] However, this will also have the effect that, if blood glucose readings from glucometres such as the MPQIDG are used, the results will be higher than the results obtained on the same samples using the YSI, if the YSI was used to measure whole blood.[83] Whole capillary blood and plasma concentrations are closely related, although whole blood readings tend to be 10–15% lower than plasma from the same specimen and the effect is hematocrit-dependent. However, the one can be calculated from the other if the hematocrit is known, but plasma eliminates the variable, hematocrit, and should therefore give more reliable results.[84]

Glucometres are convenient for measuring glycemic response and for conducting GI tests. However, the performance of only one metre, i.e. the Once Touch Ultra (OTU), has been evaluated for this purpose and it was found that the AUC and GI values were more variable and did not agree well with those obtained by the YSI. It was therefore not recommended for determining AUC or GI values in normal subjects, but the researchers stated that this does not necessarily apply to other glucometres, whose performance should be evaluated.[34]

2.4.1.2   Validity and reliability

2.4.1.2.1 Laboratory testing

- **Venous blood**

Most instruments are checked against certified serum reference material, e.g. CRM-GN2; HECTEF.[84]

A 4mmol/L lower reading in a blood sample from an antecubital vein was observed, when compared to the reading in arterialised venous blood, both at 60min after ingestion of glucose. Ambient temperature has also been found to affect blood flow rate through the forearm, thereby having a marked effect on venous blood glucose concentrations.[21] Wolever et al (2003) found significantly greater within-subject variation of blood glucose responses in laboratories that measured blood glucose in venous plasma, than those using capillary whole blood or plasma. In the 47 capillary blood subjects in an inter-laboratory study, the mean CV of the reference food (i.e. $23\pm2.1\%$) was significantly less than in the 21 venous blood subjects (i.e. 56.8 ± 4.4) ($p<0.001$). They also found by linear regression analysis, that type of blood sampling, i.e. venous vs. capillary, explained 47% of the variation in the CV of the reference food ($p<0.0001$). It was found that the use of venous plasma showed greater within-subject variation of both glycemic responses and GI values, and non-normal distribution of GI values. The recommendation of the FAO/WHO Expert consultation on carbohydrates in human nutrition was thus confirmed, i.e. that capillary rather than venous blood sampling yield the most precise and accurate GI determinations, although these researchers recommended that prospective studies be done to confirm this.[39]

Capillary blood sampling is furthermore regarded as a more valid method of blood sampling than venous blood sampling, due to the fact that there is a lack of relationship between the mean GI value for a specific food in a specific individual and the mean AUC for the reference food when capillary blood is used, whereas there is a significant correlation in the case of venous blood. When there is a strong correlation between a ratio and its denominator it indicates that the ratio does not control adequately for the denominator, which is a common problem when using ratios. This is important, because the GI is supposed to control for the glycemic responses of different subjects, which does not happen when venous blood sampling is used for GI testing.[39]

Arterialised venous blood can be used for GI determinations, but the use of normal venous blood should be discouraged.[21]

- **Capillary blood**

As mentioned before, whole capillary blood and plasma concentrations are closely related, although whole blood readings tend to be 10–15% lower than plasma from the same specimen and the effect is hematocrit-dependent. However, the one can be calculated from the other if the hematocrit is known, but plasma eliminates the variable hematocrit and should therefore give more reliable results. Theoretically, test strips using the direct-reading electrode system should be unaffected by hematocrit, especially those based on plasma.[84] Brouns et al (2005) stated that both whole capillary blood and plasma can be used, provided one or the other is used consistently.[21] Fingertip capillary blood showed the greatest sensitivity when it was compared with capillary blood from other sites, e.g. the forearm, thigh or abdomen [by researchers such as Ellison et al (2002)] and Jungheim & Koschinsky (2002) and should therefore be preferred.[21]

The CV of the YSI was <3% during a specific study period.[37] For typical clinical laboratories the CV is usually <3% [34] or at least within the level of 5%, which is usually regarded as acceptable for laboratory instruments.[35]

2.4.1.2.2 Self-Monitoring of Blood Glucose

There is generally good agreement between the glucose readings measured in capillary blood by SMBG systems or glucometres and that measured in serum or plasma by laboratory equipment. The strength of the correlation varies according to the glucose concentration and a reduced accuracy is often seen at both extremes of glucose concentration.[86] However, the MPQIDG did not show a larger deviation from the reference value at higher blood glucose readings, as was the case with other metres that were evaluated in a study, i.e. the Advantage, Glucometre Elite and Mini-Accutrend [35] and a lower CV (<3%) was observed at higher readings (>13.2mmol/L).[36] A higher CV (5.6%) was observed at very low readings (<5.5mmol/L), whereas a CV of 3.4-3.7% was seen within the normal range of 5.5-13.2mmol/L.[36] According to researchers like Widjaja et al (1999) and Lifescan (2005) the CV of analytical variation for measuring glucose using dry chemistry analyzers, such as glucometres, is typically <8%[34], although some glucometres, such as the MPQIDG using the new test strips, have a lower CV, i.e. 2.1-5.6%, depending on the range of glucose readings.[36]

Factors that may influence the results of SMBG include the following:
- Variations in hematocrit. Blood glucose readings may be markedly affected by anemia or polycythemia. Anemia may cause falsely high and polycythemia falsely low readings. The extent of this effect may vary from 4–30% for every 10% change in hematocrit, depending on which device was used.[86] Theoretically, test strips using the direct reading electrode system should be unaffected by hematocrit, especially if plasma is used, as plasma eliminates the variable hematocrit and should therefore give more reliable results.[84] The MPQIDG produced accurate results across a hematocrit range of 20–70% [36];
- Altitude [87], although this does not affect some of the newer metres, e.g. the MPQIDG.[36]
- Environmental temperature and humidity [87];
- Hypotension [87]
- Hypoxia. [87]
- TG concentrations.[87]

- Hypoglycemia and hyperglycemia. Blood glucose readings may be unreliable when a person's readings are either very low or very high [86], although this is not the case with all metres, including the MPQIDG; specifically pertaining to very high readings.[35,36]
- Dilution of the blood sample by interstitial fluid, yielding a falsely lower reading.[88]
- Hemolysis, causing a falsely higher reading.[88]
- Standing or reclining, although this does not affect some of the newer metres, e.g. MPQIDG.[36]
- Quality of lancets and collection devices that are used. A spring-loaded device with a chisel or blade type lancet should preferably be used and enough force should be used to make a good puncture or incision in the skin of the finger.[89]
- User variability.[86]
- Instrument malfunction. Fortunately this seems to be uncommon.[86]
- Defective reagent strips.[86]

Overall performance of SMBG systems is mainly a combination of the analytical performance of the instrument, user proficiency and the quality of the test strips.[87]

- **Analytical performance of the instrument**

The Diabetes Association Consensus Statement of 1987 recommended that the performance goal of all SMBG systems should achieve a total error (analytical plus user) of less than 10% at glucose concentrations ranging from 0.777–10.363mmol/L. However, this goal has apparently not been achieved for most SMBG systems and therefore it was recommended that the goal of the manufacturers of SMBG monitoring devices should be to make future SMBG systems with an analytic error of as little as +5%.[87] The metres that are used nowadays, are smaller, easier to use, yield a reading more quickly, using a smaller amount of blood and are designed to minimize user errors. This is achieved by, amongst others, the introduction of electron-mediated glucose oxidase chemistry, instead of the less reliable photo reflectance technology, which was used in older metres. With the latter metres, blood may occlude the optical window, causing erroneous readings due to interference with the optical system, and therefore has to be cleaned regularly. In addition, the blood needs to be wiped from the strip, which is an extra hassle and source or error. The American Diabetes Association reports that it has been shown that the fewer the steps that are involved, the fewer operator errors will occur. Common user errors, e.g. incorrect timing and insufficient amount of blood, have been overcome with better design of most of the new glucometres.[35]

Some metres, like the MPQIDG, have an automatic starting mechanism, which only commences once sufficient blood is placed on the strip. This feature eliminates the error of insufficient blood, which leads to falsely low readings.[35] In addition, the MPQIDG needs only 5µL of blood for a test [90], whereas some glucometres require twice as much blood. The advantages of a smaller blood sample are: a smaller gauge lancet can be used, which causes less pain and "milking" of the finger is minimized, meaning a better drop of whole capillary blood for a more accurate result. The MPQIDG also has no timing requirement, which minimizes user errors and Stenger et al (1996) reported that it is the only glucometre that is accurate and precise for monitoring blood glucose in pregnant women with diabetes, probably due to the presence of a third electrode. This meter has been clinically proven to be accurate in controlled laboratory studies and in the real world, when used for blood glucose testing by people with diabetes. Various clinical trials have demonstrated that the MPQIDG provides consistent accuracy, unaffected by the various challenging conditions that affect other glucose metres.[91]

The MPQIDG shows sufficient accuracy, as it showed the smallest deviation from the reference method (YSI) across the whole range of mean glucose levels, when compared to five other glucometres. The values also fell within the clinically acceptable zones of A and B[2], using error grid analysis.[35] However,

the precision of the MPQIDG, i.e. a CV of 8%, was not within the level of 5%, which is usually regarded as acceptable for laboratory instruments [35] or ≤3%, which is regarded as precise enough for scientific purposes, such as GI determinations.[21]

The precision (CV) of the new test strip, that was introduced after 1997 and which has been calibrated to produce capillary plasma results, as obtained by the YSI [37], is shown in Table 8. The CV of this new test strip ranged from 2.1-5.6% over a range of blood glucose readings from 2.2-26.0mmol/L, for 80 replicate tests across four lot numbers, using venous blood and fell mostly within the recommended level of 3-5%, with the only value above 5% being that for very low blood glucose readings. In addition, the accuracy of the metre, as obtained by 203 lay users, was comparable to that obtained by trained healthcare prefessionals (r=0.990, mean absolute bias: 5.6%) and the laboratory plasma method (r=0.982; mean absolute bias = 7.2%). Very good correlation was also found between the MPQIDG and the laboratory method (YSI plasma glucose)(r =0.982 and r = 0.982) respectively for lay users and trained operators and between the MPQIDG and the laboratory method (YSI whole blood glucose)(r = 0.98 and r=0.979, respectively for lay users and trained operators) (p-values were not provided).[36]

When lay users completed a questionnaire, a high rating of 5.5, out of a scale of 6, was accredited to the new test strip for ease of use. The MPQIDG also produced accurate results at high altitude, across a hematocrit range of 20–70% and additional studies showed that no clinically significant effect on the accuracy of the new test strips was found with the following: metre movement, oxygen levels, sample re-application, touching and smearing blood on the test strip and 50 drugs or endogenous substances.[36]

Table 8: Precision (CV) of the MPQIDG [36]

Mean	2.2	5.5	8.2	13.2	18.6	26.6
SD	0.12	0.2	0.29	0.47	0.52	0.56
CV (%)	**5.6**	**3.7**	**3.4**	**3.5**	**2.8**	**2.1**

N=80 each. Results in mmol/L

- **User proficiency**

The accuracy (i.e. the ability to obtain true values) and precision (i.e. the ability to obtain reproducible results) of high-quality near-patient testing devices are generally acceptable or even excellent, when used by trained laboratory technicians under carefully controlled situations.[86,88] However, when these metres are used by patients, they are not always adequate for making clinical decisions.[86] Kabadi et al (1994) found a highly significant correlation between clinic meter readings by trained laboratory personnel and laboratory venous determinations (r=0.93, p<0.00001), so much so that the former may be a reliable alternative to the latter for comparison with readings obtained by the subjects, using their own metres and test strips in the clinic, especially in the absence of a suitable clinical laboratory for venous glucose determinations.[92]

The Consensus Statement on SMBG (1987) stated that about 50–70% of individuals who have received some kind of formal training could obtain results that are within 20% of the reference method (i.e. zone A), although it has been found that performance may deteriorate over time. The most common reason for errors in SMBG is failure to comply with the instructions regarding the proper application, timing and removal of the blood sample.[86] This was confirmed by Kabadi et al (1994), who showed that "clinically acceptable user proficiency" (zone A of error grid analysis, when comparing patients' glucometre readings with both laboratory readings or the clinic meter) "in capillary blood glucose testing can be maintained in most subjects, with recurrent intensive education" and usage of their own glucometres. [92] This was furthermore confirmed by an Abbot White Paper (1997), which reported that excellent correlation was found between the MPQIDG and the laboratory method (YSI plasma glucose), and very

similar values (i.e. r=0.982 and r=0.982 respectively for lay users and trained operators) and between the MPQIDG and the laboratory method (YSI whole blood glucose) (r=0.98 and r=0.979 respectively for lay users and trained operators) (p-values were not provided).[36]

When fingerprick blood samples are obtained in accordance with the appropriate guidelines for reliable fingerprick collection and improved lancets and collection devices are used, fingerprick capillary measurements can be equivalent to conventional venous determinations. Warnick et al (1994) found fingerprick results, whether measured by a conventional enzymatic laboratory method or by a metre designed for on-site testing, to be in acceptable agreement with the accepted reference method for accuracy. Although they observed better agreement with fingerprick sampling when taken by a senior medical technologist, who was well experienced with fingerprick sampling, even an inexperienced laboratory assistant was able to obtain acceptable agreement, when the person was adequately instructed. Their recommendations for collection of fingerprick blood samples are listed in Table 9.[89]

- **Quality of test strips**

Inaccurate results may be obtained by using strips that are outdated or have been improperly stored.[86]

Users are largely dependent on the manufacturer of glucometres to ensure that each test strip is of a high quality. A user cannot be expected to both verify that a specific test strip is accurate and at the same time to use it to test a blood sample. There can be significant within-lot and lot-to-lot variation in strips and their use can be influenced negatively by environmental factors. It is therefore recommended that every effort should be made by the manufacturers of glucometres and test strips to narrow down the acceptable range specified for the control solutions that are used to check the functioning of the system and in teaching patients SMBG.[87]

Recent advances, especially the development of the so-called "reagent test strip", has made the extra-laboratory (EL) testing an inexpensive and convenient measuring of blood glucose and cholesterol possible.[88]

Table 9: Recommendations for obtaining fingerprick blood samples [89]

Operator preparation
Assemble the necessary supplies, i.e. gloves, antiseptic pads, lancets, capillaries and band-aids.

Patient preparation (which will be the same as the operator, in the case of SMBG)
He/she should sit quietly for at least five minutes before blood collection should commence. Values could change with standing or reclining. The patient can complete forms at this time or read relevant literature. If the patient must move a short distance, he/she should walk there quietly.

Check hands
Warm hands usually bleed better. If the hands are cold, the patient should rub them together or shake them vigorously for several minutes.

Select finger
The non-dominant hand and the ring finger thereof are recommended, due to the presence of fewer calluses on the latter. The middle finger might be better on women or children with small hands. Squeeze and release the chosen fingertip a few times. The "flushing of colour" into the area is an indication that there is a good flow of blood.

Cleansing
Cleanse the end of the finger with an alcohol or antiseptic pad. Dry the site thoroughly with a sterile piece of gauze or cotton wool.

Fingerprick
Pinch the end of the finger from the opposite side of the puncture site to keep the skin at the puncture site in a good condition. Preferably use a spring-loaded device with a chisel or blade type lancet and use enough force to make a good puncture or incision. With the palm of the hand facing upwards, prick the upper side corner of the chosen finger, up away from the nail bed. See to it that the lancet blade cuts across the fingerprint. The lancet should be held tightly against the skin of the finger, activated and the pressure should not be released during the puncture.

Blood collection
Remove the lancet and wipe away the first drop of blood that could be contaminated with tissue fluid or alcohol, using a sterile piece of gauze. Turn the palm down and allow drops to form and touch onto the test strips. When the collection has been completed, place a sterile pad over the puncture site and maintain pressure until the blood flow has stopped.

Stimulating blood flow
If the blood flow is too slow, the following may help:
  Lower the hand.
  Express blood down from the hand towards the finger by progressively squeezing gently and releasing the blood downward across the hand and finger in a "milking" motion. Do not squeeze the puncture site directly, as that can cause dilution with tissue fluid.
  Wipe the puncture site with gauze, as that can help to clear a developing clot and promote blood flow.
  Try to pull the cut open carefully with the fingers, in order to restore blood flow.

Precautions
Contaminated materials should be discarded safely, in accordance with accepted guidelines.

2.4.1.3   Advantages

2.4.1.3.1 Laboratory testing

- **Venous blood**

None, as venous blood collections are fairly invasive [1] and requires large blood volumes (40-50μL of blood).[83]

- **Capillary blood**

The GI was originally based on measuring glucose responses in whole capillary fingerprick blood, as this method of blood sampling is fairly simple and easy to perform, requires less training, is less invasive than conventional venous blood collections [1, 88, 91], is therefore more convenient to use [91], has less potential for negative consequences [89], are less variable than those obtained using venous blood [3] and allows for extensive GI testing on different foods.[1] Unlike venous blood determinations, readings are more cost effective and available immediately [92], unless of course the blood samples are frozen.[13]

2.4.1.3.2 Self-Monitoring of Blood Glucose

All the above-mentioned advantages of capillary blood glucose testing also apply to self-monitoring of blood glucose. Unlike venous blood determinations, readings are much more cost effective [92], even more so than capillary blood that is determined by laboratory equipment.

Blood glucose monitors are lightweight, portable and easily calibrated, although their size, shape and calibration techniques usually vary from one manufacturer to another. All metres are powered by batteries and use solid-state electronics.[87] They are inexpensive, convenient to use, require little training and yield quick results.[34] Subjects can also take blood glucose measurements in the comfort of their own homes and need not go the clinic or laboratory for blood glucose determinations [92] and blood glucose readings are available within 20sec [36,92], especially as blood is never frozen for SMBG.[90]

2.4.1.4   Limitations

2.4.1.4.1 Laboratory Testing

- **Venous Blood**

As mentioned before, obtaining venous blood is fairly invasive, difficult to perform and requires more training.[1,88,89] In addition, it is not convenient [91], has more potential for negative consequences [89], blood glucose readings are not available immediately and are more expensive [92], which does not allow for extensive GI testing.[1] In addition, a fairly large blood volume (i.e. 40–50μL) is required[83] and venous blood is not ideal for blood glucose determinations and GI tests, as body tissues, like the skin and muscle of the forearm, consume glucose, causing the concentration of glucose in a peripheral or antecubital vein to be lower than that in an artery or capillary blood.[21,61] Ambient temperature can affect blood flow rate through the forearm, thereby having a marked effect on venous blood glucose concentrations.[21] It was also found that the use of venous plasma showed greater within-subject variation of both glycemic responses and GI values, and non-normal distribution of GI values. Furthermore there is a lack of relationship between the mean GI value for a specific food in a specific individual and the mean AUC for the reference food when capillary blood is used, but not when venous blood is used, indicating that the

ratio does not control adequately for the denominator, when venous blood sampling is used for GI testing.[39] Results are also not always available immediately, if the samples are frozen.[13]

- **Capillary Blood**

More blood is required (40-50µL) for most capillary blood glucose determinations using laboratory instruments [39], as opposed to as little as 3.5µL of blood for the MPQIDG.[36] In addition, laboratory analysis of capillary blood is also more expensive than using glucometres and test strips, which does not allow for extensive GI testing.[1] Results are also not always available immediately, if the samples are frozen [13] and subjects cannot perform these tests in the comfort of their own homes.[92]

2.4.1.4.2 Self-Monitoring of Blood Glucose

For typical clinical laboratories, the CV is usually <3% [34] or at least within the level of 5%, which is usually regarded as acceptable for laboratory instruments.[35] The CV of analytical variation for the measurement of glucose is consistently ≤3% for the YSI, which is preferable for GI testing [21,34], while that for dry chemistry analyzers, such as glucometres, is typically <8% [34], although it was 2.1-5.6% over a range of blood glucose readings from 2.2-26mmol/L for the MPQIDG, using the new test strip.[36]

Since the within-instrument analytical performance of glucometres and laboratory analyzers is similar, it would seem that the higher variation in EL readings could be attributed to:

- differences in sampling due to deficiencies in training [88], e.g. regarding calibration of the metre, control solution handling and testing, pricking the finger at an appropriate place in the lateral part of the fingertip, performing a capillary whole blood glucose test by wiping away the initial drop and using an adequate second drop of blood and being monitored by the same diabetes educator and using a skills assessment checklist (Table 10). If subjects are unable to achieve clinically acceptable blood glucose readings, when compared with laboratory values, they should enter a second phase of training to assess whether they would improve with continued observation, education and counseling over a longer period. Even subjects who are proficient in their testing, should be assessed regularly in order to assess whether they maintain proficiency or become slack with time, and special emphasis should be placed on correction of technique errors (if noted) and reinforcement of proper storage and handling of test supplies. In a study performed by Kabadi et al (1994), all subjects obtained zone A acceptabitlity on an error grid analysis, when the results they obtained on their own glucometres were compared with clinic metre readings and laboratory readings [92];

- non-uniformity of calibrating instruments (refer to 2.4.1.5);

- lack of internal quality control (refer to 2.4.1.5);

- the absence of an external quality-assessment program.[88] A quality-assurance program should involve periodic monitoring of control solutions at both high and low concentrations. If such quality–assurance measurements were performed at frequent intervals, it would increase the cost of SMBG, which could create resistance to performance of quality assurance by patients, EL. In addition, testing of the instrument with control solutions does not monitor the quality of the collection procedure or proper application of blood to the test strip, which boils down to user training and monitoring. A complete quality-assurance program would evaluate the reliability of user-generated SMBG data, i.e. proper function of the metre, reagent strips and user. The development of such a program was recommended by the Panel [86,87];

- using older glucometres. Many users of older metres get inaccurate readings, due to errors that arise from user and system errors.[35] The FDA reported that about 75% of errors in SMBG could be attributed to user errors, that can mostly be blamed on designs and features that allow too much room for user errors, e.g. applying too little blood to the test strips, touching the target area, difficult timing specifications, incorrect capping of vials of test strips (which allows undesirable exposure to moisture), inadequate cleaning of optical windows of photo reflectance meters and operating in environmental conditions that do not comply to the metre's specifications e.g. regarding light, which are all more relevant for older metres.[91] However, none of these are applicable to the MPQIDG, using the new test strip.[36]

Several researchers have shown that the first four factors mentioned above definitely improve the quality and accuracy of EL blood test results.[88]

Table 10: Skills assessment checklist

Testing procedure:
Calibrate glucometre with calibrator of new box of strips (if applicable) and check that test strips have not yet expired.[90]
Open foil package by tearing at the notches in the foil and remove one end of foil packet to expose contact bars at the end of the electrode, if applicable.[90]
Insert electrode into Sensor by pushing it in gently until it stops, with contact bars facing upwards, and removing foil packet from other end of electrode packet.[90,92]
Fingerprick sampling:
Site selection.[92]
Site cleaning [92] or wash hands with soap and water and dry thoroughly.[90]
Obtain a blood sample by using a spring-loaded device with a chisel or blade type lancet and enough force to make a good puncture or incision in the skin of the finger [89], as well as appropriate technique.[90]
Obtain adequate drop.[90,92]
Softly touch blood to the target area while the display read "rdy".[90]
Do not smear sample [92], although this seemed to cause no significant difference in readings obtained, in some of the newer metres, like the MPQIDG and its new test strips.[36]
Blood drop covered area.[92]

The Panel of the Consensus Statement on SMBG recommended that the following components be incorporated into training programs:

- The subject's ability to use a SMBG device properly should be assessed by a health-care professional.
- A qualified trainer should train the subject.
- The trainer should demonstrate proper procedures and techniques and visual interpretation should be included whenever possible, in order to verify glucometre results.
- The subject should show proficiency in operating the device.
- If the subject does not show proficiency, he/she should practice until the procedure is correct.
- The principles and importance of quality control should be explained to the subject.
- The trainer should assess the subject's skills immediately.
- The trainer should also reassess the skills of the subject from time to time.
- The subject should be instructed on how to operate the system that will be used EL.[86]

2.4.1.5 Grey areas

2.4.1.5.1 Laboratory testing

- **Venous blood** (refer to 2.4.1.4)

- **Capillary blood** (refer to 2.4.1.4)

2.4.1.5.2 Self-Monitoring of Blood Glucose

Unlike diagnostic clinical chemistry laboratories, IL and EL GI testing facilities in South Africa (and many other countries world-wide) are currently not subjected to compulsory external quality assessment programs or accreditation with an appropriate authority. Relevant quality-control schemes and accreditation requirements for EL services will enhance the quality and accuracy of testing.[88]

Variability between venous and fingerprick blood glucose or cholesterol determinations can often be attributed to the greater potential of this method for technique-related variability [88,89], due to dilution of the blood sample by interstitial fluid, yielding a falsely lower reading, or hemolysis, causing a falsely higher reading.[88] However, these can be overcome by appropriate training, as mentioned above.

Many different glucometres are available on the market, of which the effective concentration range depends on the specific device, but generally covers the clinically relevant range.[86] In an independent head-to-head comparison of blood glucose metres with a reference method (YSI), all values obtained by the metres fell in the clinically acceptable zones of A or B[2], using error grid analysis.[35] A highly significant correlation was found between the clinic Companion 2 meter readings and laboratory values (r=0.93, p<0.00001) and 75% of comparisons between capillary blood glucose readings obtained by subjects, using the Companion 2, and laboratory values fell in zone A on the error grid, within the first month of training. By the end of the study period, the comparisons in all patients achieved zone A-acceptability, when comparing capillary blood glucose readings obtained by subjects, using the Companion 2, with the Companion 2 clinic meter and laboratory readings. The investigators were of the opinion that this finding could be extended to other glucometres as well.[92] The MPQIDG showed the smallest difference from the reference method (YSI Analyzer) across the entire range of mean glucose levels in a study done by Engel et al (1998). The Advantage, Elite and Mini-Accutrend showed a larger deviation from the reference value with higher blood glucose levels, although these discrepancies were not clinically significant; this was, however, not seen with the MPQIDG, Companion 2 or Lynx

glucometres. The CV (%) of the Advantage, Glucometre Elite and Mini-Accutrend were within the level of 5%, which is generally considered to be acceptable [35] or even sufficiently accurate and precise [86] for laboratory instruments. The Lynx, Companion2 and MPQIDG were above this level, but still acceptable for SMBG.[35] The new test strip of the MPQIDG has a CV of 2.1-5.6 for blood glucose readings varying from 2.2-26.6mmol/L.[36] This is very good, especially in the light that, according to the American Diabetes Association Consensus Statement, the College of American Pathologists found that the CV within systems ranged from 4 to as much as 33% [87] and the fact that, in general use, up to 50% of obtained values may vary more than 20% from the reference values.[86]

Generally, point-of-care instruments are not regarded as sufficiently accurate or precise to be used for the diagnosis of diabetes or hypercholesterolemia, mainly due to the fact that the user does often not achieve performance goals and therefore it is usually recommended that these diagnoses should be confirmed by laboratory testing. They are generally considered accurate enough for SMBG and screening purposes.[35] However, these metres can actually yield high-quality blood analyzess under ideal conditions [88] and fingerprick capillary blood glucose measurements can be equivalent to conventional venous determinations, provided blood samples are obtained according to the appropriate guidelines for reliable fingerprick collection (refer to Table 7) and improved lancets and collection devices are used.[89] This was confirmed by Kabadi et al (1994) who found a highly significant correlation between clinic meter readings and laboratory values (r=0.93, p<0.00001); to the point that they recommended that the former may be a reliable alternative to the latter for comparison with readings obtained by the subjects, using their own meters and strips in the clinic, especially in the absence of a suitable clinical laboratory for venous glucose determinations. They also showed that "clinically acceptable user proficiency in capillary blood glucose testing can be maintained in most subjects, with recurrent intensive education" and usage of their own glucometres. [92]

Often discrepancies between laboratory analyzed and point-of-care instruments can be attributed to the fact that subjects are sometimes in a non fasting status, there is sometimes a time lapse (even although it is <30min) between the fingerprick test and the withdrawal of venous blood, inadequate technique or inappropriate storage and handling of supplies and equipment by the subjects. However, the time of day, the time that has expired since the previous meal and the time difference between the fingerprick test and the venous blood glucose determinations do not seem to be important variables, as these factors did not appear to influence the comparisons between point-of-care instruments and laboratory values in the majority of the subjects in the studies of Kabadi et al (1994).[92]

The following was recommended by the Panel regarding SMBG systems:
- every effort should be made by manufacturers to develop fail-safe SMBG systems. The metres should be able to identify a faulty operation by the user, as well as to specify the nature of the problem and systems should be less dependent on user skill.[86,87] The metres should also be able to be accurate in the very high and very low blood glucose ranges that would not be affected by changes in hematocrit, and should also be manufactured in such a way that, after the occurrence of a malfunction, they cannot be used without recalibration and verification that the metre is operating correctly [86];
- manufacturers should also aim at establishing a uniform standard for the calibration and determination of the accuracy of SMBG systems [87], as well as at developing a system that requires the user to perform standard quality-control and calibration functions before accepting a sample [86];
- comparisons should periodically be made between a reading obtained by the patient with his/her SMBG device and a fasting blood sample, which was obtained at the same time and measured by a reference laboratory [87];

- adequate quality-control programs that employ simple, inexpensive and efficient methods should be developed and implemented that do calibration checks to ensure adequate metre performance; measure control solutions of known glucose concentration to evaluate the performance of test strips and metres; compare results obtained with meters with a laboratory reference method and review user technique periodically; with correction of deficiencies by a qualified health-care professional [86,87];
- the Diabetes Association Consensus Statement (1987) recommended that the future performance goal of all SMBG systems should be to achieve a total error (system plus user) of less than 10% at glucose concentrations ranging from 0.777–10.363mmol/L, 100% of the time. However, this goal has apparently not been achieved for most SMBG systems and therefore it was recommended that the goal of the manufacturers of SMBG monitoring devices should be to make future SMBG systems with an analytic error of as little as +5%.[87] SMBG measurements should be within 15% of the results of the reference measurement. Individuals who do not meet this criterion on a regular basis require further training until this goal is met.[86]

2.4.2 Other relevant concepts

2.4.2.1 Insulin Index (II)

II in a specific person is calculated as the sum of the insulin response during the food tolerance test, divided by the sum of the insulin response during the glucose or standard tolerance test, multiplied by 100 [77]. It is defined as:

$$\text{Insulin Index (II)} = \frac{IAUC_f}{\text{Mean } IAUC_s} \times \frac{100}{1}$$

where:
$IAUC_f$ (above fasting baseline) = Area under the curve above fasting baseline of a food
Mean AUC_s (above fasting baseline) = Mean area under the curve above fasting baseline of three determinations of the standard food.[69]

A good correlation was found between the mean GI and II of 42 products in healthy individuals with normal glucose tolerance. The median GI and II values of products containing added sugars were also not significantly different to that of foods containing naturally-occurring sugars.[27] Generally, the insulin response to foods consumed by healthy subjects follows the rank order of the glycemic response to those foods [27,74] and greater insulin responses are seen when high GI foods are consumed by insulin resistant individuals.[27]

Crapo et al (1977) found those subjects with higher plasma glucose responses to dextrose, and who were therefore relatively most glucose intolerant, showed greater differences in their glucose and insulin responses to the other CHO sources as well than those with lower glucose responses. Although diabetic subjects were not studied, it can be speculated that the differences between the glucose and insulin responses would be even more noticeable in diabetic subjects, necessitating differentiation between different types of CHO for therapeutic purposes in these subjects.[4] An II of foods might eventually be needed to supplement tables of GI values.[30] DeFronzo et al (1991) advised that this could also be relevant in studying the development of hyperlipidemia and artherosclerosis, as high insulin concentrations are usually associated with high blood levels of the detrimental LDL-cholesterol, TG and glucose, as well as with central obesity.[63]

2.4.2.2 Glycemic Load (GL)

There is concern about the potential for rating of foods as "good" or "bad", solely on the basis of their GI value. However, the GL can be interpreted as a measure or indicator of global dietary insulin demand, where the GL of a specific food, as defined by nutritional epidemiologists of the Harvard School of Public Health (who introduced the concept of dietary GL in 1997), is the product of that food's GI and the amount of CHO per serving.[8] Wolever & Bolognesi (1996) found that the amount of CHO alone was not significantly related to the mean glucose and insulin responses, and that the GI of the CHO explained a similar amount of the variability in glycemic responses as the amount of CHO. However, when the amount of CHO and the GI was used, approximately 90% of the variability of the observed mean glucose and insulin responses could be explained (p=0.01). Both the amount and source of CHO determined the glucose and insulin responses in lean, young healthy subjects after consuming different mixed meals with different GI values, and variation in protein and fat intake appeared to have almost no effect on glucose and insulin responses.[93] One of the advantages of the GL is that comparisons of the likely glycemic effect of realistic portion sizes of different CHO foods, with different GI values and differences in CHO density, can be made.[22] This is a measure that takes into consideration both the quantity and quality of the dietary CHO consumed and was introduced mainly to quantify the overall glycemic effect of a portion of CHO food.[68]

$$GL = \frac{CHO \text{ content per serving of food } \times \text{ GI}}{100}$$

2.4.2.3 RAG and SAG Measures

RAG (rapidly available glucose) and SAG (slowly available glucose) are both in vitro measurements that measure the amount of glucose that is released from a test food, when it is incubated for a specific period of time, together with digestive enzymes, under standardized conditions. There is a highly significant correlation (r=0.981, p<0.0001) between glycemic response, the GI values of foods and RAG (rapidly available glucose). Furthermore, a certain percentage change in RAG (which is achieved by altering the type or amount of food consumed) is associated with the same percentage change in glycemic response, which supports the hypothesis that RAG intake largely determines the magnitude of the glycemic response. If between-subject variation is taken out of the equation, 70% of the remaining variance in glycemic response can be explained by differences in the RAG content of test meals. There is no significant relationship between SAG and glycemic response, which demonstrates that the SAG fraction does not contribute to the glycemic response above that which can already be accounted for by the RAG, as it only accounts for 0.8% of the within-subject variance. It is more effective to reduce the proportion of RAG by increasing the SAG content, when the GI of a food needs to be lowered, rather than by increasing the fructose content, as the relationship between fructose and GI (or RAG) is not significant.[94] In addition, as mentioned before, the intake of too much fructose is not desirable.[26]

2.4.3 Factors influencing the glycemic response to foods and the variability of the GI of a food

2.4.3.1 Factors influencing the blood glucose response to foods

The same amount of different CHO does not necessarily cause the same glycemic and insulinemic responses.[1,4] High fibre foods were always thought to have the same effect on glycemic response as intact grains. However, it was found that glycemic response was not lowered by cereal fibre in which the cell structure had been destroyed, as whole meal bread, brown rice and brown pasta have similar GI values to their white counterparts.[1,19,95] The difference in the GI values of various foods may be explained by several factors. It would appear that the rate of absorption [96], as well as digestibility plays a major role in determining the GI of a particular food.[74] Cooked plain legumes produced the lowest rise in postprandial blood glucose levels, when compared to CHO like root vegetables, cereals and cereal products and other CHO foods like fresh peas, baked beans in tomato sauce and canned soya beans. This can partly be attributed to the fact that legumes may contain starch in a form that is more resistant to breakdown by digestive enzymes, causing them to be less digestible.[50] This was confirmed in another study, wherein it was found that each time that blood glucose readings were taken in GI tests, except in the fasting state, the mean blood glucose reading after the consumption of beans, as well as the overall mean blood glucose peak rise and IAUC were significantly lower than that for the other foods that were tested, except for spaghetti and rice, regarding the latter two aspects ($p \leq 0.05$–0.001).[32]

Different starches have different GI values and low GI foods have quite a few effects on the small bowel, possibly due to the following:
- the rate of gastric emptying, where low GI foods tend to slow down the rate of gastric emptying
- an increase in small bowel transit time when it comes to low GI foods
- the physical availability of the starch to the action of hydrolytic enzymes, where low GI foods tend to show a decrease in enzyme-nutrient interaction
- differences in the stimulation of gastrointestinal insulinogenic hormones, where low GI foods tend to slow down the absorption and stimulation of the gut hormones, Gastric Inhibitory Peptide and Glucagon-like Peptide 1.[4,97]

The slower absorption rate of low GI foods in itself leads to lower blood glucose and insulin responses. However, Torsdottir et al (1984) showed that slowing of small-intestinal absorption, rather than delayed gastric emptying, seems to be the major mechanism responsible for the fact that some CHO sources have lower GI values than others.[97]

The large bowel or colon can also have an effect on glycemic response. When CHO absorption is slowed down, it is inevitable that the delivery of CHO to the colon will be increased, although this is too little to explain the differences in glycemic responses of different CHO. However, this delivery of CHO to the colon significantly increases CHO fermentation in the colon.[97] Cummings et al (1987) have shown that the human colon contains a large bacterial population, which synthesizes many enzymes that can catabolize a wide variety of CHO, the end products of which are: bacterial mass, carbon dioxide, water and the SCFA acetate, butyrate and propionate. Hoskins and Boulding (1981) showed that, apart from the small amounts contained in the glycoprotein of the gastro-intestinal mucus, there are two major sources of CHO, which reach the colon. One of these is NSP, of which about 75% is broken down in the colon. About 75% of the energy contained in malabsorbed starch is recovered by the absorption of SCFA and the bacteria of the colon use 25% of this. According to Wolever et al (1986) low GI diets provide larger amounts of fermentable substrate to the colon, as rapidly digested foods with a high GI provide only 1–2 % of starch to the colon, whereas slowly digested foods with a low GI provide as much as 20% of their starch to the colon.[59]

Nutritional factors that are particularly known for their ability to slow down the absorption of starch include the nature of the starch, food form, dietary fibre, antinutrients, enzyme inhibitors and altered food frequency. Differences in food form and degrees of heat treatment can also affect digestibility by altering nutrient delivery to the colon. Foods high in viscous or soluble fibre or antinutrients or those that are resistant to gelatinization are digested and absorbed more slowly and can be called low GI or lente CHO foods. Certain enzyme inhibitors may also cause lente effects and certain small-intestinal effects of lente CHO may be mimicked by a change in feeding frequency.[96]

Table 11 summarizes the factors that influence glycemic responses to foods and therefore the GI and the studies that support this.

Table 11: Factors that affect glycemic responses and GI of foods [adapted from FAO/WHO, 1998[3]]

Factor	Specific food component	Effect on GI	Researchers
Amount of CHO	Not applicable (N/A)	Postprandial blood glucose and insulin levels ↑ with an ↑ in the amount of glycemic/available CHO.	Lineback, 2005 [64]
Nature of the Starch	Amylose and amylopectin	The linear nature of amylose starch causes it to create compact bundles that exclude water, making it resistant to enzymatic attack. High amylose starches also retrograde more readily on processing, making the starch molecule even more resistant to hydration and enzymatic attack. The branched nature of amylopectin starch causes it to be more open, to hydrate easily and to be more susceptible to enzymatic attack.	Jenkins et al, 1995 [97]
	Starch-nutrient interaction	There was a significant inverse correlation between the fat (r=-0.386, $p<0.01$) and protein (r=0.523, $p<0.001$) content of 62 CHO rich foods and the GI, possibly as protein increases insulin secretion, thereby lowering blood glucose levels and the GI of CHO foods or CHO sources that are consumed with protein rich foods.	Jenkins et al, 1981[1]; Brand Miller et al, 1995 [27]

Table 11/…

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
	Resistant starch (RS)	This type of starch resists digestion in the small intestine and passes into the large intestine, where it is fermented. There are three types, i.e. RS1 (in whole or partly ground grains, seeds, cereals and legumes, RS2 (in some raw starch granules, e.g. potato and green banana) and RS3 (retrograded amylose). RS causes a small ↑ in fecal bulk, which provides a substrate for the colonic micro flora and promotes the synthesis of SCFA.	FAQ/WHO, 1998 [3]; Jenkins et al, 1998 [98]
Cooking/food processing	N/A	Main effect on starches is disruption of the cellular architecture and fibrous structure of starch granules, causing the starch to gelatinize more easily, but some starch granules disrupt more easily than others. Hydration during cooking also plays a major role in ↑ starch digestibility.	Frost et al, 1994 [59]; Holt et al, 1994 [20]; Gannon et al, 1987 [61]; Jenkins et al, 1995 [97]
	Degree of starch gelatinization	In making bulgur, whole wheat is parboiled, slightly dried, milled and crushed, creating a physical restriction to water uptake that restricts starch gelatinization. Bread and Cornflakes contain mainly fully gelatinized starch and are therefore digested and absorbed more rapidly and also have a high RAG value (refer to 2.4.2.7).	Jenkins et al, 1986 [19] Englyst et al, 1999 [94]
	Particle size	Intact whole grains usually cause ↓ glycemic responses. When the processing of cereal grains involves milling or grinding, the rate of digestion and absorption is ↑ due to ↓ particle size and thus the glycemic and insulinemic responses will also be ↑ and satiety ↓. In many plant foods (e.g. minimally processed legumes and cereal grains) the encapsulation of starch and sugars within cell walls slows down the digestion and absorption of the starch/sugars therein.	Jenkins et al, 1986 [19]; Holt et al, 1994 [20]; Englyst et al, 1999 [94]

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
	Cellular structure	The cell walls of tropical fruits are softer, more easily digested, release sugars more quickly and have higher GI values than temperate climate fruits, which have tougher cell walls and lower GI values.	Brand Miller et al, 1995 [27]
	Food form	This is partly responsible for the low glycemic response of legumes. Spaghetti has a low RAG and GI value, due to the dense food matrix of the very hard wheat (Triticum durum) it is made from, which hinders enzymatic hydrolysis of the starch. White bread has a similar GI to whole meal bread in healthy and diabetic subjects, thus food form may be more important than fibre in determining glycemic response.	Frost et al, 1994 [50]; Englyst et al, 1999 [94]; Jenkins et al, 1981[1]; Jenkins et al, 1983 [32]
Cooking/food processing	Parboiling	Parboiling of wheat and rice ↓ glycemic response, as it seems to ↓ gelatinization. The mean GI values of long grain white parboiled rice (GI=67±5; white bread=100) and instant rice (GI=65±5; white bread=100), as tested in diabetic subjects, were significantly less than that of regular long grain white rice (GI=83±5; white bread=100; $p<0.01$). Reducing the cooking time of rice to 5min vs. the recommended 15–25min significantly reduced the GI of regular ($p<0.01$), but not parboiled rice. Most of these differences in GI can be attributed to the differences in the availability of the starch to amylolitic hydrolysis.	Jenkins et al, 1986 [19]; Wolever et al, 1986 [53]
	Cold extrusion	This is used in the production of pasta products and ↓ glycemic response.	Frost et al, 1994 [59]
	Extrusion, flaking and popping	All of these ↑ glycemic and insulinemic responses, due to faster digestion and absorption.	Frost et al, 1994 [59]; Holt et al, 1994 [20]

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
Nature of mono- and disaccha-rides	N/A	The sweetness of fruit is mainly determined by a mixture of glucose, sucrose and fructose, which all have different glycemic effects. The average fructose content of temperate climate fruits is higher and the average sucrose content is lower than that of tropical fruits, leading to lower GI values for temperate climate fruits. However, the total glucose content of both types of fruit is similar.	Brand Miller et al, 1995 [27]
	Glucose	Glucose or dextrose caused some of the highest blood glucose and insulin responses of a number of CHO rich foods and is usually, for GI testing, allocated a value of 100.	Crapo et al, 1977 [4]; Wolever et al, 1991 [2]
	Fructose	Fructose has a low GI value and mean blood glucose, urinary glucose and HbA1c values were lower in both type 1 and type 2 diabetic subjects on a high fructose diet in comparison to a low fructose diet, although not statistically significant throughout. Forbes et al (1993) stated that the normal daily consumption of fructose is 10g and at a daily intake of >20g, VLDL-cholesterol, insulin and uric acid levels can be raised and gastrointestinal side effects can be experienced by susceptible persons. Fructose is also a reducing sugar and, as it is therefore an initial substrate in the Maillard reaction, which can also occur in vivo, may contribute to protein cross-linking in human tissues such as the lens of the eye and ultimately ageing. Added fructose as sweetener should not be recommended, but naturally occurring fructose in fruit, vegetables and honey is permissible.	Jenkins et al, 1981 [1]; Bantle et al, 1992 [99]; Franz et al, 1994 [18]; Cummings et al, 1997 [26]
	Lactose	Lactose is a beta-linked disaccharide comprising glucose and galactose and has a low GI, probably since galactose has a low GI, as glucose has a high GI.	Mahan & Estcott-Stump, 2004 [5]; Foster-Powell et al, 2002 [22]
	Polyols	Sorbitol, mannitol and xylitol are common polyols that have a lower glycemic response than sucrose and other CHO, but they can cause gastrointestinal discomfort when consumed in large amounts, i.e. ≥50g. Their energy values are all 3.5 calories/g, as their stool excretion was negligible, indicating that the digestion and absorption rate from the small intestine was appreciable.	Franz et al, 1994 [39]

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
Nature of mono- and di-saccharides	Sucrose	Canned fruit and dairy products containing added sugars produced higher GI values than their unsweetened counterparts, but lower GI values than that of bread, which is sugar free. If sucrose is added to low GI foods, the resulting GI is ↑, but if it is added to high GI foods, the resulting GI will be ↓, as table sugar has an intermediate GI value. The sugar content of 62 CHO rich foods was not related to glycemic response, although absorption might have been faster. There was no correlation between the sugar content of 15 foods and their GI values in diabetic subjects, as the substitution of 18g marmalade for 26% of the starch in bread caused no significant alteration in glycemic response and a 40% ↓ in glycemic response was seen after the consumption of All-Bran compared with Cornflakes, in spite of the fact that 36% of the CHO in All-bran is sugar, whereas only 9% of the CHO in Cornflakes is sugar.	Brand Miller et al, 1995 [27]; Jenkins et al, 1981 [1]; Jenkins et al, 1984 [100]; Jenkins et al, 1983 [32]
Other food components and factors	Protein	In a study conducted on 5–7 diabetic subjects, no correlation was found between total protein content of foods and GI, when the glycemic response to whole meal bread was not significantly altered by the addition of 89g cottage cheese (12.1g protein) or 250mL low fat milk (8.3g protein). Variation in protein content of typical mixed meals had a negligible effect on the blood glucose and insulin responses of eight healthy subjects. The GI ↓ effect of protein on CHO foods seems to only become significant when there is >25g protein per 50g CHO serving. When type 2 diabetic subjects ingested 50g protein with 50g glucose, the glycemic response was ↓, but the insulinemic response was ↑, in comparison to when 50g protein or 50g glucose was given. However this could be attributed to the fact that double the amount of macronutrients were given.	Jenkins et al, 1983 [32]; Wolever et al, 1996 [93]; Nuttall et al, 1984 [58]

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
Other food components and factors	Fat	There was no correlation between total fat content of foods and GI, when the glycemic response to whole meal bread in 5–7 diabetic subjects was not significantly altered by the addition of 89g cottage cheese or 250mL low fat milk (5g fat). Variation in fat content of typical mixed meals had a negligible effect on the blood glucose and insulin responses of 8 healthy subjects. Fat seems to only start altering the GI of CHO rich foods significantly, when there is >25g fat per 50g CHO serving.	Jenkins et al, 1983 [32]; Wolever et al, 1996 [93]; Nuttall et al, 1984 [58]
	Dietary fibre	*Soluble fibre* includes pectins, gums, mucilages and some hemicelluloses, as well as tragacanth, konjac mannan and psyllium. These all ↓ gastric emptying rate and therefore ↓ the glycemic response to that food and its GI. Soluble fibre can also delay nutrient absorption by forming a viscous gel, which impairs the transfer of glucose to the absorptive surface of the small intestine. Unlike tropical fruits, temperate climate fruits contain a higher proportion of viscous soluble fibres, causing them to have lower GI values. *Insoluble fibre* includes cellulose, lignin and hemicelluloses. Wheat bran, sugar beet and ispaghula are all types of insoluble fibre and Cherbut (1994) found that of these, wheat bran had the smallest water holding capacity. Sugar beet and ispaghula fibre can increase gastrointestinal volume, due to their high water-holding capacity, which is one of the factors that control gastrointestinal motility. They also have a blood glucose flattening effect, due to the motor action of the fibre and a shorter glucose contact time with the absorbing surface. *Other non-digestible and partially digestible CHO.* Phillips et al (1995) showed that CHO which escape absorption in the small intestine may increase fecal bulk, as shown by altered colonic micro flora, increased fecal nitrogen losses and SCFA synthesis, especially butyrate, which have many health benefits.	Franz et al, 1994 [18]; Cherbut, 1995 [101]; Jenkins et al, 1983 [95]; Foster-Powell, 2002; Jenkins, 1982 [102]; Brand Miller et al, 1995 [27] Franz et al, 1994 [18]; Cherbut, 1995 [101] Jenkins et al, 1998 [98]

Table 11 (continued)

Factor	Specific food component	Effect on GI	Researchers
	Antinutrients	The difference in the GI values of various food items is also affected by the food content of antinutrients such as phytic acid, polyphenols (tannins), lectins and saponins, which may all limit the rate of digestion. The low GI values and ↓ glycemic responses of most legumes can partly be explained by their high antinutrient content.	Banzal et al, 1997 [77]; Thompson, 1988 [103]; Jenkins et al, 1995 [97]; Frost et al, 1994 [59]
	Acidity or organic acids or salts	High acidity slows down the rate of gastric emptying and therefore ↓ the glycemic response to such a food or meal, as well as its GI. Bread products that contain sodium propionate (e.g. sourdough bread) ↓ blood glucose and insulin responses in healthy individuals, probably due to ↓ gastric emptying rate.	Foster-Powell et al, 2002 [22]; Liljeberg et al, 1998 [104]; Liljeberg et al, 1996 [105]
	Degree of ripeness	The degree of ripeness of fruits (e.g. bananas) plays a major role in glycemic response and therefore the GI of the food.	Hermansen et al, 1992 [62]
	Enzyme inhibitors	Enzyme inhibitors occur naturally in some foods and can ↓ the digestion and absorption of foods in the digestive tract. Legumes are some of the richest food sources of enzyme inhibitors and produce a very flat glycemic response, as they may limit starch digestion. They are, however sensitive to heat and, to a varying degree, their activity may survive cooking. The medication, Acarbose, is an enzyme inhibitor.	Krause & Mahan, 2004 [5]; Jenkins et al, 1995 [97]; Jenkins, 1982 [102]
	Osmolality	High osmolality slows down the rate of gastric emptying and therefore ↓ the glycemic response to and GI of such a food or meal.	Foster-Powell et al, 2002 [22]
	Malabsorption of CHO	Lower blood glucose responses to the consumption of low GI foods could also be attributed to CHO malabsorption, due to the slow rate at which some foods are digested. However, Jenkins et al (1982) showed that 13% of the CHO present in lentils was malabsorbed in comparison with 6% of that in whole meal bread, using breath hydrogen analysis. However, this small difference in CHO malabsorption does not explain the 72% difference in the GI values between the two starches.	Jenkins et al, 1983 [32]
	Chewing	Muir et al (1992) found different glycemic responses with different degrees of chewing.	Frost et al, 1994 [59]
	Altered food frequency	Data on meal patterns (gorging vs. nibbling) are sparse and do not consistently demonstrate any significant differences in blood glucose and insulin response.	Lineback, 2005 [64]

2.4.3.2   Factors influencing the variability of the GI values of foods

One of the major problems in GI research and one of the main criticisms against the use of GI is the so-called lack of standardized methodology amongst different researchers in determining the GI. Several factors can have an effect on the variability of glycemic responses and are listed below. Differences in GI values of similar foods reported by different investigators could be due to real differences in structure or digestibility of foods that appear similar, variation in methodology or the effects of random variation.[2] It was difficult to know how much each of these effects contribute to the variation in GI values, because the performance of GI methodology had not been assessed before the inter-laboratory study, which main objective was to determine the magnitude of variation of the GI values of the same foods determined by experienced investigators using their usual procedures in different laboratories (world-wide), which were in line with the procedures recommended by the FAO/WHO, 1998.[39] Since the inter-laboratory study had been conducted, an international committee for the standardization of GI testing methodology has been appointed by ILSI. The findings and recommendations of which were published recently and which are included below.[21]

Methodological issues (which can influence the accuracy and/or precision of GI results obtained):

- Subject characteristics [2,21,23]
- Within and between subject variability [23,80,81,82]
- Number of subjects used [21,23]
- Preparation of subjects prior to testing [21,81]
- Time of day that tests are conducted [21]
- Variation in FBG levels [2]
- Choice of standard food, number of repetitions of the standard food and effect thereof on mean GI values obtained [2, 21,23,56]
- Definition of 50g available/glycemic CHO [21,23]
- Variation in food portion size [1, 2, 21]
- Preparation of test foods [23]
- Volume and type of drinks consumed with test meals [1,21,23,106]
- Length of time of consumption of test food [21]
- Method, frequency and length of time of blood glucose sampling [2, 21,23]
- Day-to-day variation in glycemic responses [39]
- Different methods of calculating AUC and GI values [2, 21,23]
- Biochemical assay [50]
- Interval between test days [4]
- Re-utilization of test results of the standard/reference food [21]
- Randomization and how it is done [21]
- Outliers [21]
- Measuring instrument [21]

International standardization of GI methodology can reduce the variation in GI values obtained substantially.

- **Subject characteristics**

Although it has been stated by Wolever et al (1991) that age, gender, glucose tolerance status, dose and timing of insulin or oral hypoglycemic agents, the degree of diabetes control and FBG value on the day of the test, especially in type 1 diabetic subjects, can all have an effect on absolute glycemic responses, they

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added that all these factors appear to influence the response to all foods similarly. Therefore, if they are controlled adequately, they only have small effects on the resulting GI values of the foods.[2] In spite of this, some researchers have still stated that there are many subject characteristics that can affect the glycemic response to a given food, including health status, type and treatment of Diabetes Mellitus, BMI, age, gender, ethnicity and background knowledge of GI-studies.[23, 82] However, Wolever et al (2003) found in an inter-laboratory study that variables like age, gender, ethnicity or race, BMI, plasma or whole blood glucose and mean AUC after consumption of the reference food, accounted for only 3% of the variation in the CV of the reference food. These subject characteristics might have contributed to the highly significant differences in absolute glycemic responses between subjects, but these factors had no significant effect on the GI values that were obtained in the inter-laboratory study.[39]

Age
Fukagawa (1990) has shown that physical activity tended to decrease with age, both of which may affect glucose tolerance [23] and Tessari (2000) found that diet changed with increasing age. However, Wolever et al (1988) found no significant difference between GI values obtained in children vs. adults, both with type 1 diabetes.[2,21,23]

Ethnicity
When rural African vs. normal Western subjects were used as test subjects, it had no significant effect on mean GI values.[1] Walker et al (1984) demonstrated that there was no significant difference in blood glucose responses between different races[23,82], although Summerson and co-workers (1992) were able to show race-related differences in the control of diabetes in adults.[23]

Gender
Nuttall et al (1985) found the glucose response to a mixed meal to be different in healthy men, when compared to healthy women. Blood glucose levels of healthy women were raised for 1h after breakfast and dropped below the fasting baseline during the following 3h. In healthy men, however, this biphasic response was not present. The investigators were unsure whether this gender difference is also present in diabetic subjects.[61] Contradictory to this, Rasmussen et al (1992) did not find a significant influence of gender on glycemic and insulinemic responses in middle-aged male and female type 2 diabetic subjects.[82] Wolever et al (2002) also found no differences in glycemic responses between males and females.[21]

Recommendation: It was concluded that the common practice to include both genders in GI studies should be maintained.[21]

Body Mass Index (BMI)
Castillo et al (1994) showed that obese subjects might have altered glucose tolerance due to the insulin resistance that is commonly associated with central or abdominal obesity. They therefore recommended that only subjects with a normal BMI range (18.5–24.9 kg/m^2) be used as healthy test subjects for GI tests. However, it was previously recommended by Marion (1984) that, as about 80% of type 2 diabetic subjects are obese or have a history of obesity when diabetes is diagnosed, a reference BMI of 20–35 kg/m^2 will be more representative of the general type 2 diabetic population when choosing diabetic test subjects to use in GI tests.[23] However, as mentioned before, BMI was one of the subject characteristics that might have contributed to the highly significant differences in absolute glycemic responses between subjects in the inter-laboratory study, but had no significant effect on the GI values that were obtained.[39]

Health status
When determining the GI of a specific food, subjects from the healthy population or type 1 or type 2 diabetic subjects can be included for determining the GI of a specific food.[1,2,22,23,30,32]

In 1983 Jenkins et al determined the GI values of foods and meals, using groups of 5–7 diabetic volunteers. 50g CHO portions (as calculated from FCT) from 15 different CHO rich foods or CHO and protein meals were consumed after an overnight fast. The area under the 3h blood glucose response curve was calculated using a specific formula and expressed as a percentage of the mean AUC when an equivalent amount of CHO was taken as white bread. The GI values of the foods were also calculated as the mean of the percentage expression in each volunteer and were found to be significantly related (r=0.756, p<0.01) to the GI values obtained on the same foods by Jenkins et al (1981) when the GI values of 62 foods were determined in healthy individuals over 2h, with glucose as the reference food. The AUC values of the 3h tests on 15 foods in diabetic subjects were also significantly related to the AUC values of the 2h tests on the same foods in healthy volunteers (r=0.753; p<0.01).[1,32] In a prospective comparison of the GI values of 22 foods tested in healthy and diabetic subjects, a regression equation also showed that the GI values obtained in the healthy subjects were virtually identical to that obtained in the diabetic subjects (r=0.92, N=19, P<0.001).[56] Jenkins et al (1986) found no significant differences in the GI values of 7 CHO foods between type 1 and type 2 diabetic subjects (r=0.96, p<0.01).[19]

Wolever et al (1986) recommended that type 2 diabetic subjects that are used to conduct GI tests should be well controlled, as poorly controlled diabetic subjects show larger variability in measurement.[53] Franz et al (2000) recommended that the glucose tolerance status of subjects should be determined by a standard 2h GTT, before subjects can be classified as either healthy or diabetic and that HbA1c concentrations, that show blood glucose control over the preceding three months, should be measured and should be within the acceptable range of 7–8% to ensure that diabetic subjects are well-controlled.[23] Venter et al (2003) recommended that the serum and urine creatinine concentrations should be in the normal range to ensure that subjects have normal renal function. They also recommended that type 2 diabetics who are used as test subjects should be treated with diet alone or diet and metformin rather than sulphonylureas, as the UK Prospective Diabetes Study Group (1998) found that intensive glucose control with metformin seems to decrease the risk of complications in overweight diabetic patients, is associated with less weight gain and fewer hypoglycemic attacks than are sulphonylureas.[23]

Recommendation: Subject characteristics that have been examined specifically and were found to have no significant effect on mean GI values: normal vs. diabetic subjects [1,32,56], type 1 vs. type 2 diabetic subjects [19,53], type 2 diabetic subjects on oral agents vs. type 2 diabetic subjects on insulin [19], type 2 diabetic subjects in good vs. poor metabolic control [53] and children vs. adults with type 1 diabetes, as found by Wolever et al (1988).[2,21] However, in spite of this, and in spite of the fact that it has been shown that type 2 diabetic subjects give very consistent GI results [32] and display significantly less within-subject variation (CV 15-16%) than subjects with type 1 diabetes (CV 29%), or healthy individuals (CV 25%), Brouns et al (2005) recommended that only healthy individuals be used as test subjects for GI tests.[21] If a specific food or formula feed is developed for a specific target population and labeling purposes, the health status or specific characteristics of subjects included in the study should preferably be in agreement with those of the target population, e.g. diabetic patients or athletes, etc.[23]

- **Within and between subject variability**

There is concern about the widespread use of the GI in clinical practice, as its' inherent reliability is questioned by some unresolved issues. The calculated AUC, using venous plasma glucose concentrations, after ingestion of 75g glucose was greater than that following white bread, which is consistent with other GI research. The intra-individual CV in healthy male individuals was higher for glucose than for white bread and both were higher than that reported by Wolever et al (1985) [80] (refer to Table 9). The inter-individual CV for both standards was also unacceptably high, i.e. 50% for white bread and 68% for glucose, respectively, and once again higher for glucose than white bread. They recommended further

investigations in order to understand the mechanisms for the large intra- and inter-individual variation in the calculation of the GI, before it can be accepted in clinical practice.[80] However, the high CV can primarily be explained by the fact that venous blood was used, as capillary blood was shown to yield smaller SD values in an inter-laboratory study.[39]

Nell (2001) and Kruger et al (2003) conducted similar studies on healthy female and type 2 diabetic subjects, respectively, the results of which are also displayed in Table 12.[81,82] Although there is variation between individual subjects in the glycemic response to a food, as well as in the GI of that food to a certain extent, some studies show greater inter- than intra-individual variation in healthy subjects [80] and type 2 diabetic subjects using glucose as reference food [82], whereas others show greater inter- than intra-individual variation in healthy subjects using white bread as reference food.[81] Some, however, show greater intra- than inter-individual variation in type 2 diabetic subjects using white bread as reference food.[82] However, it is evident from Table 12 that the method of blood sampling and method of calculation can have an effect on the CV obtained.

Table 12: Intra- and inter-individual CV for plasma glucose after oral glucose and white bread in different studies, using different types of subjects and different methods of calculating AUC

Researchers	Type of subjects	Type of blood sampling	Intra-individual CV (%) (glucose)	Intra-individual CV (%) (white bread)	Inter-individual CV (%) (glucose)	Inter-individual CV (%) (white bread)
Aginsky et al, 2000 [80]	Healthy	Venous	46 ± 10.2	34.8 ± 5	68	50
Nell, 2001 [81]	Healthy	Venous	45 (IAUC) 21.4 (AUC_{min})	63.8 (IAUC) 36.2 (AUC_{min})	57.2 (IAUC) 37.2 (AUC_{min})	71.0 (IAUC) 43.9 (AUC_{min})
Kruger et al, 2003 [82]	Type 2-diabetic	Venous	11.6 (IAUC) 8.8 (AUC_{min}) 10.0 (AUC_0)	27.9 (IAUC) 21.8 (AUC_{min}) 10.6 (AUC_0)	19.9 (IAUC) 17 (AUC_{min}) 15.3 (AUC_0)	23.1 (IAUC) 21.4 (AUC_{min}) 10.0 (AUC_0)
Wolever et al, 1996 [107]	Healthy	Capillary	21.4 (IAUC)	12.7 (IAUC)		
Wolever et al, 1985 [56]	Healthy	Capillary	25 ± 12 (IAUC)			
Wolever et al, 1985 [56]	Type 2-diabetic (not on insulin)			16 ± 7 (IAUC)		
Wolever et al, 1985 [56]	Type 2-diabetic (on insulin)			15 ± 4 (IAUC)		
Wolever et al, 1985 [56]	Type 1 diabetic			29 ± 19 (IAUC)		

Within subject variation

Blood glucose responses within subjects can vary considerably from day to day.[3] When the same subject tests the same food, e.g. glucose or white bread on different occasions, the variability of the subject's glycemic response can be expressed as the coefficient of variance (CV). Wolever et al (1985) reported a mean CV for the IAUC of 25% in eleven healthy subjects taking 50g glucose on four to 15 occasions (Table 10). They also found varying mean CV values in different types of diabetic subjects taking 50g CHO from white bread on 5–35 occasions. Type 2 diabetic subjects showed less variation (mean CV 16% for those not on insulin and 14.9% for those on insulin) from day to day than either healthy (mean CV 25%) or type 1 diabetic subjects (mean CV 29.1%). Type 1 diabetic subjects showed almost twice the variation of type 2 diabetic subjects.[56]

Table 13: Differences between CV of repeated tests of the standard food (Wolever et al, 1985)[56]

Subjects	Average repeated tests of standard food	CV (%)	SD
Normal	8	25	12
Type 2 (not on insulin)	8	16	7
Type 2 (on insulin)	11	15	4
Type 1	9	29	19

Wolever et al (1996) compared the variability of glycemic responses after white bread vs. oral glucose in healthy subjects and found that 70% (7 out of 10) had a 2h CV higher than 7% after a 75g glucose drink and 70% had a 2h CV less than 7% after white bread. The mean CV of 2h blood glucose after oral glucose (19.2$\pm$2.8%) was 2-3 times greater than after bread (5.2 $\pm$ 0.8%) (p<0.01). This could possibly be due to the fact that a larger dose of CHO was fed, i.e. 75g instead of the usual 50g CHO load.[107] However, this was not confirmed by Kruger et al (2003), who examined the variability of glycemic responses after white bread vs. oral glucose in type 2 diabetic subjects. However, this study was conducted using 50g available CHO from glucose or white bread.[82]

Between subject variation

Between subject variation implies that one subject responds differently to another subject when the same food is tested on different occasions. Different treatments of diabetic subjects could have an effect on FBG concentrations. However, Wolever et al (1985) found the intra-individual variation in glycemic response in type 2 diabetic subjects on insulin to be virtually identical to that in type 2 diabetic subjects on oral hypoglycemic agents or diet alone. The mean GI values of rice tested in type 1 and type 2 diabetic subjects by Wolever et al (1986) were also similar (82$\pm$22 compared with 74$\pm$19) and the reproducibility 22 months later in the same group of subjects was excellent (81$\pm$15 compared with 83$\pm$15).[56] Gannon and Nuttall (1987) concluded that medication (insulin and oral hypoglycemic agents) might have varying effects on the human body from day to day, which could influence glycemic and insulin responses between different diabetic subjects.[82]

Rasmussen et al (1992) also studied within and between subject variation of both glycemic and insulinemic responses, when type 2 diabetic subjects who were treated with diet and oral hypoglycemic agents took a starchy meal. The subjects took 45g available CHO as white bread (90g) over a 7-day period. They reported a mean intra-individual CV of 19% and a mean inter-individual CV of 33% for glycemic areas. They concluded that most of the variation of glycemic responses in type 2 diabetic subjects (not on insulin) was attributable to between subject variation. However, this was due to the fact that they compared the absolute glycemic responses. It is important not to confuse the terms GI and glycemic response. The GI reduces the between subject variation significantly, as it does not measure the absolute glycemic response to a food, but rather the relative glycemic response, as a subject's response is indexed against his or her response to a standard.[108]

In a recent inter-laboratory study, seven experienced GI testing laboratories from around the world all tested the same centrally distributed foods, according to the laboratories' normal in-house testing procedures, which were in line with the FAO/WHO recommended method for GI testing.[3] The five laboratories using fingerprick capillary blood samples for GI testing showed less between subject variation than the laboratories using venous samples.[39]

Recommendation: Subject characteristics that have been examined specifically and have been found to have no significant effect on mean GI values are the following: normal vs. diabetic subjects [1,32,56], type 1 diabetic subjects vs. type 2 diabetic subjects [19,53], type 2 diabetic subjects on oral agents vs. type 2 diabetic subjects of insulin [19], type 2 diabetic subjects in good vs. poor metabolic control [53] and children with type 1 diabetes vs. adults with type 1 diabetes (found by Wolever et al, 1988).[2,21]

- **Number of subjects used**

To determine the GI of a food, six or more subjects should be used.[3] It was recently shown with Berger's power calculations that were based on the smallest SD, that at least 24 subjects are necessary to determine the mean GI of white bread in order to have an 80% chance that the GI of the bread will not differ by more than 10%, in both healthy individuals[81] and type 2 diabetic patients.[82] However, Kruger et al (2003) showed that larger groups of 24–28 subjects should be used[82] and Nell (2001) showed that even larger groups of 24–90 subjects should be used for foods to be consistently classified on a scale of 0–100 as having a low (0–55%), moderate (56–69%), or high (70+%) GI, using glucose as standard/reference.[81]

The power calculations done by Nell (2001) and Kruger et al (2003) were based on GI tests done on venous blood, which yield substantially higher SD values in GI testing than capillary blood.[39] Better power and more precision could possibly be attained if more subjects are used, but at a higher cost. Calculations done by Brouns et al (2005), using the data of a recent inter-laboratory study [39] revealed that using ten subjects provides a reasonable degree of power and precision for most purposes of GI determinations. Large improvements in power and precision would require 2-3 times more subjects and can be used to detect small differences in GI.[21]

Recommendation: It was recommended that at least 10 subjects be used for GI tests to provide a sufficient statistical power.[21,106]

- **Preparation of subjects prior to testing**

Food consumption
Vorster et al (1990) recommended that subjects ought to be prepared for GI tests three days before the actual GI tests will be conducted, by prescribing a high CHO (60% of total energy) diet, with the rest of the energy coming from protein and fat (20% each), as this type of diet "ensures optimal substrate induction of enzyme synthesis and activation" and helps to prevent ketogenesis and gluconeogenesis, which may occur when CHO has been restricted for a while. They also recommended that subjects consume a weight-maintenance diet during the course of the study.[23]

They furthermore recommended that subjects consume a standard pre-evening meal with 50% of the total energy coming from CHO, 30% from fat and 20% from protein to help prevent potential second-meal effects, as shown by Gresse & Vorster (1992).[23] The GI of the pre-test meal (night before a GI test is conducted) should not be too low, as the glycemic response to a second meal (e.g. breakfast) can be improved by decreasing the GI of the fist meal (e.g. supper), as shown by Wolever et al (1988) and Thorburn et al (1993).[21,59] Brouns et al (2005) reported that the consumption of dietary RS on the day before a GI test is performed, might also affect the result.[21] However, the GI of the pre-test meal should

also not be too high, as Gannon et al (1987) showed that a high GI meal can cause a large insulin response and increased peripheral glucose uptake, inducing hypoglycemia, increased FFA and relative insulin resistance.[59] It would therefore seem that the GI of the pre-test meal (night before a GI test is conducted) should preferably be intermediate.[59] However, Campbell et al (2003) found that strict control of meal consumption on the night before a GI test is conducted caused more variation than "uncontrolled" tests, possibly due to the added stress caused by the stricter measures.[38]

Recommendation: Brouns et al (2005) therefore recommended that each subject consume a meal of his/her choice on the evening before a GI test is conducted and that this meal should preferably be consumed on the evening before each test. [21]

Alcohol consumption
Shelmet et al (1988) showed that 0.75g ethanol per kg over 30min caused acute insulin resistance in six healthy, overnight fasted males. It also decreased total body fat oxidation by 79%, protein oxidation by 39% and almost completely nullified the 249% rise in CHO oxidation seen in controls after the same amount (0.5g per kg over 5min) of glucose infusion.[42] It was also found that when five healthy, overnight fasted males consumed 48g of alcohol, gluconeogenesis was decreased by 45% vs. the placebo in the 5h after alcohol ingestion.[43] However, the effect that the consumption of alcohol has on the metabolism of human beings on the day after consumption has not been investigated yet[21] and Campbell et al (2003) found that abstaining from alcohol during the day before a GI test is conducted caused more variation than "uncontrolled" tests, possibly due to the added stress caused by the stricter measures.[38]

Recommendation: There seems to be no need to abstain from consuming alcohol on the day before a GI test is conducted.[21]

Caffeine consumption
Consumption of 5 mg/kg caffeine by 18 healthy, fit adult males caused a significantly greater increase in insulin concentration during a GTT [41] and 3 mg/kg caffeine, which was intravenously administered to 12 healthy, overnight fasted subjects, decreased insulin sensitivity by 15% ($p<0.05$), caused a significant ($p<0.05$) increase in plasma FFA and increased plasma epinephrine levels significantly ($p<0.0005$). It also caused smaller, significant increases in plasma norepinephrine ($p<0.02$) and blood pressure ($p<0.001$). This can mainly be due to the fact that caffeine is a methyl-xanthine derivative and an adenosine receptor antagonist [40] and adenosine plays an important role in the effect of insulin activity on glucose disposal and caffeine inhibits this function.[41]

Medication
Recommendation: Healthy subjects: no drugs should be taken that may affect glucose tolerance.[23]
Diabetic subjects (type 1 and 2) who use medication should take their normal dose of insulin or oral hypoglycemic agent after the FBG sample was taken and 5–10 minutes before consumption of the test meal is commenced.[2]

Activity
Improved insulin sensitivity has been shown for up to two days after acute physical activity was done, but not for five days [44] and it was also found that acute physical exercise increased glucose uptake by muscles during exercise, as well as on the following day, possibly to replenish muscle glycogen stores.[45] This supported the suggestion by Fukagawa et al (1990), that change in activity might influence peripheral insulin sensitivity, thereby having an effect on the glycemic and insulinemic responses of test subjects.[81] However, when exercise on the day before conducting GI tests was strictly controlled, GI results were actually more variable than in subjects whose activity levels were not controlled, possibly due to the added stress invoked by stricter measures.[38]

Recommendation: Subjects should be advised not to change their physical activity patterns during periods of taking part in GI tests [81], as vigorous physical activity on the day before "uncontrolled tests" yielded lower blood glucose readings at 90min.[38] Venter et al (2003) also recommended that usual physical activity of subjects should be sustained [23] and unusual vigorous physical activity on the day before GI tests are conducted should be discouraged.[21]

Smoking
Campbell et al (2003) did not allow smoking on the morning of conducting GI tests in both controlled and "uncontrolled" tests. Although the smokers who partook in the tests reported that they craved for cigarettes during the tests, similar glycemic response values were observed for the controlled and "uncontrolled" tests.[38] Cigarette smoking may cause acute insulin resistance, as found by Attvall et al (1993) and Frati et al (1996).[21]

Recommendation: It has been recommended that smoking should be prohibited on the morning of conducting a GI test.[21]

Length of overnight fast
In a study in which the results of GI tests in which the length of the overnight fast was strictly controlled were compared to "uncontrolled" tests, the variability of results were less in the "uncontrolled" tests, possibly due to the added stress that was caused by the stricter measures.[38] The exact length of the overnight fast may, however, be important, as it was found by Klein et al (1993) and Samra et al (1996) that the supposed steady state that exists after an overnight fast is actually a time of significant changes in the body, e.g. falling plasma insulin concentrations and increasing lipolysis.[21]

Recommendation: Subjects should be studied in the morning at breakfast time, after an overnight fast of 10–14h.[21]

- **Time of day that tests are conducted**

It was found that the difference in glycemic response between two breakfast cereals that were studied in eight healthy individuals in the morning after a 10–12h overnight fast, as well as at lunchtime (12:00) after a standard breakfast had been eaten, were significantly greater in the morning than at lunchtime, in spite of the fact that physical activity was restricted. It was also found that glycemic responses at breakfast were less variable than those at lunch.[60]

Recommendation: Subjects that are used for GI tests should be studied in the morning, on separate days, after a 10–12h overnight fast.[3] This has been confirmed by Brouns et al (2005), who recommended that all GI tests should be done in the morning at breakfast time, after an overnight fast of 10-14h, in order to be comparable with the GI values of other foods in GI tables. This is especially important to limit intra-individual variation, due to time of day and meal influences.[21]

- **Variation in FBG levels**

As mentioned before, the FBG value on the day of the test can have an effect on the variability of glycemic responses, especially in type 1 diabetic subjects.[56] Wolever et al (1986) found that the GI values in seven type 2 diabetic subjects with FBG levels below 8.3mmol/L were not significantly different to those in six type 2 diabetic subjects with FBG levels above 8.3mmol/L (r=0.911; p<0.01).[53]

Oleerton et al (1999) found that the daily biological variability in humans accounted for 14 of the 15% total variability in fasting plasma glucose readings. Kruger et al (2003) found a variation in fasting plasma

glucose concentrations of about 14–20%, which is in line with that found in other studies. They concluded that this is probably due to within subject biological factors, such as the dawn phenomenon, as subjects were asked to keep their activity levels constant during the time of the study and the meal that was taken on the evening before each test was kept the same. In addition, exclusion criteria were: HbA1c values >8%, impaired renal function as determined by serum creatinine and creatinine clearance tests, smoking, and alcohol intake of more than 6% of total daily kJ. Although lifestyle changes can affect fasting plasma glucose readings, this was unlikely to have happened during the eight weeks that the study was in progress.[82]

Recommendation: FBG appears to influence the response to all foods similarly and therefore, if FBG is standardized, it only has a small effect on the resulting GI values of the foods. Type 1 diabetic subjects should therefore use their normal dose of insulin at all times, including for GI tests, as this helps to stabilize blood glucose levels.[2]

- **Choice of standard food, number of repetitions of the standard food and effect thereof on mean GI values obtained**

Choice of standard food
Originally 50g glucose, dissolved in water was used as the standard food to determine the GI and was given a value of 100.[1] White bread was used as standard food at a later stage due to concerns of delayed gastric emptying because of the excessive sweetness and the osmotic effect of a glucose solution.[2,32] It is the opinion of Brand Miller et al (1995) that an index where glucose is taken as 100 is more logical and easier to explain to patients, than an index where white bread is taken as 100.[27] Vorster et al (1990) regarded glucose as the ideal standard in healthy subjects, as long as care is taken to avoid an osmotic effect in the gut by dissolving the glucose in an adequate volume of water.[23]

Glucose as standard food seemed also seemed to be more variable (2-3 times) in glycemic responses observed in healthy individuals and it was therefore suggested that starchy meals may lead to more precise assessment of CHO tolerance.[107] However, the magnitude of these findings was investigated in three recent studies in healthy male [80] and female [81] subjects, as well as in type 2 diabetic subjects [82], respectively. Aginsky et al (2000) found glucose to be more variable than white bread [80], which is similar to what Wolever et al (1996) found.[107] However, Nell (2001) found both the intra- and inter-individual CV of IAUC and AUC minimum (AUC_{min}) of glucose to be less variable than white bread and recommended that oral glucose be used as the standard food in GI determinations, instead of white bread.[81] Kruger et al (2003) also found the CV of the IAUC and AUC_{min} to be significantly higher for white bread than for glucose in a group of type 2 diabetic subjects. It was therefore concluded that, when IAUC, which is usually used in GI calculations, is used the within and between subject blood glucose concentrations tend to be less variable after a glucose test meal than after a starch test meal, e.g. white bread.[82] Surprisingly enough, the mean CV of the AUC values of repeated tests on bread (27.7±5) taken by ten healthy subjects from one center in the inter-laboratory study did not differ significantly from their repeated tests of glucose (23.1±4.3), although the bread values were more variable.[39]

Glucose should be purchased in bulk, if it is used as standard food. Fifty grams glucose powder should be weighed out in separate portions and dissolved in 200-250mL (1 cup) water. Glucose solutions should also preferably be served at the same temperature.[23] However, if monohydrated glucose is used as reference food, 55g should be used, due to the fact that 1.1g of this form of glucose actually contains only 1.0g glucose.[21]

Truswell et al (1992) were of the opinion that, if white bread is used as standard food, it should be standardized as far as possible, as it is not always consistent and can go stale, causing it to lose water

when standing at usual indoor temperatures.[106] Venter et al (2003) suggested that, in order to avoid differences in the quality and quantity of the CHO load, all bread used for GI tests should come from the same batch and supplier. They also recommended that bread crusts be removed, as suggested by Robinson et al (1986), due to the influence of the Maillard reaction on the availability of the CHO from the crust. They furthermore suggested that, if white bread is used as standard food, each sample should provide 50g available CHO as determined by FCT.[23] The draft regulations of the Dept of Health) (Directorate Food Control, SA) specify that all foods used for GI determinations should be analyzed by a SANAS accredited laboratory, according to a specified protocol in order to determine the amount of glycemic/available CHO in the food.[31]

Recommendation: Although the variability of the GI values for white bread from different cities worldwide was similar to those for the other foods in the inter-laboratory study (which supports the validity of using white bread as reference food), glucose is a more logical and easily standardized reference food for international use. It is therefore recommended, for international standardization, that GI values of foods be expressed relative to glucose = 100.[21,39] Brouns et al (2005) advised that, for practical purposes, it is acceptable to use other foods as reference foods for GI tests, e.g. white bread, provided they have been calibrated against glucose and the conditions of the preparation of these foods are standardized.[21] GI values obtained using white bread as standard can be adjusted to yield GI values using glucose as standard by multiplying by the factor of 70/100 [27] or dividing by 1.4.[39]

Number of repetitions of the standard food and effect thereof on mean GI values obtained
The mean IAUC of at least three repeated trials of the standard/ reference food should be used to calculate the GI [2,56], as the mean of three trials of the reference food is more likely to be a true reflection of how a subject's body responds to the standard food than the result of a single trial. Since the response of a specific subject's body to the reference food is used to calculate the GI value of every food that is tested thereafter, an unrepresentative value for the IAUC of the reference food will affect the GI values of all foods tested and may cause that subject's mean GI values of foods to differ from that of other subjects. Wolever et al (2003) showed with a mathematical model that, using the mean of three trials of the reference food compared to one trial of the reference food in order to calculate GI values, resulted in more normally distributed GI values, less variability, lower SD values and a lower mean value.[39]

When the GI values of foods are determined, the IAUC of the standard or reference food is used as the denominator of every other food that is tested. Although precision of GI tests will be improved if test subjects perform repeated measurements of both the reference food and test foods, repeating all measurements adds undesirable cost. However, variation in the responses to the reference food has a more marked effect on the mean GI values obtained than the variation in the GI values of the test foods, as the former is used to calculate the GI values of all the latter.[21]

Recommendation: It was recommended that the reference food should be tested on at least two occasions, as "the margin of error of the estimate of mean GI decreased substantially from one to two reference food measurements". There is very little benefit in taking more than three to four measurements of the IAUC of the reference food, as the reduction in the margin or error from one to two repetitions of the standard food is statistically significant, whereas there are no statistically significant differences among the margins of error for other numbers of repetitions of the reference food.[21]

Definition of 50g available CHO portion of food

It was recommended that a 50g available or glycemic CHO portion of a food is used when the GI is determined, which, according to the AOAC (Association of Official Analytical Chemists) method, is measured as total CHO minus dietary fibre.[3] However, most research institutes in the world obtain the amount of available CHO in food by subtracting "dietary fibre" from total CHO, which is not necessarily equivalent to the amount of *in vivo* available CHO in that particular food and may therefore lead to an over-estimation of the amount of available CHO in foods that contain indigestible CHO, which is not being detected as dietary fibre. The method for determining dietary fibre does not detect the amount of fibre in resistant starch RS1 and RS2 and therefore these will mistakenly be included as glycemic CHO.[81]

According to Venter et al (2003), FCT are generally used to obtain the nutritional composition of different test foods, to ensure that 50g of available CHO is ingested by test subjects and that, if the available CHO in the product cannot be obtained from the FCT, this should be determined by having a nutritional analysis done by a research institute.[23] According to the draft regulations of the Department of Health (Directorate Food Control, SA), the available CHO in a product should be determined by a SANAS accredited laboratory, according to the specifications in the regulations.[31]

Venter et al (2003) were of the opinion and reported that this had been confirmed by Brand Miller et al (2001), that RS is "available CHO" and should be included in the 50g CHO portion, as it is available for digestion in the human colon.[23] There are various ways of defining and measuring CHO, dietary fibre and RS in different countries. The portion size of food containing 50g available CHO may therefore vary in different laboratories, depending on the definitions used. However, only CHO that are available to the body for energy, i.e. that yield glucose and is known as available or glycemic CHO, should be included in the portion of CHO that is tested for GI and, according to Wolever et al (2003), RS, in addition to non-starch polysaccharides (NSP), should therefore not be included in the 50g available CHO test portion for GI tests.[39]

Since standardized methods to measure RS were not yet available when the inter-laboratory study was conducted and the impact of the exclusion of RS on the GI was unknown, barley, which has a high RS content, was chosen in this study to investigate its effect on GI tests. The high RS content of the barley resulted in a larger portion size (93.9g) when the amount of RS was subtracted, as opposed to 79.6g when it was included in the available or glycemic CHO portion. The GI value for the bigger portion size was 13% higher, i.e. 44 as opposed to 39 (glucose = 100), but the difference was not significant.[39] Granfeldt et al (1995) showed that the glycemic response observed after consumption of a 45g total starch portion containing only 15% of RS was identical to the response after a 55% larger portion size in a food containing 35% RS, when the RS content was subtracted from the total amount of CHO. This is probably due to the fact that the food factors that cause intrinsic RS formation also reduce the *in vivo* availability of most of the starch. Until recently, the AOAC analytical dietary fibre method could not measure RS3 accurately, leading to an underestimation of the total RS content of many foods. However, AOAC recognized a new method recently, as discovered by McCleary & Monaghan (2002), which can measure RS3 specifically and accurately. Measuring available CHO is therefore no longer a problem for most common foods.[21]

Recommendation: Some CHO foods contain indigestible CHO that is interpreted by the body as dietary fibre, but is not detected as such by the classical dietary fibre analytical method, e.g. products that contain fructo-oligo-saccharides and the like. Therefore the available CHO should be determined by using approved specific analytical methods to analyze for available starch, mono- and di-saccharides, malto-oligosaccharides like maltodextrin and sugar alcohols.[21]

- **Variation in food portion size**

Simple increases in meal size will not invalidate GI tables based on 50g CHO portions, as the increase in GI is smaller than expected at CHO portions in excess of 50g.[1] Wolever & Bolognesi (1996) showed that increasing the amount of CHO ingested increases glycemic response in a dose-response manner in healthy subjects. However, there is a noticeable tendency for all dose response curves to flatten above 50g CHO [2,21,39], although there is no significant difference in GI at 25g portions. When 25g available or glycemic CHO portions are used for GI tests, the IAUC can be doubled. This is justified by the fact that there is a proven linear dose-response relationship between the IAUC and the amount of available or glycemic CHO consumed in the range 25-50g.[27]

Recommendation: Most published GI values are based on a 50g available CHO portion of food.[1,22,27,30] Foods should preferably be weighed dry to determine the test portions, as the moisture content of cooked foods show large variation.[2] Vorster et al (1990) stated that the only exception to a test portion containing 50g available CHO is where the volume of a food that is low in CHO dictates a smaller load such as a 25g CHO portion.[23] However, when less than 10g CHO is consumed, significant changes in blood glucose levels may not be detected. Therefore 25-50g available CHO is recommended for GI tests, preferably 50g.[21] This has been confirmed by Lineback (2005), who stated that it is not practical to determine the GI using a 50g available CHO portion with many foods and ingredients, as in the case of polyols, as these foods contain little CHO and will lead to the consumption of an unreasonably large portion of test food.[64] Less than 25g available CHO (but more than 10g) could be considered for foods that contain very little available CHO, but cannot be recommended due to lack of data and further research is needed in this regard.[21]

- **Preparation of test foods**

Recommendation: Test foods should be given in random order on separate days and each test portion should provide 50g of glycemic or available CHO. Test foods should preferably be purchased in bulk and selected from the same batch so as to ensure uniformity of shelf life and similarity of management during production. Test foods that must be consumed in a cooked state should be prepared beforehand and frozen in portions in plastic bags or sealed containers at –18 to –30 °C. Required foods should be removed from the freezer on the night before a GI test will be conducted, thawed at room temperature and reheated in a microwave, if necessary, at specified times.[81] A digital scale should be used to weigh out precise portions of dry foods that contain 50g CHO each. Standardized equipment, cooking methods and utensils should be used in the preparation of cooked food products.[23] However, it should be remembered that freezing could lower the GI of some foods.[22,30]

- **Volume and type of drinks consumed with test meals**

An accompaniment is usually given with dry test foods, as it might be unpleasant to consume dry foods alone.[1] This accompaniment should be low in energy, very low in CHO [106] and kept the same (regarding type and volume) for different foods that are evaluated.[21,106] The drink is for rehydration and comfort of the test subjects, seeing that all GI tests are conducted in the morning after an overnight fast [21] and the volume of the drink should be standardized, in order to ensure that meal volume is not a variable.[4,21] It has therefore been recommended that a standardized amount of drink, such as 250mL (one cup), be given with all test meals, ignoring the water that is included in the test food. Tests conducted on the reference foods used for GI testing should be either 50g glucose diluted into 250mL water or a portion of white bread containing 50g available/glycemic CHO given with 250mL of fluid.[21]

The type of drink should be kept the same, as the consumption of 375 mg caffeine caused a significant increase in C-peptide and insulin, as well as a 24% increase in glucose AUC [41] and caffeine also caused an acute decrease in insulin sensitivity in humans.[40] Although water is mostly the accompanying drink in GI testing, coffee and tea are also sometimes used. Young & Wolever (1998) have shown that coffee and tea did not affect the IAUC significantly, although variation in volume influenced the pattern of blood glucose response.[21]

Recommendation: It was recommended that a standard amount of 250mL water should be taken with all test foods, including reference foods. In the case of glucose, 50g should be diluted into 250mL water. As a second step, non-energetic or stimulant beverages like coffee or tea may be taken with the test food instead of water, as long as the same volume and type of drink is taken during the different GI test sessions of each subject.[21]

- **Length of time of consumption of test food**

It has been shown that there is a linear relationship between the CHO content of a test drink and rate of gastric emptying, as well as that the osmolarity of a test drink does not seem to play an important role, but that the CHO content does. However, most GI tests are done on 50g glycemic CHO. In spite of this, fluids seem to have a strong effect of initial volume on gastric emptying rate and therefore Brouns et al (1998) have stated that ingestion time should be standardized.[21]

Recommendation: Fluids should be ingested within 5–10min and semi-solid ingestion should take place within 10–20min, depending on the type and taste of the food. However, most test foods should be consumed within 10–15min, depending on the type of food.[21]

- **Method, frequency and length of time of blood glucose sampling**

Method of blood sampling
Refer to 2.4.1.1.

Frequency of blood sampling
In a study of healthy and diabetic volunteers, the GI values determined on the same test foods correlated closely (r=0.836, p<0.001). In a prospective comparison of the GI values of 22 foods tested in healthy and diabetic subjects, a regression equation also showed that the GI values obtained in the healthy subjects were virtually identical to that obtained in the diabetic subjects (r=0.92, N=19, p<0.001).[56] Usually blood glucose measurements are taken after an overnight fast (0min) and at 15, 30, 45, 60, 90 and 120min after the first bite of test food or sip of test drink in subjects without diabetes and therefore the test lasts up to 2h. It is taken at 30min intervals and additionally at 150 and 180min in diabetic subjects and therefore the test lasts up to 3h.[2,21] The first bite of food or sip of drink is set as time 0 and the next blood sample should be taken at exactly 15min afterwards. Wolever et al (2004) found that when blood samples were taken less frequently, e.g. every 30min instead of every 15min or for less than 2h in healthy subjects, it had a significant effect on the mean and variation of the resulting AUC, which also seemed to increase as the frequency and duration of blood sampling decreased.[21]

Recommendation: Blood glucose measurements should be taken after an overnight fast (0) and at 15, 30, 45, 60, 90 and 120min after the first bite of test food or sip of test drink in subjects without diabetes and at 30min intervals and additionally at 150 and 180min in diabetic subjects.[2,21] The first bite of food or sip of drink is set as time 0 and the next blood sample should be taken at exactly 15min afterwards (healthy individuals) [21] and 30min afterwards (diabetic individuals).[2]

Length of time of blood sampling
The length of time during which testing is done post-ingestion of the test food may play a significant role. Granfeldt et al (1991) recommended that more studies are needed to evaluate whether the 2h time period commonly used for healthy individuals has to be modified in certain subjects. This is due to the fact that, amongst others, the GI of pasta products, as calculated using the 2h AUC in healthy elderly men, were not significantly different from the reference, in this case white bread, in spite of clear differences in the glycemic response curve, due to a substantially higher insulin response after the consumption of white bread.[21]

Recommendation: Until more research has been done in this regard, blood glucose measurements should be taken after an overnight fast (0) and at 15min intervals during the first hour and at 30min intervals during the second hour and thus up to 2h (healthy subjects). It should be taken at 30min intervals during the first 2h and additionally at 150 and 180min and thus op to 3h (diabetic subjects).[2,21]

- **Day-to-day variation in glycemic responses**

There is considerable variation in blood glucose responses from day-to-day within subjects.[3] In an inter-laboratory study, the between-laboratory variation in GI was mainly attributed to this random, day-to-day variation in glycemic responses within subjects.[39]

Recommendation: The most effective strategy for improving the precision of measurements of GI values of foods would be to find ways to reduce the within-subject variability in blood glucose responses.[39] Exercise on the day before a GI test is conducted and the pre-test meal [21,23] need to be standardized [23,38, 81], as well as medication [2,23] taken by the subject and the length of the overnight fast. Smoking should also be prohibited on the morning of conducting a GI test.[21] However, all of these factors should be controlled in a non-stressful manner, as it has been found that the added stress caused by stricter control of GI tests increased variability in GI values.[38]

Also refer to Subject characteristics (pages 49-51) and Preparation of subjects prior to testing (pages 54-56).

- **Different methods to calculate the AUC and GI**

Wolever et al (1985) reported a large difference in the GI values of the same food, determined by different investigators, whereas if the same method was used to calculate the GI, the GI values were very similar, suggesting that the different GI values could be attributed to different methods of data analysis rather than differences in responses to foods.[56] This was confirmed by Venter et al (2003), who are of the opinion that the main source of error in determining the GI could be the method of calculating the AUC.[23]

There are different methods to calculate the AUC (refer to Figure 3).

AUC (above fasting baseline) (IAUC)
The incremental area under the curve was originally defined as the area under the blood glucose response curve, using the fasting glucose value as baseline.[1] This can be calculated geometrically, using the trapezoid rule.[3]

The formula is as follows:
At/2 + At + (B-A)t/2 + Bt + (C-B)t/2 + Ct + (D–C)t/2 + Dt + (E–D)t/2 …..etc. where A,B,C,D and E represent positive blood glucose increments and t is the time interval between blood samples. If the blood

glucose increment D is positive (i.e. greater than the fasting value or baseline) and E is negative (i.e. less than the fasting value or baseline) only the area above the fasting value or baseline (between D and E) is used. If the value E occurs t min after value D, a straight line drawn between points D and E crosses the baseline at time T after D, where T<t. Thus the area above the curve between D and E is given by DT/2. Because T/t=D/(D+{E}) (where {E}=absolute value of E), therefore T=Dt/(D+(E)) and thus DT/2=D2t/2(D+{E}). The overall equation simplifies to: Area = (A+B+C+D/2)t+D2t/2(D+{E}).[2]

This equation ignores all the areas under the FBG value and it is the opinion of Venter et al (2003) that it will therefore not always reflect the glucose response to specific foods accurately, as in an abnormal physiological condition, as seen in hypoglycemic non-diabetic subjects, an undershoot of the baseline occurs after an initial high glucose concentration. Therefore, this method will not always give a true representation of the glucose response to specific foods.[23]

(a)

(b)

(c)

(d)

(e)
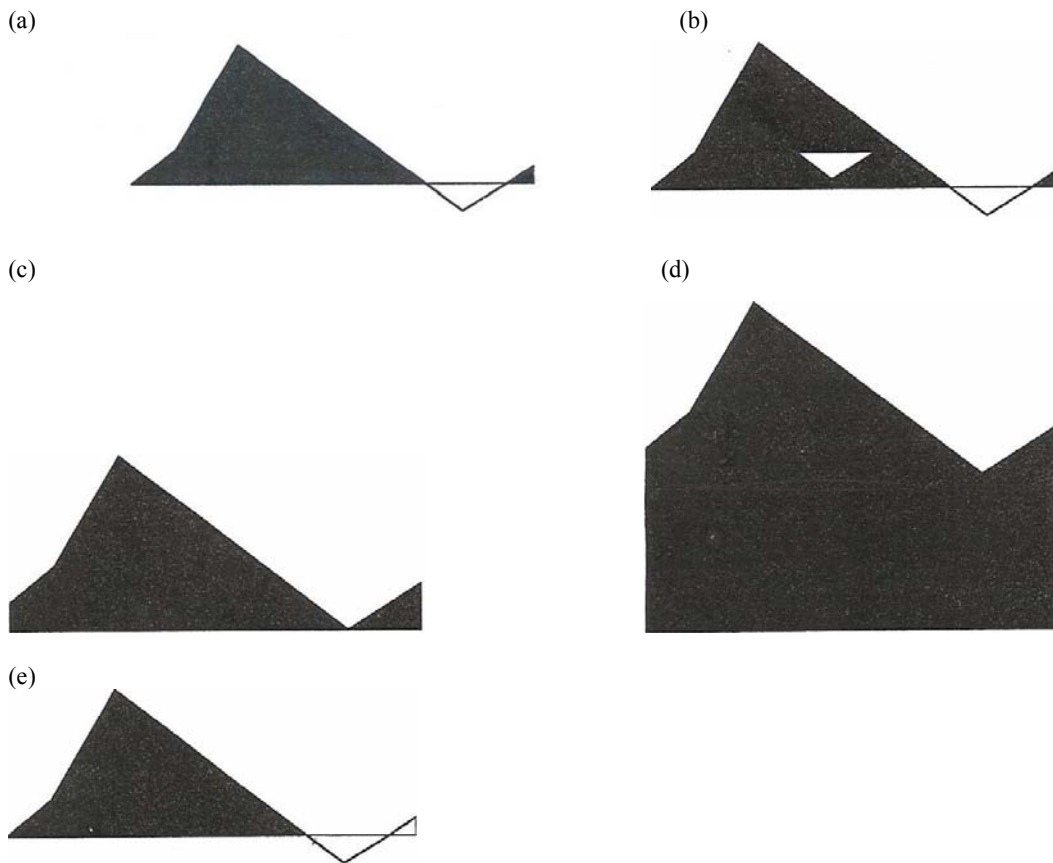
Figure 3: Different methods to calculate the AUC
(a) IAUC, (b) Net incremental AUC, (c) AUC$_{min}$, (d) AUC$_0$ and (e) Incremental AUC$_{cut}$.

Net incremental AUC

The net incremental AUC is a variation of Wolever's IAUC method and was used by several researchers, i.e. Bantle et al (1983), Nuttall et al (1983), Gannon et al (1986) and Laine et al (1987). In this method the area under the FBG is subtracted from the area above the FBG curve. According to Wolever (1990), a difference between the IAUC above baseline and the net incremental AUC will only be seen in cases where the postprandial blood glucose concentration drops below the fasting value.[21]

Incremental area above the lowest glucose value as baseline (AUC$_{min}$)

Vorster and co-workers (1990) proposed an AUC with the lowest or minimum blood glucose reading as baseline to calculate the GI. They had shown that the sharp rise in blood glucose levels, when glucose was used as the standard/reference food, led to reactive hypoglycemia at approximately 90min in many healthy subjects. This was, however not prevalent when slowly absorbed or low GI CHO was consumed. Therefore, in healthy subjects who experience hypoglycemia or blood glucose levels below the fasting level, the methods that ignore the areas below fasting baseline will not reflect the true picture, in their opinion. In this AUC$_{min}$ method, hypoglycemia is regarded as a physiological undesirable state, just like hyperglycemia is. When blood glucose levels remain above the fasting value (as is mostly the case in diabetic subjects) the AUC$_{min}$ method will give the same results as in the IAUC of Wolever et al (1991).[23]

Recently, Nell (2001) found the AUC$_{min}$ method to show less variation than the IAUC method and suggested that the former method of calculating the GI is a more relevant physiological method than the latter method.[81] However, a data set of five foods tested in 47 healthy subjects who took part in an inter-laboratory study [39] was recently used to compare the performance of some of these methods of calculating AUC. It was found that GI values based on AUC$_{min}$ correlated significantly with the subjects' glycemic response to the reference food (in this case glucose), which suggested that this is not a good method for GI determinations, as GI values obtained depend on the glucose tolerance status of the subject.[21]

Total AUC (AUC$_0$)

AUC$_0$ has been criticized as insensitive for detecting differences between the postprandial glycemic responses of different foods and meals.[21,61] This method includes the whole area under the blood glucose response curve down to a blood glucose concentration of 0. Variations in AUC$_0$ due to variation in FBG are thus not due to the test meal consumed.[21]

Incremental area under the curve until first return to baseline (Incremental AUC$_{cut}$)

As mentioned before, a data set of five foods tested in 47 healthy subjects, who took part in an inter-laboratory study [39], was recently used to compare the performance of some of the methods of calculating AUC. Incremental AUC$_{cut}$ yielded GI values, which agreed well with the recommended method, i.e. IAUC. However, the SD values of the GI values were greater than for the IAUC method and though small, the difference was present for all five foods, as well as statistically significant, using the analysis of variance (two-tailed t-test).[21]

Recommendation: The FAO/WHO Expert Consultation on carbohydrates in human nutrition suggested that the AUC (above fasting baseline) or IAUC be used for GI tests, as most of the GI data that are available have been calculated using this formula.[3] Brouns et al (2005) confirmed this and added that the GI should be calculated as the mean of the individual ratios, i.e. f:r, where f is an individual subjects' IAUC after consumption of the test food and r is the average of the IAUC for the same subject after consumption of 2-3 repetitions of the reference food. Seeing that f:r and F:R do not yield the exact same result, the GI should preferably be calculated using f:r and not as a ratio of the means, i.e. F:R, where F is the mean incremental AUC for a group of subjects after consumption of the test food and R is the mean

for the same group of subjects of the average of the IAUC after consumption of 2-3 repetitions of the reference food.[21]

- **Biochemical assay**

O'Dea et al (1980) and Jenkins et al (1982) showed that the glycemic responses of foods can be predicted from the rate at which foods are digested in vitro.[50] However, these procedures for measurement of starch digestion generally do not predict metabolic responses adequately, especially when the food form and the rate of gastric emptying play a significant role.[106]

- **Interval between test days**

It has been recommended that there should be an interval of at least one day between GI tests.[4] This was confirmed by Brouns et al (2005), who advised that a washout period of one day might not be enough to prevent the food tested in a first session from influencing the glycemic response of the subject to the food tested on a subsequent session.[21]

Recommendation: It was recommended that a washout period of two days between GI tests is sufficient, as the impact that a food tested in a first session will have on the metabolism of the subject two days later is negligible.[21]

- **Re-utilization of test results of the standard/reference food**

Recommendation: Values obtained by having subjects conduct tests on the reference food for one study can be used as reference values in another subsequent study on two conditions:
- The subjects must not undergo physiological changes between two studies, e.g. significant weight change, change in medical treatment or physical activity, as these can cause important changes in their FBG levels or their glycemic responses to foods.[21]
- Wolever et al (1985) reported that the AUC of the reference food (glucose) differed by 0–23% in healthy subjects when they ingested two rice meals 2y apart.[56] It has therefore been recommended that the reference food be tested every 2–3 months by each test subject or for every six foods that are tested by the subject, whichever is more frequent. If a test of the reference food is always done at the start and finish of each study, the last reference test of the first study can be used as the first reference test of the subsequent study.[21]

- **Randomization and how it is done**

Foods for testing should ideally be randomized in blocks of up to six foods, with a reference food test scheduled at the start and finish of the block. The reference trial at the end of the first block can be used as the first reference trial of the next block of test foods. However, randomization is not the only approach to conducting GI tests and, although important in parallel-design clinical trials of drugs, is difficult or even impossible to apply in studies in which the GI values of foods need to be determined.[21]

- **Outliers**

Wolever et al (1991) recommended that, should a subject's GI value for a specific food be an outlier, i.e. >2 SD from the mean, and it is not due to large variability of the repeated reference foods, the test food may be repeated again. The outlier can then be discarded if two further test results yield a GI value similar to that for the rest of the group, but should be suspected to be real if a repeated test of the food is similar to the original result.[2] If outliers are excluded, it should be stated and results including the

outlying points(s) should be given, as well as the position(s) thereof in relation to the rest of the data, i.e. SD from the mean including the outlier(s), so that the impact of removing the outlier(s) on the results and conclusions, as well as the reasonableness of excluding the outlier(s) can be assessed by other researchers. According to Altman (1991), a value can be regarded as suspicious and removed from the data set, if it does not seem to be plausible or if a mistake in the detemination thereof is evident.[21]

Recommendation: An outlier could be removed from the data set under certain circumstances and for reasons of practicability, without having to reassess that particular GI value.[21]

- **Measuring instrument**

Abbott/Medisense metres are examples of strip-test systems that use the YSI reference method to calibrate the test-strips.[84] In the decade from 1993-2003 most glucose metres were calibrated to a capillary whole blood glucose technique, such as the YSI. However, from 1997, some manufacturers, e.g. Abbott/Medisense, changed their calibration method from capillary whole blood to capillary plasma, as the strips-test methods generally respond to glucose concentration in plasma which diffuses into the test strip reaction zone, even though whole blood is used for the test.[37,109] This explains the improvement in the performance of glucometres after 1997 when compared to the YSI (a reference method), which CV was <3% during an overall study period [37] and which is preferable for GI testing.[21]
For typical clinical laboratories, the CV is usually <3% [34] or at least within the level of 5%, which is usually regarded as acceptable for laboratory instruments.[35]

Recommendation: Blood glucose concentrations can be determined by enzymatic determination in capillary whole blood or plasma samples or by enzymatic methods that are normally used in blood glucose metres. However, the precision and accuracy of the method that is used should be evaluated.[21]

2.5   Need for standardized measurement

The FAO/WHO recommended a standardized method of doing GI tests in 1998.[3]   However, it was decided by seven experienced GI investigators in different international centers that an evaluation of the recommended method was required. Consequently an inter-laboratory study was conducted to determine the magnitude and sources of variation of GI values obtained on four centrally provided foods and white bread, which had to be obtained locally by each investigator. This study found that the GI values of foods were more precisely and accurately determined when capillary blood sampling was used, instead of venous blood sampling, with a mean between-laboratory standard deviation of 9.0.[39]

Since the inter-laboratory study had been conducted, an international committee for the standardization of GI testing methodology was appointed by ILSI, the findings and recommendations of which were published recently [21] and included in 2.4.3.2.

2.6   Conclusion from literature

The literature study revealed the following:
- another system is needed to classify CHO rich foods instead of using the terms simple and complex CHO, which has become obsolete and should not be used anymore.[3,23] Jenkins et al (1981) proposed a classification of CHO rich foods based on the effect of specific foods on blood glucose levels, in comparison to a standard. This is called the glycemic index (GI)[1] and has also been recommended as a useful indicator of the impact different foods have on blood glucose levels and to be used to compare foods of similar composition within food groups [3];

- it has been shown in prospective studies that the GI reduced the risk of diabetes [7,8], CVD [9] and cancer [10] and clinical studies have shown that the GI improved glycemic control in diabetic subjects [11] and caused a significant reduction in total cholesterol.[75] The GI is also useful for sports performance [17] and memory [21];
- many factors can influence the GI values of foods (refer to Table 11);
- many factors can influence the variability of the GI values of foods (refer to 2.4.3.2);
- the OTU glucometre was recently evaluated against a reference technique or gold standard (YSI) by comparing the IAUC and GI values of seven potato meals in healthy subjects. The OTU showed more variation and did not agree well with the values obtained using the YSI. The OTU was therefore not recommended for determining AUC or GI values in healthy subjects. The researchers, however, concluded that this does not necessarily apply to other glucometres and recommended that the performance of these meters should be evaluated [34];
- Abbott/ Medisense metres are examples of strip-test systems that use the YSI reference method to calibrate the test-strips.[84] The MPQIDG has been found in an independent head to head comparison conducted by the International Diabetes Institute (Melbourne, Australia) on many glucometres, to show the smallest difference from the reference method (YSI) across a whole range of mean glucose levels.[35] In the decade from 1993-2003 most glucose metres were calibrated to a capillary whole blood glucose technique, such as the YSI. However, from 1997, some manufacturers, e.g. Abbott/Medisense, changed their calibration method from capillary whole blood to capillary plasma, as the strips-test methods generally respond to glucose concentration in plasma which diffuses into the test strip reaction zone, even although whole blood is used for the test. This explains the improvement in performance of near-patient testing systems after 1997 when compared to the YSI (a reference method); CV was < 3% during an overall study period [37] and which is preferable for GI tests [21];
- the CV of the new test strip of the MPQIDG ranged from 2.1-5.6% over a range of blood glucose readings from 2.2-26mmol/L, for 80 replicate tests across four lot numbers and fell mostly within the recommended level of 3-5%, with the only value above 5% being that for very low blood glucose readings. The accuracy of the metre, as obtained by 203 lay users, was comparable to that obtained by the trained healthcare professionals (*r*=0.990, mean absolute bias = 5.6%) and the laboratory plasma method *(r*=0.982; mean absolute bias = 7.2%). Very good correlation was also found between the MPQIDG and the laboratory method (YSI plasma glucose)(*r* =0.982 and *r* = 0.982) respectively for lay users and trained operators and between the MPQIDG and the laboratory method (YSI whole blood glucose) (*r*=0.98 and *r*=0.979 respectively for lay users and trained operators) (no p-values were provided). The MPQIDG also produced accurate results at high altitude, across a hematocrit range of 20–70% and additional studies showed that no clinically significant effect on the accuracy of the new test strips was found with metre movement, oxygen levels, sample re-application, touching and smearing blood on the test strip and 50 drugs or endogenous substances [36];
- it has been found that "clinically acceptable user proficiency in determining capillary blood glucose can be maintained in most subjects, with recurrent intensive education during follow-up clinic visits".[92] In addition it has been found that the MPQIDG, with the microflo test strip, is unique in the sense that it has been designed to minimize user errors. This meter has been clinically proven to be accurate in controlled laboratory studies, as well as in the real world for blood glucose testing by diabetic individuals. "Findings from various clinical trials demonstrate that the MPQIDG provides consistent accuracy, unaffected by the various challenging conditions that affect other glucose meters" [91];
- the results of an inter-laboratory study showed that the observed between-laboratory variation in GI could mainly be attributed to random, day-to-day variation of blood glucose responses within subjects. Finding ways to reduce within-subject variation of glycemic responses would therefore

probably be the most effective strategy to improve the precision of measurement of GI values.[39]

3. RESEARCH DESIGN AND METHODOLOGY

3.1 Aim of the study

The overall aim of the study (including Part 1 and 2) was to compare the mean GI values of foods (as determined EL, using a mixed group of subjects and the MPQIDG) to the mean GI values of the same foods (as determined IL, using a healthy group of subjects and laboratory equipment), in order to determine whether:

- EL testing is an acceptable alternative for IL testing, and
- there is a significant difference between the mean GI values obtained, using the two methods.

The research project was conducted in two parts. In Part 1 of the study, the absolute blood glucose readings, AUC and GI AUC values obtained IL on specific foods by a group of healthy subjects, using the YSI (gold standard), were compared to the absolute blood glucose readings, AUC and GI values obtained IL on the same foods by the same group of healthy subjects, using the MPQIDG. The mean GI values of these foods, as determined IL in a group of healthy subjects, using YSI were also compared to the mean GI values of the same foods, as had previously been obtained (for food companies) EL by a mixed group of subjects (i.e. diabetic and healthy subjects), using the MPQIDG.

It was decided to also compare the mean GI values of five other foods, as determined EL by a mixed group of subjects, using the MPQIDG, to the mean GI values of the same foods, as had been obtained IL by experienced, international GI testing laboratories, each using a group of healthy subjects and laboratory equipment, which formed part of an inter-laboratory study [39] (Part 2 of the study). The results of the latter study, using healthy subjects IL and laboratory equipment, were thus used as a gold standard, to which the EL results of Part 2 of the study were compared.

3.2 Part 1

3.2.1 Background information

Intra-laboratory (IL): In some GI testing centers, subjects eat a standard dinner [21,23] in the laboratory and sleep in beds provided in the laboratory. In other centers, subjects can eat a standard dinner at home and sleep in their own beds the night before a GI test is conducted. They then go to the laboratory for the GI test on the morning of the test. The latter protocol was followed during the IL part of Part 1 of the study. GI tests are also usually conducted IL, in either healthy or diabetic subjects (either type 1 or type 2)[22,30], using laboratory equipment, such as the YSI [39], and the international protocol for GI testing.[3]

Extra-laboratory (EL): In EL tests the subjects eat a standard dinner [21,23] at home and sleep in their own beds the night before a GI test is conducted. They then conduct the GI test in the comfort of their own home, using the MPQIDG and the international protocol for GI testing.[3]

3.2.2 Research problem

What was the degree of variance and was there a significant difference between the mean GI values obtained when a group of healthy subjects (Group 1) did three glucose (reference food) and two food (i.e. Muesli and Apple juice) determinations IL, using the YSI (i.e. gold standard), and the mean GI values of the same two foods (Muesli and Apple juice), obtained in the following ways (refer to Table 14):
Group 1, IL, using the MPQIDG (for sub-problems 3.2.3.1 and 3.2.3.2, respectively)
Group 2 (same group of healthy subjects as Group 1), EL, using the MPQIDG (as a control) and

Groups 3 and 4 (mixed groups of subjects, i.e. healthy, type 1 and type 2 diabetic subjects) who tested Muesli and Apple juice, respectively, EL, for food companies, using the MPQIDG? These two foods were chosen as they are easy to consume IL, do not require any cooking, their GI values have both been determined internationally by other researchers [22,30] and one is a liquid and the other a solid food.

Table 14: Summary of methodology for Part 1 of the study

Group	Subjects	Technique	IL/EL[*]	Test foods	Research problem and sub-problems
1	Healthy 4 Male 8 Female	YSI[+] & MPQIDG[++]	IL	3 Glucose M[$] A[$$]	3.2.2.1 3.2.2.2
2	Healthy 4 Male 8 Female	MPQIDG	EL	3 Glucose M A	3.2.2.1
3	Mixed **Male:** 1 type 1 Diab.[#] 1 type 2 Diab. 1 healthy **Female:** 1 type 1 Diab. 2 type 2 Diab. 4 healthy	MPQIDG	EL	3 Glucose M	3.2.2.1
4	Mixed **Male:** 1 type 1 Diab. 1 type 2 Diab. **Female:** 1 type 1 Diab. 2 type 2 Diab. 3 healthy	MPQIDG	EL	3 Glucose A	3.2.2.1

[*] IL: Intra-Laboratory
     EL: Extra-Laboratory
[+] YSI: Yellow Springs International Analyser
[++] MPQIDG: Medisense Precision QID Glucometre
[$] Muesli
[$$] Apple juice
[#] Diabetic subject(s)

3.2.3 Sub-problems

The following sub-problems were formulated:
3.2.3.1 What was the degree of correlation between the absolute blood glucose readings obtained IL on two food products (i.e. Muesli and Apple juice) and three glucose determinations, using a group of healthy subjects (i.e. Group 1), and the YSI and MPQIDG
3.2.3.2 Was there agreement and what was the correlation between the GI values of Muesli and Apple juice and the AUC values (above the fasting baseline) of Glucose 1, 2 and 3, Muesli and Apple juice in the individual subjects, as determined IL in a group of healthy subjects (Group 1), using the YSI, when compared to the GI and AUC values of the same two foods, that were determined IL in the same group of healthy subjects, using the MPQIDG?

3.2.3.3 How did the GI values of the two foods that were determined in the mentioned four ways compare when using the three methods of calculating the AUC (IAUC, AUC_{min} and AUC_0)?

3.2.4 Hypotheses

The following research hypotheses were formulated:

3.2.4.1 There would be a significant correlation ($p \leq 0.05$) between the actual blood glucose readings as obtained IL, when a group of healthy subjects (Group 1), and the YSI and MPQIDG were used.

3.2.4.2 There would be a significant correlation ($p \leq 0.05$) and agreement between the GI and AUC values as obtained IL, when a group of healthy subjects (Group 1), and the YSI and MPQIDG were used.

3.2.4.3 There would be a significant difference ($p \leq 0.05$) between the mean GI values of the two foods that were determined in the mentioned four ways, using the IAUC calculation, when compared to the mean GI values of the two foods, using the other two most commonly used methods of calculating the AUC (AUC_{min} and AUC_0).

3.2.5 Study design

3.2.5.1 Type of Study
Quasi-experimental, controlled trial (including a historical element).

3.2.5.2 Study Population
Internationally, 8-12 subjects are usually used for GI tests [2,3,39].

3.2.5.3 Inclusion criteria
- Age: Persons from ages 18-70y.[2,21]
- Gender: Male and female subjects.[21]
- BMI: Persons with a BMI of 18–35kg/m^2.[23]
- Blood glucose control: Only persons that had good blood glucose control [2] and who had been consulted by a dietician (in the case of diabetic subjects) were used in this study.
- Exercise: Any person, whether he/she exercised regularly or not at all, were included in the study, as long as he/she did it consistently.[23]
- IL part of the study: healthy subjects.[22]
  EL part of the study: Healthy, type 1 and type 2 diabetic subjects.[1,19,32,53,56]

3.2.5.4 Exclusion Criteria
- Age: Persons younger than 18 or older than 70y.[2,21]
- BMI: Persons with a BMI of less than 18 or more than 35kg/m^2.[23]
- Persons whose blood glucose was completely out of control [2] and who had never been consulted by a dietician.
- Exercise: Any person with an erratic exercise program.

3.2.5.5 Study group
- Twelve healthy (29-54y old) individuals [2,3,21] who complied with the inclusion criteria were used for the purposes of the IL part of the study. Subjects were recruited from patients, who were consulted in the clinical practice of the investigator, as well as from volunteers, who were interested in partaking in the study.
- Healthy, type 2 and type 1 diabetic subjects [1,19,32,53,56] were used during the EL part of the study.

3.2.6 Materials and methods

The protocol for all testing activities (IL and EL of Part 1 of the study) was standardized according to the international protocol for GI testing [3,21], which allowed for:

- Standardized techniques, i.e. the subjects were trained in using the MPQIDG, lancet and test strips (Addendum 3).
- Measures to reduce the effect of day-to-day within-subject variation in glycemic responses, i.e. control had to be taken over lifestyle-confounding factors. Subjects were requested to standardize their lifestyle, e.g. they were asked to standardize their consumption of caffeine[40,41] (by consuming either decaffeinated drinks at all times or a standard amount of caffeine containing drinks), alcohol [42,43] (by consuming either no alcohol or a standard volume of alcohol on the evening before all GI tests) and medication [23] (by either consuming medication like vitamin tablets, thyroid tablets, diabetic medication, etc. every day or not at all, and refraining from conducting a GI test if they took other medication like antibiotics, headache tablets, etc.). They also had to standardize their exercise habits [23] (by keeping to the same exercise program, especially on the day before all GI tests were conducted, with the last exercise to be done by 12h00), sleeping habits [46] (by trying to go to bed at the same time every evening) and smoking habits [21] (by either smoking the same number of cigarettes the day before a test or not smoking at all). Female subjects were advised to not conduct a GI test during their menstrual period, as hormonal fluctuations can affect blood glucose response.[47] All subjects were advised to not conduct a GI test if they have an infection in their bodies, e.g. a cold, toothache, etc, as infections usually affect blood glucose levels.[5,48]
- Involvement of subjects, by having them choose a standard meal [21] which they had to provide and cook themselves on the night before all GI tests were conducted, of which about 50% of the total energy preferably had to come from CHO, 30% from fat and 20% from protein, to help prevent potential second-meal effects.[23] They were asked to consume the meal before 20h00 on the night before testing, although they were also allowed to have a small snack and drink, e.g. a fruit or a biscuit, if preferred and coffee or tea before 22h00. All subjects, however, had to decide beforehand whether he/she wanted to take only coffee or tea, or the small snack as well, and had to follow the same procedure for every test.

IL testing

Subjects had to be in a fasting state on the morning of the tests, i.e. only water should have been taken after 22h00 on the previous night. They came to the laboratory of Du Buisson and Partners (Pathologists, Nelspruit) at 7h30, for the tests to commence at 8h00, on 5-6 consecutive Tuesdays or Saturdays during the Autumn/beginning Winter of 2001, depending on the schedules of the subjects and were therefore studied after an 10h fast. They were required to sign informed consent (Addendum 1) and had to fill out a form (Addendum 4), recording the meal, snack (if any) and drink(s) they had the night before. The food (i.e. glucose or Muesli) or drink (i.e. Apple juice) that the subjects had to consume on the mornings of the tests was obtained from the local supermarket or pharmacy by the investigator and was supplied to them. Du Buisson and Partners Pathologists sponsored the minor laboratory equipment and medical staff. The YSI was borrowed from the Department of Animal and Wild Life Science, Faculty of Natural and Agricultural Sciences (UP) and the solutions necessary for the operation of this instrument were supplied by the investigator. Novo Nordisk, Pharmaceutical Company sponsored the glucometres and test strips.

On these test days, a previously measured out portion of food/drink, which contained 50g glycemic/available CHO (total CHO minus dietary fibre) was taken by the subjects, spread out over the first 15min, after a fasting blood sample (and a second one as a control) was obtained. The sample of glucose was always mixed with one cup of water (250mL). One cup of water was also given with Muesli,

according to the international protocol for GI testing.[3,21] A blood glucose sample for both YSI and MPQIDG was obtained every 15min, until two consecutive values were equal to or below the starting value, but not longer than 2h in healthy individuals. This was done for glucose, on three separate occasions, and for the two foods, i.e. Muesli and Apple juice, on two separate occasions. The glucose tests were alternated with the food tests.[21]

On the mornings of the tests, the blood samples were obtained by the subjects themselves in the laboratory of Du Buisson and Partners, using a lancet to prick the finger. Four to five drops of blood were placed in a clearly marked grey-top micro glucose tube, containing fluoride oxalate anticoagulant, after which the tube was capped and rolled in the hand to mix the blood with the anticoagulant, and placed in one of two specially marked trays for each subject. The subjects also had to place one drop of blood, preferably from the same "hole" in the finger on a test strip of the MPQIDG, to obtain an immediate reading (20sec turn over time), at the same time that the blood samples were obtained for analysis by YSI. One FBG sample was obtained, as well as a second sample for control purposes, for analysis by YSI and MPQIDG. After consumption of the test food/drink, the subjects had to obtain another blood glucose value for analysis by YSI and MPQIDG.

Once each subject had a few blood samples in his/her tray, the tray with samples was taken by him/her to the medical technologist on duty, as the blood glucose content of the whole capillary blood was determined by laboratory personnel of Du Buisson and Partners, using the STAT2300 YSI glucose analyzer (YSI or gold standard). The medical technologist transferred the samples into the other specially marked tray for a specific subject and returned the subject's tray to him/her, so that he/she could place more samples for YSI therein, as the blood samples were obtained by the subject him/herself. The medical technologist analyzed blood samples throughout each morning that tests were conducted at Du Buisson and Partners, and therefore the samples had a short turn over time, i.e. 15min maximum for any one sample. The medical technologists were instructed to continuously roll the grey-top tubes of all the subjects in the hand to mix the blood with the anticoagulant, while the samples were awaiting analysis.

One of the medical technologists of Du Buisson and Partners underwent training in the operation of the YSI at the Department of Animal and Wild Life Science, Faculty of Natural and Agricultural Sciences (UP) and she instructed the other medical technologists accordingly. The YSI was put through a set-up process by the medical technologist on duty and was subsequently placed on "standby", until commencement of the analysis procedure of the blood samples, as obtained by the test subjects, on the mornings of the tests. Upon reception of the first blood samples of the test subjects, the medical technologist on duty labeled the blood samples with a specific code for each test subject and pressed "run", in order to change the YSI from "standby" mode into the operational mode. She/he then followed the following procedure, in order to obtain a reading for a specific blood sample, using the YSI:
Pressed "sample" button.
Typed in the code of the specific blood sample.
Opened the grey-top tube, placed the tube containing the sample of blood under the sipper, which had appeared in the mean time, so that the sipper was submerged in the blood.
Pressed "sample" again and waited for the sipper to "sip up" some of the blood.
Waited for the result to be printed [110] and attached this to the specific subject's specially designed form (Addendum 5), on which the medical technologist also wrote all the blood glucose readings, as determined by YSI, of each test subject for each food.
Waited for the YSI to indicate that it was ready to analyze another blood sample.

The subjects recorded the blood glucose readings of MPQIDG themselves on a specially designed form (Addendum 6). At the end of each morning of conducting GI tests in the laboratory of Du Buisson and Partners, all the forms containing the food records (Addendum 4) of the subjects, as well as the forms

containing the blood glucose readings of the subjects (Addenda 5 and 6), as determined by the MPQIDG and YSI, were collected by the investigator and taken to the offices of the investigator, who captured the data.

EL testing

Subjects had to be in a fasting state on the mornings of the tests, i.e. only water should have been taken after 22h00 on the previous night. The EL tests (Group 2) were done in the subjects' own time, during the same two calendar months that the IL tests were conducted, except for the EL tests that had been done by the mixed groups of subjects (Groups 3 and 4) for food companies, before the commencement of this study. However, they were asked to start all GI tests at approximately the same time on the mornings that GI tests were conducted in order to control the fasting time in a non-stressful manner.[38] They were asked to fill out a form (Addendum 4), recording the meal, snack (if any) and drinks they had the night before. The food (i.e. glucose or M) or drink (i.e. A) that the subjects had to consume on the morning of the tests, was obtained from the local supermarket or pharmacy by the investigator and the glucometres and test strips were sponsored by Novo Nordisk, Pharmaceutical Company. All of there were supplied to the subjects in advance.

On all these test days, a previously measured out portion of food/drink, which contained 50g glycemic/available CHO (total CHO minus dietary fibre) was taken by the subjects, spread out over the first 15min, after a fasting blood sample (and a second one as a control) was obtained. The sample of glucose was always mixed with one cup of water (250mL). One cup of water was also given with the Muesli, according to the international protocol for GI testing.[3,21] Instructions in this regard were given to the test subjects in writing. This was done for glucose, on three separate occasions, and for the two foods, i.e. Muesli and Apple juice, on two separate occasions.

On the mornings of the EL testing, the subjects themselves obtained the blood samples, in the comfort of their own homes, using a lancet to prick the finger. They had to place one drop of blood on a test strip of the MPQIDG, to obtain an immediate fasting capillary blood glucose reading (20sec turn over time), and another fasting value was obtained, as a control. After consumption of the test food/drink, blood glucose values for the MPQIDG were obtained every 15min, until two consecutive values were equal to or below the starting value, but not longer than 2h in healthy individuals or 3h in diabetic subjects. All these blood glucose readings were filled out thoroughly on a specially designed form (Addendum 6) and were faxed to the investigator by the subjects, upon completion of each test. The investigator captured the data.

The protocol for testing activities, regarding standardized techniques (Addendum 3), measures to reduce the effect of day-to-day within-subject variation in glycemic responses and involvement of subjects, which was described in 3.2.6, was also followed for the EL tests that were conducted for food companies, before the commencement of this study.

Blinding

No blinding was done in any of the tests, as this is not the international protocol in GI testing.[21] The foods (Muesli and Apple juice) were alternated with the glucose (reference food) tests.

Bias

Possible sources of bias:
- Selecting bias: Convenience sampling of patients and volunteers (however, this corresponds with the international protocol).[3,21]
- Subject bias: Compliance of test subjects. The procedures were explained to the subjects by the investigator and they were asked to sign informed consent. However, the investigator had to assume, in good faith, that the subjects would keep to the recommended procedures.

3.2.7   Statistical analysis

The statistical analysis was done by the Department of Statistics of the Medical Research Council using the SAS System for the Main Frame, Release 8.2, running under CMS. The graphs were drawn using Excel for Windows, 1998 and Grapher, Version 4, developed by Golden Software, 2002.

Descriptive and inferential statistical techniques were used.
The formula of Pearson [111] was used to determine the correlation between:
- the actual blood glucose readings obtained IL by YSI and MPQIDG for the three glucose determinations, and Muesli and Apple juice;
- the actual blood glucose readings obtained IL by YSI and MPQIDG for the three glucose determinations and Muesli and Apple juice, after outliers were identified (using regression analysis)[112] and removed, where necessary.
Statistical significance was set at $p = 0.05$.

The analysis of variance (two tailed t-test) was used to compare:
- the means of the absolute blood glucose readings obtained IL by YSI and MPQIDG for the three glucose determinations, and Muesli and Apple juice, respectively.
Statistical significance was set at $p = 0.05$.

The GI values of the two foods (M and A) were determined by dividing the IAUC for the food/drink by the average of the IAUC values for each subjects' three glucose value determinations for each IL test of each healthy subject (Group 1), using YSI and MPQIDG, respectively. The GI values of the two foods were also determined in this manner for each EL test, using the MPQIDG value of the subjects (Groups 2, 3 and 4). The GI obtained in each person was used to calculate the mean GI values of the two foods; showing the standard error (SE) and confidence interval (CI) values in tables.

The analysis of variance (ANOVA) was used to compare the mean GI values obtained IL for the two foods, using YSI (Group 1) with:
- the mean GI values obtained for the two foods (Group 1), using MPQIDG, IL,
- the mean GI values obtained for the two foods (Group 2), using MPQIDG, EL and
- the mean GI values obtained for the two foods (Groups 3 and 4), using MPQIDG, EL.
Statistical significance was set at $p = 0.05$.

Respectively, the formula of Pearson [111] was used to determine the correlation between the under mentioned, and the formula of Lin [113] was used to determine whether there was reproducibility (or agreement) between the:
- GI and AUC values obtained IL for the two foods in each subject of Group 1 using the YSI and the MPQIDG, respectively, and
- GI and AUC values obtained IL for the two foods in each subject of Group 1 using the YSI and the MPQIDG in the laboratory, after outliers were identified (using regression analysis) and removed, where necessary.
Statistical significance was set at $p = 0.05$.

The analysis of variance (two tailed t-test) was used to compare:
- the means of the AUC readings obtained IL by YSI and MPQIDG for the three glucose determinations, and Muesli and Apple juice, respectively;
- the means of the AUC readings obtained IL by Ysi and MPQIDG for the three glucose determinations, and Muesli and Apple juice (respectively), after removal of outliers;
Statistical significance was set at $p = 0.05$.

The AUC for each IL test of each healthy subject (Group 1), as well as each EL test of each of the other subjects (Groups 2, 3 and 4) was calculated, using the three methods of calculation [21] and the GI values obtained were compared, using the analysis of variance (two-tailed t-test) (p = 0.05).

Correlation coefficient
Correlation analysis is the statistical tool that is used "to describe the degree to which one variable is linearly related to another". A number of measures for describing the association and strength of association between two variables have been developed by statisticians. In this thesis only the **coefficient of determination** (r^2) and Pearson's **coefficient of correlation** (r) [111], as well as **Lin's Concordance Correlation coefficient** (r_c) [1113] were used.

- **Coefficient of determination** *(r^2)*

The sample coefficient of determination is "a measure of the degree of linear association between x and y" and is symbolized by r^2, where:

$$r^2 = \frac{1 - \sum (y_i - \hat{y}_i)^2}{\sum (y_i - y\text{ mean of }y_i)^2}$$

where:
$\hat{y}_i$ = the regression value of y_i
Mean of y_i = mean of the y's

When there is perfect correlation, the value of r^2 = +1 and every observed value of Y lies on the estimating line. However, when there is no correlation, the sample coefficient of determination is 0 and the points could e.g. lie randomly around a horizontal regression line. These are the two extremes. In most of the problems encountered by researchers, r^2 lies somewhere between these two extremes of 0 and 1, where r^2 close to 1 indicates a strong linear relationship between x and y, while r^2 near 0 indicates little linear relationship between these two variables. It is also important to realize that r^2 measures only the strength of a linear relationship between two variables, and not e.g. when the x and y values lie on a parabola.

To calculate r^2 with the abovementioned equation, a series of tedious calculations must be done. To bypass these, statisticians have developed a short-cut equation, using values that have already been determined in the regression analysis. r^2 calculated by the shortcut method:

$$r^2 = \frac{a\sum y_i + b\sum x_i y_i - n \text{ mean of } y^2}{\sum y_i^2 - n \text{ Mean of } y^2}$$

where:
r^2 = sample coefficient of determination
a = y-intercept
b = slope of the best-fitting estimating line
n = number of data points
x_i = values of the independent variable
y_i = values of the dependent variable
Mean of y = mean of the observed values of the dependent variable

If e.g. $r^2 = 0.8$, it tells us that 80% of the variation in Y can be explained by the regression line.

- **Pearson's correlation coefficient** (r)

When dealing with samples, the sample coefficient of correlation is r and is calculated as follows:

$$r = \sqrt{r^2}$$

However, r is nothing more than the $\sqrt{r^2}$ and its meaning can therefore not be interpreted directly, as with r^2. r can be either positive or negative and the sign of r indicates the direction of the relationship between the two variables x and y. If y increases as x increases, there is a positive relationship and r will lie between 0 and +1. If, however, y decreases as x increases, an inverse or negative relationship exists and r will fall between 0 and -1.[111]

The calculated Pearson's correlation coefficient was interpreted as follows:
$r \leq 0.5$ reflects poor correlation
$r = 0.6$ reflects fair correlation
$r = 0.7$ reflects acceptable correlation
$r = 0.8$ reflects good correlation and
$r = 0.9$ reflects very good correlation

- **Lin's concordance correlation coefficient** (r_c)

Lin reports that, according to Westgard & Hunt (1973) and Bauer & Kennedy (1981), when a new instrument or assay is developed, it is important to evaluate whether the substitute can reproduce the results produced by a traditional gold-standard instrument. "Such validation processes are often evaluated by using the Pearson correlation coefficient, the paired t-test, the least squares analysis of slope (=1) and intercept (=0), coefficient of variation or the intraclass correlation coefficient". However, none of these alone can fully assess the desired reproducibility characteristics, i.e. whether the measurements fall on a 45° line through the origin, within a tolerable error. The Pearson correlation coefficient e.g. measures the strength of a linear relationship between two variables, but is unable to detect departure from the 45° line.

To measure reproducibility or agreement between paired readings, Lin proposed the Concordance correlation coefficient, which is calculated as follows:

$$r_c = \frac{2 \sum x_i . y_i}{\sum x_i^2 + \sum y_i^2 + (n-1)(\text{mean of x} - \text{mean of y})^2}$$

The concordance correlation coefficient has the following advantages:
- It evaluates the degree to which paired data fall on the 45° line.
- It contains measurements of accuracy and precision.
- Any departure from the 45° line yield an $r_c < 1$, even if $r = 1$.
- It is simple to use.
- "Its estimate using the sample counterparts is consistent and has asymptotic normality for bivariate normal data. However, its statistical properties (consistency and asymptotic normality) can be much improved by using the inverse hyperbolic tangent transformation (Z-transformation)".
- It is robust vs. samples from the uniform and Poisson distributions, even with small sample sizes.

- It can even be utilized to evaluate agreements among more than two readings, although the multiple-reading formula has to be adjusted slightly.[113]

The calculated Lin's concordance correlation coefficient was interpreted as follows:

$r_c \leq 0.5$ indicates poor reproducibility or agreement

$r_c = 0.6$ indicates fair reproducibility or agreement

$r_c = 0.7$ indicates acceptable reproducibility or agreement

$r_c = 0.8$ indicates good reproducibility or agreement

$r_c = 0.9$ indicates high reproducibility or agreement

NOTE: It was decided to use Lin's concordance correlation coefficient to test agreement, instead of Bland and Altman [114], upon recommendation from the statistician. The two methods are equivalent to each other, in that they both include the correlation coefficient, as well as the difference between the means obtained. In testing agreement it is important to not only look at the correlation coefficient, which cannot fully assess the desired reproducibility characteristics, but to also look at whether there is reproducibility or agreement, i.e. the formula used must be able to detect departure from the 45° line. Lin's concordance correlation coefficient is able to do the latter, is simple to use and is also a more recent method than Bland and Altman. [114]

Regression Analysis

Correlation analysis is often used together with regression analysis to measure how well the regression line explains the variation of y, which is the dependent variable. After calculation of *r*-values, regression analysis was used to identify outliers, by observing studentized residuals. If a studentized residual was >2, the observation was considered to be an outlier.[112]

The smaller the p-value, the lesser is the probability to call a difference significant, when in fact no difference exists.

The Glycemic Index (GI)

The Glycemic Index (GI) of a food is:

$$\frac{AUC_f \text{ (above fasting baseline)}}{\text{Mean } AUC_g \text{ (above fasting baseline)}} \times \frac{100}{1}$$

where:

AUC_f (above fasting baseline) = Area under the curve above fasting baseline of a food

Mean AUC_g (above fasting baseline) = Mean area under the curve above fasting baseline of 3 glucose determinations [1].

Area under the curve (AUC) above fasting baseline (IAUC)

Wolever defined the incremental area under the curve as the area under the blood glucose response curve, using the fasting glucose value as baseline.[2] This can be calculated geometrically, using the trapezoid rule.[3]

The formula is as follows:

At/2 + At + (B - A)t/2 + Bt + (C - B)t/2 + Ct + (D – C)t/2 + Dt + (E – D)t/2 …..etc., where A,B,C,D and E represent positive blood glucose increments and t is the time interval between blood samples. If the blood glucose increment D is positive (i.e. greater than the fasting value or baseline) and E is negative (i.e. less than the fasting value of baseline) only the area above the fasting value of baseline (between D and E) is used. If the value E occurs t min after value D, a straight line drawn between points D and E

crosses the baseline at time T after D, where T< t. Thus the area above the curve between D and E is given by DT/2. Because T/t = D/(D+{E}) (where {E} = absolute value of E), therefore T = Dt/(D + (E)) and thus DT/2 = D2t/2(D + {E}). The overall equation simplifies to: Area = (A + B + C + D/2)t + D2t/2(D+{E}).[2]

Incremental area above the lowest glucose value as baseline (AUC$_{min}$)
Vorster and co-workers (1990) proposed an AUC with the lowest or minimum blood glucose reading as baseline to calculate the GI.[23]

The formula is as follows:
AUC= {[(Z-L) +(A-L)]/2 + [(A-L)+(B-L)]/2 + [(B-L)+(C-L)]/2 + [(C-L)+(D-L)]/2 + [(D-L)+(E-L)]/2 + [(E-L)+(F-L)/2] +[(F-L)+(G-L)] /2+[(G-L)+(H-G)]/2}t.
L = Lowest or minimum blood glucose reading.
Z represents the fasting or initial blood glucose sample

Total AUC (AUC$_0$)
This method includes the whole area under the blood glucose response curve down to a blood glucose concentration of 0. Variations in AUC$_0$ due to variation in FBG are thus not due to the test meal consumed.[21]
The formula is as follows:
AUC = [(Z+A)/2 + (A+B)/2 + (B+C)/2 + (C+D)/2 +(D+E)/2 + (E+F)/2 + (F+G)/2 + (G+H)/2]t.
Z represents the fasting or initial blood glucose sample and A to H represents the subsequent blood glucose samples taken at every 15min time increment, which is represented by t.

Standard Deviation (SD)
The population SD or σ is the square root of the population variance. The SD is therefore the square root of the average of the squared distances of all possible observations from their mean. The formula for the standard deviation is:

$$\sigma = \frac{\Sigma (x - \mu)^2}{N - 1}$$

where:
x = the observation
μ = the population mean
N = the total number of elements in the population
Σ = the symbol for the sum of all the (x - μ)^2, or all the values x^2
σ = the population standard deviation [111]

Standard Error (SE)
SE is calculated as follows:

$$\frac{\sigma}{\sqrt{n}}$$

The SE indicates the variation in a mean.

Confidence Interval (CI) values

Usually the 95% confidence interval is used and is calculated by the following formula:

mean of y $\pm$ 1.96 $\quad \sqrt{\quad}$ SD

$\quad\quad\quad\quad\quad\quad\quad\quad$ n

If the number of subjects that were used are 30 or more, the normal distribution is used. However, if the number of subjects that used were less than 30, the critical values of the t distribution should be read off a table and used instead of 1.96 in the above formula.

Two-tailed t-test

The analysis of variance (two-tailed t-test) was used to determine whether there is a significant difference between two means, taking into consideration the standard deviations and number of subjects used.

ANOVA procedure

The ANOVA procedure (the SAS System; the GLM procedure, Release 8.2) was used to determine whether there is a significant difference between more than two means.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Faculty of Health Sciences (UP) and the Pretoria Academic Hospital (Ethics number: S 80/2001) and all subjects signed informed consent (Addendum 1).

3.3 Part 2

3.3.1 Background information (Multi-centre study of Wolever et al, 2003) [39]

In 2001 an inter-laboratory study was conducted by Wolever et al (2003) [39] to assess the international method for GI testing recommended by the FAO/WHO [3], as well as to determine the magnitude and sources of variation of GI values obtained by experienced investigators; testing the same foods in different GI testing centres around the world. Four centrally provided foods (Rice, Spaghetti, Barley and Instant Potato) were sent to each laboratory partaking in the study and White bread had to be obtained locally by each of the laboratories. Five capillary and two venous GI testing laboratories took part in the study and the blood glucose response of the above mentioned five foods and three determinations of a reference food were determined in 8–12 healthy subjects by each of seven GI testing centres, using laboratory equipment (such as the YSI and which is regarded as gold standard). The GI values were calculated [39], using the IAUC, as recommended by the FAO/WHO.[3] One of the principal investigators suggested that the investigator test the same foods and offered to send them to the investigator in order to determine whether there is a significant difference between the GI values obtained on the five foods by experienced international capillary laboratories (using healthy subjects and laboratory equipment) (gold standard), and the GI values obtained EL on the same foods by the investigator, using a mixed group of subjects and the MPQIDG. This is the currently way GI testing laboratories compare their performance in GI testing to that of other international GI testing laboratories.

3.3.2 Research problem

What was the degree of variance between the mean GI values obtained EL (mixed group of subjects and MPQIDG) and the weighted mean GI values obtained IL (by a group of healthy subjects from five experienced capillary GI testing centres in the inter-laboratory study, using laboratory equipment) [39] on three glucose (reference food) and five food (i.e. Rice, Barley, Spaghetti, Instant Potato and White bread) determinations? [39]

3.3.3 Sub-problems

The following sub-problems were formulated:
3.3.3.1 What was the degree of variance between the mean GI values of each of these five foods obtained in each of the five capillary laboratories in the inter-laboratory study, using healthy subjects and laboratory equipment [39], when compared to the weighted mean GI values of the rest of the capillary laboratories, including the EL results on a group of mixed subjects using the MPQIDG (in order to simulate participation in the inter-laboratory study)?

3.3.3.2 How did the SE, the 95% CI limits and the deviation from the group mean GI value of each of the five foods [as determined by each of the five capillary laboratories in the inter-laboratory study using healthy subjects and laboratory equipment [39], as well as by the investigator in the current EL study, using a mixed group of subjects (i.e. healthy and diabetic subjects) and the MPQIDG] compare to the weighted means of the rest of the laboratories for these parameters?

3.3.4 Hypotheses

The following research hypotheses were formulated:

3.3.4.1 There would be no significant difference between the mean GI values of the five foods obtained when a mixed group of subjects (i.e. healthy and type 2 diabetic subjects) did three glucose (reference food) and five food (i.e. Rice, Barley, Spaghetti, Instant Potato and White Bread) determinations using the MPQIDG, EL, and the weighted mean GI values obtained when a group of healthy subjects from five experienced capillary GI testing centres in the inter-laboratory study use laboratory equipment to test the same foods IL.[39]

3.3.4.2 The SE, 95% CI and deviation from group mean GI value of each of the five foods [as determined by each of the five capillary laboratories in an inter-laboratory study using healthy subjects and laboratory equipment, as well as by the investigator in the current EL study, using a mixed group of subjects (i.e. healthy, type 1 and type 2 diabetic subjects) and the MPQIDG] would be less than or equal to the weighted means of the rest of the laboratories for these parameters.

3.3.5 Study design

3.3.5.1 Type of study
Quasi-experimental, controlled trial (including a historical element).

3.3.5.2 Population
Internationally, 8-12 subjects are usually used for GI tests.[2,3,39]

3.3.5.3 Inclusion criteria
- Age: Persons from ages 18-70y.[2,21]
- Gender: Male and female subjects.[21]
- BMI: Persons with a BMI of 18–35kg/m^2.[23]
- Blood glucose control: Only persons that had good blood glucose control [2] and who had been consulted by a dietician (in the case of diabetic subjects) were used in the study.
- Exercise: Any person, whether he/she exercised regularly or not at all was included in the study, as long as he/she did it consistently.[23]
- Healthy and type 2 diabetic subjects.[1,19,32,53,56]

3.3.5.4 Exclusion criteria
- Age: Persons younger than 18 or older than 70y.[2]
- BMI: Persons with a BMI of less than 18 or more than 35.[23]
- Persons whose blood glucose was completely out of control [2] and who had never been consulted by a dietician.
- Exercise: Any person with an erratic exercise program.[23]

3.3.5.5 Study group
- Five healthy (30-43y old) individuals and five type 2 diabetic (30-62y old) individuals, who complied with the inclusion criteria, were used for the purposes of the EL part of the study. Subjects were recruited from patients, who were consulted in the clinical practice of the investigator, as well as from volunteers, who were interested in partaking in the study.

- In the inter-laboratory study, which had been conducted IL and with which the results of the study were compared, 8-12 healthy subjects were used by each international laboratory for the GI tests.[39]

3.3.6 Materials and methods

The protocol for testing activities, regarding standardized techniques (Addendum 3), measures to reduce the effect of day-to-day within-subject variation in glycemic responses and involvement of subjects, which was described in 3.2.5, was also followed for all the EL tests of Part 2 of the study and this information was supplied to the test subjects before the study commenced. The pre-test meal had to be consumed before 20h00, on the evening before all GI tests, which the subjects had to cook and provide themselves. However, they were also allowed to have a small snack and drink, e.g. a fruit or a biscuit, if desired and coffee or tea before 22h00, but each subject had to decide beforehand whether he/she wanted to take only coffee or tea or the small snack as well and follow the same procedure for every test.

Subjects had to be in a fasting state on the morning of the tests, i.e. only water should have been taken after 22h00 on the previous night. The EL tests were done in the subjects' own time, over a period of two months during the Autumn/beginning Winter of 2002. However, they were asked to start all GI tests at approximately the same time on the mornings that GI tests were conducted, in order to control the fasting time in a non-stressful manner.[38] They were required to sign informed consent (Addendum 2) and to fill out a form (Addendum 4), recording the meal, snack (if any) and drinks they had the night before. The food (i.e. Glucose, Barley, Spaghetti or instant potato) that the subjects had to consume on the mornings of the tests was obtained from the chief investigator of the inter-laboratory study and the White bread was obtained from the local supermarket. Novo Nordisk, Pharmaceutical Company sponsored the glucometres and test strips. All of these were supplied to the subjects in advance.

On all these test days, a previously measured out portion of centrally provided food or local white bread or glucose powder, which contained 50g glycemic/available CHO (total CHO minus dietary fibre) and which was also used in an inter-laboratory study [39], was prepared by the subjects themselves in the way prescribed for the inter-laboratory study and which was printed on the test forms (Addendum 6). The food was taken by each subject, spread out over the first 15min, after a fasting blood sample (and a second one as a control) was obtained. The sample of Glucose was always mixed with one cup of water (250mL) and one cup (250mL) of water was also allowed with the White bread, cooked Rice, cooked Barley, cooked Spaghetti and Instant Potato samples, according to international protocol for GI testing.[3] This was done for glucose, on three separate occasions, and for the five foods on five separate occasions. The glucose was taken at the commencement of the study, in the middle and again at the end of the study.

On the mornings of all these EL tests, the subjects themselves obtained the blood samples, in the comfort of their own homes, using a lancet to prick the finger. They had to place one drop of blood on a test strip of the MPQIDG, to obtain an immediate fasting capillary blood glucose reading (20sec turn over time), and another fasting value was obtained, as a control. After consumption of the test food/drink, blood glucose values for the MPQIDG were obtained every 15min, until two consecutive values were equal to or below the starting value, but not longer than 2h in healthy individuals or 3h in diabetic subjects. All these blood glucose readings were filled out thoroughly on a specially designed form (Addendum 6) and were faxed to the investigator by the subjects, upon completion of each test. The investigator captured the data.

The GI tests on these five foods formed part of an inter-laboratory study and all the laboratories that took part in this study conducted their tests in their own laboratories using laboratory equipment (such as the YSI) and 8 -10 healthy individuals during 2001.[39]

Blinding

No blinding was done in any of the tests, as this is not the international protocol in GI testing.[21] The foods were alternated with the glucose (reference food) tests.

Bias

Possible sources of bias:

- Selecting bias: Convenience sampling of patients and volunteers [however, this is similar to the international protocol.[3,21]]

- Subject bias: Compliance of test subjects. The procedures were explained to the subjects by the investigator and they were asked to sign informed consent. However, the investigator had to assume, in good faith, that the subjects would keep to the recommended procedures.

3.3.7   Statistical analysis

The statistical analysis was done by the Department of Statistics of the Medical Research Council, using the SAS System for the Main Frame, Release 8.2, running under CMS. The graphs were drawn using Excel for Windows, 1998.

Descriptive and inferential statistical techniques were used.

The IAUC for each EL test of each subject was calculated. The IAUC for each of the foods of each subject was divided by the averages of the IAUC for each subject's glucose values, and the GI obtained in each person was used to calculate the mean GI values of the five foods; showing the SD and CI values in tables.

- The analysis of variance (two tailed t-test) was used to compare the weighted mean GI values for each of the five foods obtained by all five capillary laboratories using laboratory equipment to the mean GI values of the same foods obtained EL by a group of mixed subjects using the MPQIDG. The mean GI values of all five capillary laboraties, as well as EL, were also compared using ANOVA. Statistical significance was set at p=0. 05.

- The analysis of variance (two–tailed t-test) was also used to compare the mean GI values of each of five foods obtained IL by each of the five capillary laboratories using laboratory equipment to the weighted mean GI values of the same foods obtained by the rest of the laboratories, including the EL results. Statistical significance was set at p=0. 05.

- The SE, CI width and deviation from group mean GI value of each of the five foods, as determined by each of the five capillary laboratories in the inter-laboratory study [39] were calculated, as well that of the EL study using a mixed group of subjects and the MPQIDG. These values were compared to the weighted means of the rest of the laboratories for these parameters.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Faculty of Health Sciences (UP) and the Pretoria Academic Hospital (Ethics number: S 80/2001) and all subjects signed informed consent (Addendum 2).

4. RESULTS AND DISCUSSION

In this chapter the results of the sub-problems will be presented and discussed first and thereafter the results of the research problem per se (separately for Part 1 and 2 of the study).

4.1 Part 1 of the Study

A group of 12 trained, healthy subjects (four male and eight female), aged 29-54y (41$\pm$7), BMI 18-30 kg/m^2 (24$\pm$4), were recruited and tested IL (Group 1) under well-controlled conditions.[2,3,21] Table 15 shows the age and BMI of the subjects that partook in Part 1 of the study.

Table 15: Characteristics of subjects (N=12) who partook in Part 1 of the study

Subject	Age (y)	BMI (kg/m^2)
A1	41	20
A2	42	24
A3	42	28
A4	50	28
A5	54	20
A6	37	23
A7	43	30
A8	44	20
A9	34	26
A10	36	27
A11	29	18
A12	40	22
Mean	**41**	**24**
SD	7	4

4.1.1 Sub-problem

What was the degree of correlation between the absolute blood glucose readings obtained IL on two food products (i.e. Muesli and Apple juice) and three glucose determinations, using a group of healthy subjects (i.e. Group 1), and the YSI and MPQIDG?

The actual blood glucose readings on the YSI and MPQIDG for three glucose determinations, one Muesli and one Apple juice determination, at each time (i.e. at 0, 15, 30, 45, 60, 75, 90, 105 and 120min of the different test days, for the 12 subjects) were compared. The coefficient of determination (r^2) and Pearson's correlation coefficient values (r-values) were determined (Table 16).

Table 16: Pearson's correlation coefficient values (*r*-values) between the absolute blood glucose readings, as done on the YSI and MPQIDG

Product	Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Glucose 1	0.3	0.1	0.7	**0.9**	**0.9**	**0.9**	**0.9**	**0.8**	**0.8**
Glucose 2	0.4	0.5	**0.97**	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**	**0.9**
Glucose 3	0.5	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**
Muesli	0.4	**0.8**	**0.9**	**0.95**	**0.9**	0.6	0.5	**0.8**	0.7
Apple juice	0.02	0.3	0.6	**0.8**	**0.8**	0.7	0.4	0.2	**0.9**

Bold print indicates good or very good correlation

Regression analysis was used to identify outliers, by observing studentized residuals. When the studentized residual was >2, the observation was regarded as an outlier.[112] The subjects whose blood glucose readings for specific foods at specific times were identified as outliers are shown in Table 17.

Table 17: Outliers on absolute blood glucose readings for specific foods at specific time intervals

Product	Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Glucose 1	A12	A12	A4; A6	A4	A10; A11	A10	A1	-	A10
Glucose 2	A3	-	A8	-	A10	A10	-	A1	A6; A10
Glucose 3	A4	-	A5	A7	A9; A12	A5; A12	A7; A8	-	-
Muesli	A6	A12	A8	-	A4	A7	A7	A7; A11	A2
Apple juice	-	A4	A7	A8; A9	A10	A7	-	A10	-

Out of the 45 time intervals (i.e. nine times for each of the five products), subjects A10 and A12 were identified as outliers on seven occasions each, subject A7 on six occasions, subject A4 on five occasions, subject A1 on four occasions, subjects A6, A8 and A9 on three occasions each, subjects A5 and A11 on two occasions each and subjects A2 and A3 on one occasion each. On nine out of the 45 occasions no outliers were identified.

After removal of outliers [112], the coefficient of determination (r^2) and Pearson's correlation coefficient (*r*) were re-calculated and the final *r*-values are shown in Table 18 (p-values in brackets).

Table 18: Pearson's correlation coefficient values (*r*-values) between the absolute blood glucose readings, as done on the YSI and MPQIDG, after removal of outliers

Product	Time (min)								
	0	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**
Glucose 1	0.4 (0.28)	0.1 (0.8)	**0.8 (0.006)**	**0.97 (<0.0001)**	**0.98 (<0.0001)**	**0.95 (0.0002)**	**0.9 (0.0005)**	**0.8 (0.0006)**	**0.9 (0.004)**
Glucose 2	0.03 (0.92)	0.5 (0.11)	**0.98 (<0.0001)**	**0.8 (0.004)**	**0.9 (<0.0001)**	**0.9 (0.004)**	**0.8 (0.003)**	**0.9 (0.001)**	**0.9 (<0.0001)**
Glucose 3	**0.6 (0.05)**	**0.9 (<0.0001)**	**0.9 (0.0002)**	**0.95 (<0.0001)**	**0.9 (0.0002)**	**0.95 (<0.0001)**	**0.97 (<0.0001)**	**0.9 (<0.0001)**	**0.9 (0.0009)**
Muesli	0.3 (0.45)	**0.8 (0.0085)**	**0.9 (0.0004)**	**0.95 (<0.0001)**	**0.96 (<0.0001)**	**0.8 (0.0495)**	0.7 (0.11)	**0.9 (0.01)**	**0.9 (0.024)**
Apple	0.02 (0.95)	**0.7 (0.01)**	**0.8 (0.002)**	**0.9 (0.005)**	**0.9 (0.0005)**	**0.8 (0.01)**	0.4 (0.2454)	0.5 (0.24)	**0.9 (0.01)**

Bold print indicates significant correlation (p≤0.05)

There was a good to very good correlation between most (36 out of 45 readings) of the blood glucose readings (p≤0.05), except for all the FBG readings, as well as the 15min readings of Glucose 1 and 2, the 90min reading of Muesli and 90 and 105min readings of Apple juice. Out of the 45 time intervals, 26 showed a very good correlation (r=0.9; $p<0.02$) between the absolute blood glucose readings obtained on the two instruments and of these 12 were highly significant ($p<0.0001$). Eight out of the 45 time intervals showed a significant correlation (*r*=0.8; $p<0.04$), one showed a fair correlation (*r* =0.6; p=0.05), two showed an acceptable correlation (*r*=0.7; p=0.1) and nine out of the 45 time intervals showed a poor correlation (*r*≤0.5; p>0.1). The correlation (*r)* between all of the determinations at time intervals 30, 45, 60, 75 and 120min was good to very good (*r*=0.8–0.9) and significant (p≤0.05).

There was poor correlation of blood glucose readings between the two instruments at 0min (fasting) for all the foods tested (except for Glucose 3, after removal of outliers)(Table 18). No mention is made in the literature that FBG readings obtained on the MPQIDG correlate poorly with that of the YSI. In fact, Engel et al (1998) found that the MPQIDG yielded the smallest difference from the reference method (YSI) across the entire range of mean glucose levels, when compared to the Advantage, Elite, Mini-Accutrend, Lynx and Companion 2. The MPQIDG, as well as the Lynx and Companion 2 showed no tendency to show a larger deviation from the reference value with higher blood glucose readings, as did the Advantage, Elite and Mini-Accutrend. All values obtained by all the glucose metres fell within the clinically acceptable zones of A and B[2], using error grid analysis, which is considered clinically acceptable.[35] Excellent correlation was reported between the MPQIDG using the new test strips and the laboratory method (YSI plasma glucose)(*r*=0.982 and *r*=0.982), respectively for lay users and trained operators, and between the MPQIDG and the laboratory method (YSI whole blood glucose)(*r*=0.98 and *r*=0.979), respectively for lay users and trained operators (no p-values were provided).[36]

Velangi et al (2003), who also evaluated a glucometre for determining the glycemic response of foods, found a good correlation (*r*=0.89; $p<0.001$) for the individual blood samples measured by the OTU glucometre when compared to the YSI.[34] They found a non-significant increase (p=0.55) in difference between the OTU and the YSI as glucose concentration increased, whereas the investigator found an improvement in the correlation as the blood glucose readings increased. Nevertheless, 92.1% of the OTU values were within zone A and 100% within zones A and B, which could be considered clinically acceptable, after adjusting glucose values for the 12.7% calibration difference.[34]

The reason for the poor correlation of blood glucose readings between the two instruments at 0min (fasting) for all the foods tested (except for Glucose 3, after removal of outliers) (Table 18), could possibly be due to the fact that the test subjects' hands were still cold at the commencement of all the tests, as Part 1 of the study was conducted during the late Autumn/beginning Winter. Warm water was used to improve blood flow, but it is possible that the test subjects' hands were not very warm by the time they took the fasting readings of each test and that their hands warmed up with each warming of the hands before a subsequent reading was taken. Their hands probably also warmed up as the outside temperature increased from 08h00-10h00, seeing that no heater was used in the room. This is confirmed by the fact that there was very good correlation (*r*=0.9; p≤0.05) at 120min, when the tests stopped and at which time the readings were very similar to the fasting readings.

The test subjects were also not used to giving 50μL of blood (about five drops of blood) for a blood glucose reading when using the YSI. This could have caused them to exert too much pressure on the finger to obtain a blood sample for the YSI, especially when their hands were still cold early in the morning, leading to unrealistically low readings on the latter instrument, as dilution of the blood sample by interstitial fluid can yield false lower readings.[88] This could possibly make the MPQIDG a better instrument to obtain blood glucose samples for GI testing, as only one drop of blood (3.5μL) is needed, which minimizes squeezing of the finger to obtain enough blood. However, the CV of the new test strips

of the MPQIDG varies from 2.1-5.6% for blood glucose readings ranging from 2.2-26.6mmol/L, with the highest CV for the lowest readings [36], whereas the CV of YSI is consistently <3%, which is preferable for GI testing.[21]

A reason for the poor correlation of blood glucose readings at 15min (Glucose 1 and 2; Table 18) could possibly be due to the fact that the test subjects were not yet proficient in obtaining blood samples for the YSI, as mentioned before. They were, however, conversant with obtaining a drop of blood (3.5 μL) for the blood glucose determination using the MPQIDG, as all of them have used this instrument before.

A reason for the poor correlation at 90min for M and 90min and 105min for A is not clear. It could have been been due to human factors, such as faulty operation of the YSI by the specific technician that was on duty that day or the test subjects starting to tire towards the end of the study.

All the actual blood glucose readings (598 in total) obtained using the YSI and MPQIDG for three glucose determinations, one Muesli and one Apple juice determination, at all time intervals (i.e. at 0, 15, 30, 45, 60, 75, 90, 105 and 120min of the different test days, for the group of healthy subjects, i.e. Group 1) were also compared and the means determined. The coefficient of determination (r^2), Pearson's correlation coefficient value (r-value) and p-value were determined, as well as Lin's concordance correlation coefficient, which shows agreement. The means were compared using the analysis of variance (two-tailed t-test) (Table 19). These calculations were done to assess the internal measurement consistency of the equipment.

Table 19: Means of all the absolute blood glucose readings for the group of healthy subjects (Group 1) for all five food products

	MPQIDG	YSI
Means (mmol/L)	6.0	5.1
SD	1.6	1.4
95% CI	4.1-4.5	3.6-3.9

Regression analysis was not used to identify outliers, as the data set was so large that identifying and removing outliers would probably not have made a difference to the results.

All the absolute blood glucose readings, obtained using the YSI, were consistently lower than the readings obtained using the MPQIDG. The mean and SE of all the individual blood glucose readings measured by MPQIDG (6.0±0.065 mmol/L), was significantly higher than that measured by YSI, (5.1±0.057) (p=0.0000). However, the Pearson correlation coefficient for all 598 readings was r =0.9 (p<0.0001), indicating very good correlation and Lin's concordance correlation coefficient was r_c=0.8, indicating good agreement or reproducibility.

The finding that the absolute blood glucose readings, obtained using the YSI were consistently lower than the reading obtained using the MPQIDG could possibly be due to the fact that the new test strips of the MPQIDG were calibrated to yield plasma results as determined by the YSI [36,37], whereas whole capillary blood was used on the YSI in this study. It is well known that capillary plasma, as measured by YSI, yields blood glucose readings that are 10-15% higher than readings obtained using whole capillaryblood.[84] The packed cell volume must be known in order to calculate the one from the other.[21] However, the GI values should not have been affected by the fact that the YSI yields lower blood glucose readings than the MPQIDG, as the GI is a calculated index value (refer to 3.5.7).

In conclusion

There was a fair to very good and significant (r=0.6–0.98; p≤0.05) correlation between the absolute blood glucose readings, as obtained on the YSI and MPQIDG, after removal of outliers, at all the time points, except for at 0min (all but one product), at 15min (two of the test products), at 90min (Muesli and Apple juice) and at 105min (Apple juice). There was a very good correlarion (r=0.9;p<0.0001) and good greement (r_c=0.8) between all the absolute blood glucose readings at all time points, as obtained on the YSI and MPQIDG (without having outliers removed).

4.1.2 Sub-problem

Was there agreement and what was the correlation between the GI values of Muesli and Apple juice and the AUC values (above the fasting baseline) of Glucose 1, 2 and 3, Muesli and Apple juice in the individual subjects, as determined IL in a group of healthy subjects (Group 1), using YSI, when compared to the GI and AUC values of the same two foods, that were determined IL in the same group of healthy subjects, using the MPQIDG?

4.1.2.1 GI Values

Tables 20-23 summarize the GI values of the two foods (Muesli and Apple juice), as determined IL in Group 1, using the YSI and MPQIDG, before and after removal of outliers,

- **Muesli**

When the GI values of Muesli, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 20), Pearson's correlation coefficient was r=0.6, indicating a fair correlation and Lin's concordance correlation coefficient (which tests reproducibility/agreement) for the GI values was r_c=0.6, indicating a fair reproducibility.

The fair correlation (r=0.6) and fair agreement (r_c=0.6) between the GI values of Muesli could possibly be due to the fact that there was a poor correlation between the absolute blood glucose readings at 0min of Glucose 1, 2 and Muesli, as well as at 15min of Glucose 1 and 2, for the reasons explained above.

Table 20: GI values of Muesli, as determined IL by a group of healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	GI values of Muesli using MPQIDG, IL (Group 1)	GI values of Muesli using YSI, IL (Group 1)
A1	40.0	55.0
A2	76.0	33.0
A3	39.0	27.0
A4	51.0	43.0
A5	14.0	15.0
A6	68.0	63.0
A7	43.0	46.0
A8	48.0	22.0
A9	25.0	23.0
A10	76.0	45.0
A11	71.0	76.0
A12	67.0	54.0
MEAN (GI)	**51.5**	**41.8**
SE	5.9	5.3
Subjects	12	12
95% CI	38.5–64.5	30.1–53.5

Regression analysis was used to identify outliers, by observing studentized residuals.[112] The following four subjects were identified as outliers: subjects A1, A2, A8 and A10.

The mean GI values of Muesli tested by the two different methods were compared, using the analysis of variance (paired t-test). The mean GI values and SE changed from 51.5 ± 5.9 for the MPQIDG and 41.8 ± 5.3 for the YSI (before removal of outliers) to 47.3 ± 7.4 and 43.4 ± 7.4, respectively after removal of outliers. The former values (before removal of the outliers) did not differ significantly from the latter values (after removal of outliers)($p=0.69386$ for the MPQIDG and $p=0.84372$ for the YSI) (Tables 20 and 21).

When the mean GI values of Muesli, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared after removal of outliers (Table 21 and Figure 4), Pearson's correlation coefficient was $r=0.9$ ($p=0.0003$), indicating a very good and significant correlation and Lin's concordance correlation coefficient was $r_c=0.9$, which indicates a high reproducibility/agreement (refer to Table 24).

Table 21: GI values of Muesli, as determined IL by a group of healthy subjects (Group 1) using the MPQIDG and YSI , after removal of outliers

Subject ID	GI values of Muesli using MPQIDG, IL (Group 1)	GI values of Muesli using YSI, IL (Group 1)
A3	39.0	27.0
A4	51.0	43.0
A5	14.0	15.0
A6	68.0	63.0
A7	43.0	46.0
A9	25.0	23.0
A11	71.0	76.0
A12	67.0	54.0
MEAN (GI)	**47.3**	**43.4**
SE	7.4	7.4
Subjects	8	8
95% CI	30.9-63.6	27.1-59.6
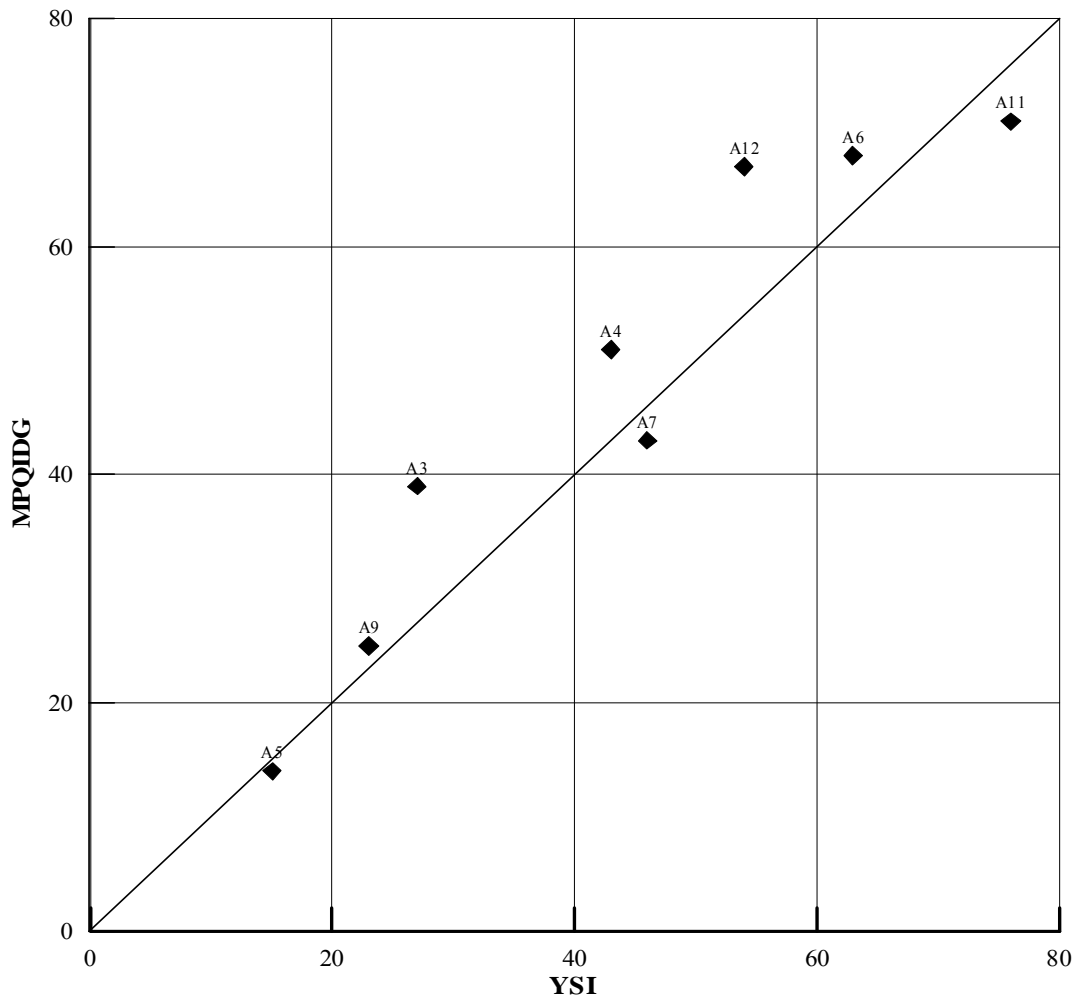
Figure 4: GI values of Muesli as determined by the MPQIDG vs. YSI, after removal of outliers

- **Apple juice**

When the GI values of the Apple juice, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 22), Pearson's correlation coefficient was $r=0.5$, indicating a poor correlation and Lin's concordance correlation coefficient (which tests reproducibility/agreement), for the above GI values was $r_c=0.5$, also indicating a poor reproducibility/agreement.

The poor correlation ($r = 0.5$) and poor agreement ($r_c = 0.5$) between the GI values of the Apple juice, as determined IL by Group 1, using the YSI and MPQIDG could possibly be due to the fact that there was a poor correlation between the blood glucose readings at 0min of Glucose 1 and 2 and Apple juice, as well as at 90min and 105min for Apple juice, for the reasons explained above.

Table 22: Comparison of the GI values of Apple juice, as determined IL by a group of healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	GI values of Apple juice using MPQIDG, IL (Group 1)	GI values of Apple juice using YSI, IL (Group 1)
A1	29	32
A2	66	76
A3	47	41
A4	42	57
A5	27	30
A6	33	31
A7	41	57
A8	62	37
A9	28	43
A10	41	42
A11	28	45
A12	58	41
MEAN (GI)	**41.8**	**44.3**
SE	4	3.8
Subjects	12	12
95% CI	33-50.7	35.9-52.8

Regression analysis was used to identify outliers, by observing studentized residuals.[112] The following two subjects were identified as outliers: subjects A8 and A12. As far as outliers were concerned for the GI values of the two foods (Muesli and Apple juice), subject A8 was the only subject that was identified as an outlier with the GI determinations of both Muesli and Apple juice. All the other subjects were identified as outliers only once, i.e. subjects A1, A2, A10 and A12.

The mean GI values of Apple juice tested by the two different methods were compared, using the analysis of variance (two-tailed t-test). The mean GI values and SE changed from 41.8 ± 4 for the MPQIDG and 44.3 ± 3.8 for the YSI (before removal of outliers) to 38.2 ± 3.8 and 45.4 ± 3.2, respectively after removal of outliers The former values (before removal of outliers) did not differ significantly from the latter values (after removal of outliers)(p=0.55524 for the MPQIDG and p=0.8435 for the YSI).

When the GI values of the Apple juice, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared after removal of outliers (Table 23 and Figure 5), Pearson's correlation coefficient was r=0.8 (p=0.004), indicating a good and significant correlation and Lin's concordance correlation coefficient (which tests reproducibility/agreement) was r_c=0.7, which reflects an acceptable reproducibility (refer to Table 24).

Table 23: Comparison of the GI values of Apple juice, as determined IL by a group of healthy subjects (Group 1) using the MPQIDG and YSI, after removal of outliers

Subject ID	GI values of Apple juice using MPQIDG, IL (Group 1)	GI values of Apple juice using YSI, IL (Group 1)
A1	29	32
A2	66	76
A3	47	41
A4	42	57
A5	27	30
A6	33	31
A7	41	57
A9	28	43
A10	41	42
A11	28	45
MEAN (GI)	**38.2**	**45.4**
SE	3.8	3.2
Subjects	10	10
95% CI	30.3-44.5	35.2-48.2
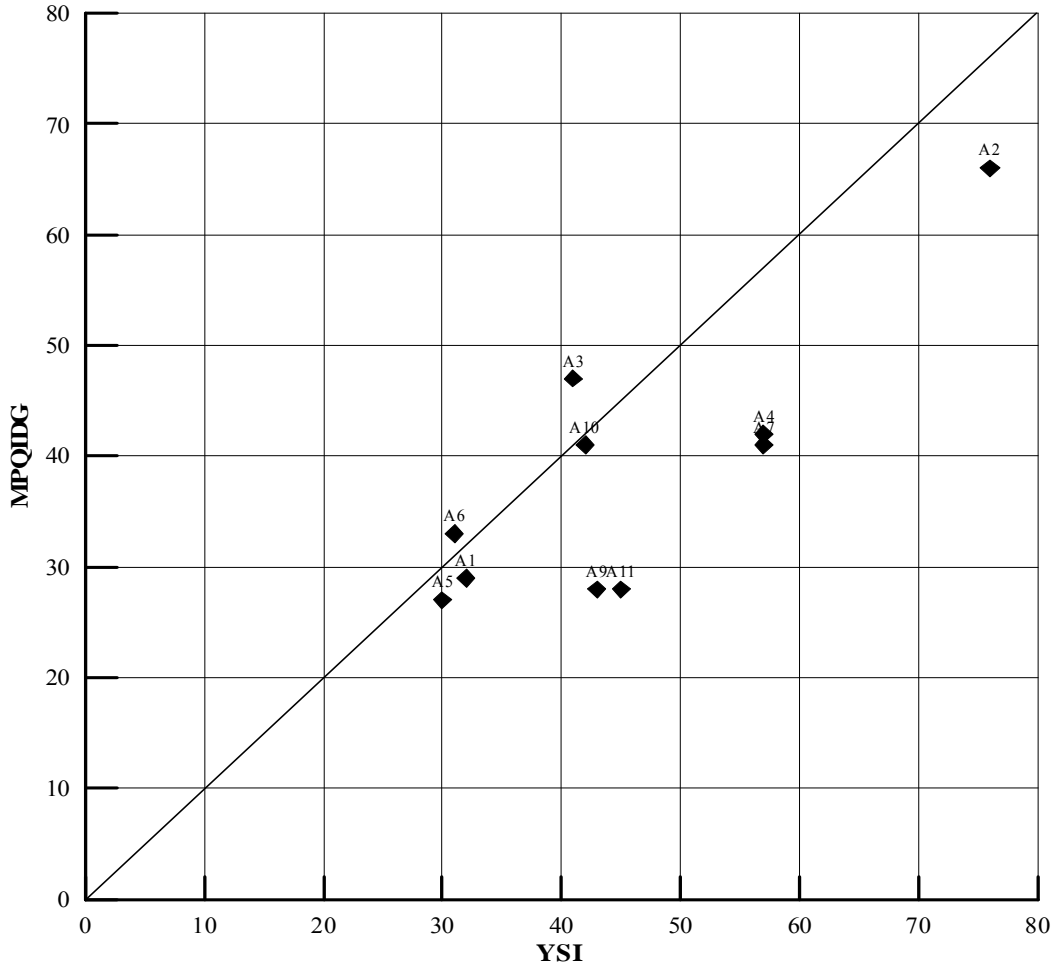
Figure 5: GI values of Apple juice as determined by the MPQIDG vs. YSI, after removal of outliers

Table 24 is a summary of Pearson's correlation coefficient (r) (p-value), as well as Lin's concordance correlation coefficient (r_c) (which shows reproducibility/agreement) between the two instruments for the GI values of Muesli and Apple juice, after removal of outliers. The correlations seem to be good and significant and the reproducibility high and acceptable for Muesli and Apple juice, respectively.

Table 24: Pearson's correlation coefficient (r), p-values and Lin's concordance correlation coefficient (r_c), for the GI values of Muesli and Apple juice, after removal of outliers

Food	r	p-value	r_c
Muesli	0.9	0.0003	0.9
Apple juice	0.8	0.0043	0.7

Velangi et al (2003) found the mean GI values, as obtained by the YSI, to be greater than the mean GI values, as obtained using the One Touch Ultra (OTU) glucometre. When the mean GI values of eight foods, as determined by YSI, were compared to the mean GI values, as obtained using the OTU, there was a significant heterogeneity between the mean GI values, as obtained using the YSI, whereas the mean GI values of the different foods were very similar, as obtained using the OTU and did not differ significantly (Table 25).[34]

Table 25: Mean GI values ($\pm$ SEM) of the different test meals as determined by YSI and OTU [34]

Products	Glycemic Index (GI)	
	YSI	OTU
White Bread	71*	71
Russett potato	77$\pm$9*	57$\pm$10
Instant potato	88$\pm$8	81$\pm$12
Prince Edward Island potato	73$\pm$5*	69$\pm$6
French Fries	64$\pm$6*	67$\pm$6
Red potato (hot)	89$\pm$7	80$\pm$9
Red potato (cold)	56$\pm$5	61$\pm$5
White potato	72$\pm$8*	69$\pm$7
Anova: effect of food#	p=0.004	p=0.11

* Indicates a significant difference ($p \leq 0.05$)
# Significance of heterogeneity of means
SEM refers to standard error of the mean

However, the results of this study, using the MPQIDG IL in comparison with the YSI (Group 1), were different (refer to Tables 21 and 23).

In conclusion
There was a good and significant correlation (r=0.8; p=0.0043) and acceptable agreement (r_c=0.7) between the GI values of Apple juice, whereas there was a very good, significant correlation (r=0.9; p=0.0003) and very good agreement between the GI values of Muesli, using the YSI and MPQIDG.

4.1.2.2 AUC values

As the **Glycemic Index (GI)** of a food is an index, i.e.:

$$\frac{\text{AUC}_f \text{ (above fasting baseline)}}{\text{Av. AUC}_g \text{ (above fasting baseline)}} \times \frac{100}{1}$$

where:
AUC_f (above fasting baseline) = Area under the curve above fasting baseline of a food
Av. AUC_g (above fasting baseline) = Average Area under the curve above fasting baseline of 3 glucose determinations.

It was decided to also investigate whether there was agreement (as well as what the correlation was) between the AUC (above fasting baseline) of the three glucose determinations, Muesli and Apple juice in the individual subjects, as determined IL in a group of healthy subjects (Group 1), using the YSI, when it was compared to the AUC (above fasting baseline) of the same three glucose determinations, Muesli and Apple juice, that were determined IL in the same group of healthy subjects, using the MPQIDG. This was also done by Velangi et al (2005), who evaluated a glucose metre for determining the glycemic responses of foods.[34]

Tables 26–30 summarize the AUC values for the three glucose determinations and the two food determinations (Muesli and Apple juice), as determined IL in Group 1, using the YSI and MPQIDG, before and after removal of outliers (where applicable).

- **Glucose 1**

When the AUC values of Glucose 1, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 26 and Figure 6), the mean AUC $\pm$SE as measured by MPQIDG (242$\pm$21.7) was higher than that measured by YSI (229$\pm$29.7) but the difference was not significant (p=0.69301). Pearson's correlation coefficient was r=0.7 (p=0.0081), indicating an acceptable and significant correlation and Lin's concordance correlation coefficient was r_c=0.7, indicating an acceptable reproducibility (Table 31).

Table 26: AUC (above fasting baseline) values of Glucose 1, as determined IL in a group of healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	AUC (above fasting baseline) of Glucose 1, using MPQIDG, IL (Group 1)	AUC (above fasting baseline) of Glucose 1, using YSI, IL (Group 1)
A1	345.6	283.9
A2	213.8	171.6
A3	143.3	125.8
A4	255.1	137.1
A5	147.3	167.4
A6	302.4	173.5
A7	260.5	256.9
A8	249.8	281.9
A9	148.5	167.3
A10	210.1	242.9
A11	257.9	235.3
A12	371.3	506.1
Mean	242	229
SE	21.7	29.7
Subejcts	12	12
95% CI	191.2-293.1	158.8-299.5

Regression analysis was used to identify outliers, by observing studentized residuals.[112] No outliers were identified.
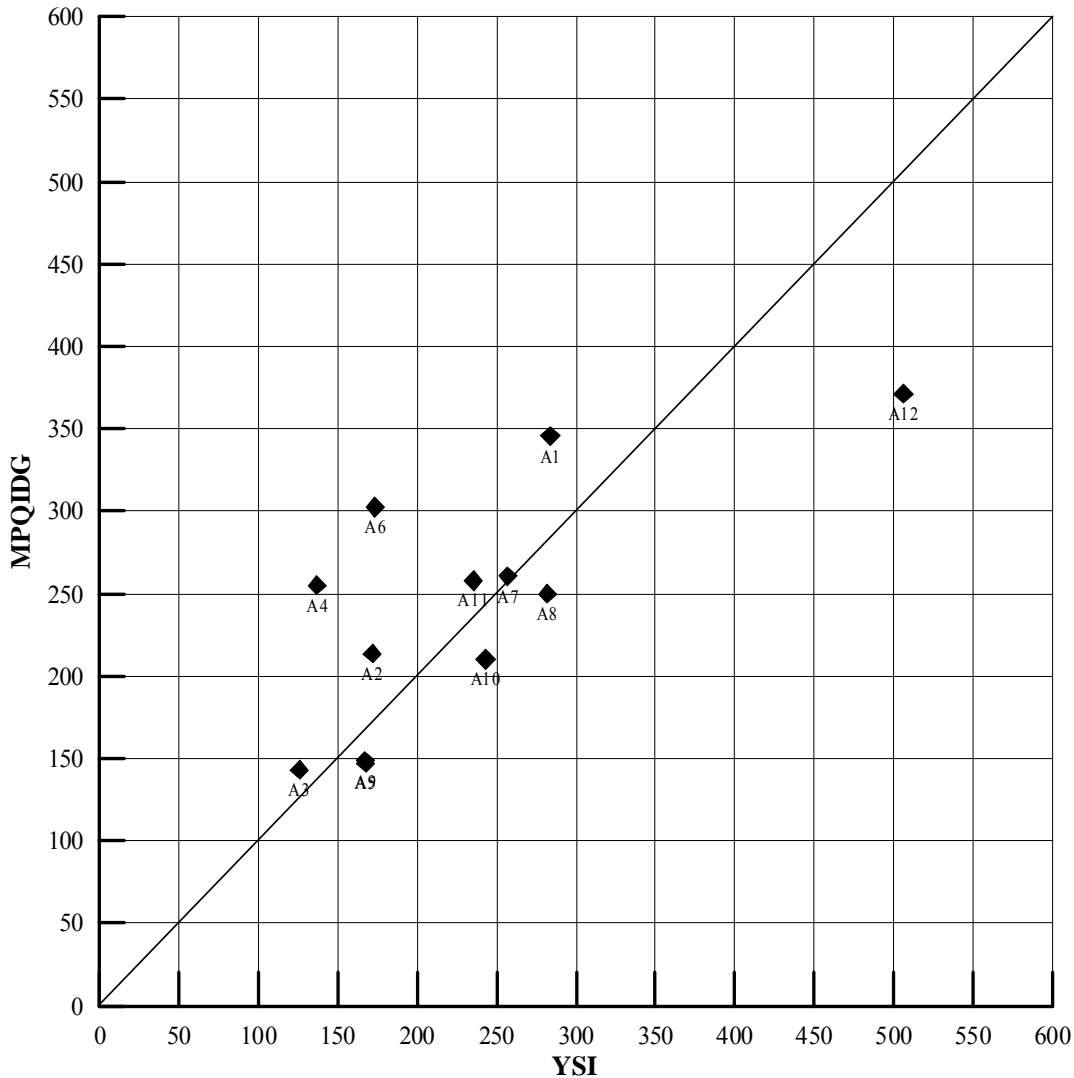
Figure 6: AUC (above fasting baseline) values of Glucose 1 as determined by the MPQIDG vs. YSI

The acceptable, though significant correlation (r=0.7; p=0.0081) and acceptable reproducibility (r_c=0.7) between the AUC values of Glucose 1, using the two instruments could probably be due to the fact that there was poor correlation at 0min and 15min of the absolute blood glucose readings of Glucose 1 (r=0.4; p=0.2834 and r=0.1; p=0.8045, respectively, after removal of outliers)(Table 18). The tendency for the mean AUC_{MPQIDG} (Glucose 1) to be higher than the mean AUC_{YSI} could possibly be due to the fact that plasma values are usually 10-15% higher than whole blood glucose values [84] and this was not taken into consideration when the AUC readings were compared.

- **Glucose 2**

When the AUC values of Glucose 2, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 27), the mean AUC $\pm$ SE as measured by MPQIDG, (229$\pm$29.7) was higher than that measured by YSI (191$\pm$21.4), but the difference was not significant (p=0.32818). Pearson's correlation coefficient was r=0.9 (p<0.0001), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient was r_c=0.8, indicating a good reproducibility/agreement (Table 31).

Table 27: AUC (above fasting baseline) values of Glucose 2 as determined IL in healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	AUC (above fasting baseline) of Glucose 2 using MPQIDG, IL (Group 1)	AUC (above fasting baseline) of Glucose 2 using YSI, IL (Group 1)
A1	224.3	156.7
A2	201.9	138.7
A3	122.4	126.8
A4	334.9	276.4
A5	255.5	188.8
A6	324.0	239.9
A7	401.9	332.0
A8	89.7	139.4
A9	93.1	86.1
A10	141.0	152.5
A11	243.3	173.6
A12	314.3	277.1
Mean	229	191
SE	29.7	21.4
Subejcts	12	12
95% CI	158.7-299.0	140.0-241.3

Regression analysis was used to identify outliers, by observing studentized residuals.[112] The AUC values of subject 8 were identified as an outlier. When the AUC values of Glucose 2 were compared after removal of the outlier (Figure 7), the mean AUC $\pm$ SE as measured by MPQIDG (242$\pm$28) was higher than that measured by YSI (195$\pm$21.9), but the difference was not significant (p=0.20838). Pearson's correlation coefficient for the AUC values of Glucose 2 was r=0.9 (p<0.0001), indicating very good and highly significant correlation and Lin's concordance correlation coefficient (which tests reproducibility or agreement) was r_c=0.8, which reflects a good reproducibility or agreement. The removal of the outlier had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient.

Figure 7: AUC (above fasting baseline) values of Glucose 2 as determined by the MPQIDG vs. YSI, after removal of the outlier

The very good and highly significant correlation (r=0.9; p<0.0001) between the AUC values of Glucose 2 and the good agreement (r_c=0.8), using the two instruments, before removal of outliers, could probably be due to the fact that the subjects were more efficient at obtaining the larger samples of blood for the YSI by the time they tested Glucose 2, in comparison to Glucose 1. The correlation of the absolute blood glucose readings at 15min for Glucose 2 was better than for Glucose 1, although still poor (r=0.5; p=0.1061 and r=0.1; p=0.8045, respectively)(Table 18), although the correlation of the absolute blood glucose readings at 0min for Glucose 1 was better than that of Glucose 2 (r=0.4; p=0.2834 and r=0.03; p=0.923, respectively)(Table 18). The correlation between the AUC values for Glucose 2 was very good and highly significant, in spite of the fact that the correlation of the absolute blood glucose readings at 0min and 15min of Glucose 2 was poor (r=0.03; p=0.923; r=0.5; p=0.1061). Removing the outlier from the data set had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient. The tendency for the mean AUC_{MPQIDG} (Glucose 2) to be higher than the mean AUC_{YSI} could possibly be due to the fact that plasma values are usually 10-15% higher than whole blood glucose values [84] and this was not taken into consideration when the AUC readings were compared.

- **Glucose 3**

When the AUC values of Glucose 3, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 28), the mean AUC $\pm$ SE as measured by MPQIDG (224$\pm$28.6) was higher than that measured by YSI (179$\pm$32.6) but the difference was not significant (p=0.32818). Pearson's correlation coefficient was r=0.9 (p<0.0001), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient was r_c=0.9, indicating a high reproducibility/agreement (Table 31).

Table 28: AUC (above fasting baseline) values of Glucose 3 as determined IL in a group of healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	AUC (above fasting baseline) of Glucose 3 using MPQIDG, IL (Group 1)	AUC (above fasting baseline) of Glucose 3 using YSI, IL (Group 1)
A1	262.2	200.9
A2	152.1	158.4
A3	162.8	113.7
A4	230.4	111.2
A5	204.1	121.5
A6	225.9	182.3
A7	290.8	198.6
A8	249.7	210.0
A9	55.3	41.2
A10	177.9	151.3
A11	206.7	160.4
A12	471.0	504.0
Mean	224	179
SE	28.6	32.6
Subejcts	12	12
95% CI	156.5-291.7	102.4-256.5

Regression analysis was used to identify outliers, by observing studentized residuals.[112] The AUC values of subject A4 were identified as an outlier.

When the AUC values of Glucose 3, were compared after removal of the outlier (Figure 8), the mean AUC $\pm$ SE as measured by MPQIDG (224$\pm$30.0) was higher than that measured by YSI (186$\pm$33.5) but the difference was not significant (p=0.43311). Pearson's correlation coefficient was r=0.9 (p<0.0001), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient (which tests reproducibility/agreement) was r_c=0.9, indicating a high reproducibility or agreement. The removal of the outlier had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient.

Figure 8: AUC (above fasting baseline) values of Glucose 3 as determined by the MPQIDG vs. YSI, after removal of the outlier

The very good and highly significant correlation ($r = 0.9$; p < 0.0001) between the AUC values of Glucose 3 and the very good agreement (r_c=0.9), using the two instruments, before removal of the outlier, could probably be due to the fact that the subjects were even more efficient at obtaining the larger samples of blood for the YSI by the time they tested Glucose 3 (in comparison to Glucose 1 and 2). The correlation of the absolute blood glucose readings at 15min for Glucose 3 was better than for Glucose 1 and 2 and very good and highly significant (r=0.9; p<0.0001). The correlation of the absolute blood glucose readings at 0min of Glucose 3 was fair and almost significant (r=0.6; p=0.0508) and better than that of Glucose 1 and 2. The correlation between the AUC values of Glucose 3 was very good and highly significant, in spite of the fact that the correlation of the absolute blood glucose readings at 0min for Glucose 3 was only fair (r=0.6; p=0.0508)(Table 18). Removal of the outlier from the data set had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient. The tendency for the mean AUC_{MPQIDG} (Glucose 3) to be higher than the mean AUC_{YSI} could possibly be due

to the fact that plasma values are usually 10-15% higher than whole blood glucose values [84] and this was not taken into consideration when the AUC readings were compared.

- **Muesli**

When the AUC values of Muesli, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 29), the mean AUC $\pm$ SE as measured by MPQIDG (124$\pm$19.3) was higher than that measured by YSI (92$\pm$17.3) but the difference was not significant (p=0.24290). Pearson's correlation coefficient was r=0.9 (p=0.0002), indicating a very good and significant correlation and Lin's concordance correlation coefficient was r_c=0.9, indicating a high reproducibility or agreement (Table 31).

Table 29: AUC (above fasting baseline) values of Muesli as determined IL in a group of healthy subjects (Group 1) using the MPQIDG and YSI

Subject ID	AUC (above fasting baseline) of Muesli using MPQIDG, IL (Group 1)	AUC (above fasting baseline) of Muesli using YSI, IL (Group 1)
A1	110.2	118.6
A2	144	51.1
A3	55.6	44.0
A4	139.5	75.0
A5	28.5	36.7
A6	192.8	125.8
A7	135.3	121.8
A8	94.3	45.4
A9	24.9	22.6
A10	134.4	82.4
A11	166.5	144.4
A12	256.4	233.5
Mean	124	92
SE	19.3	17.3
Subejcts	12	12
95% CI	77.7-169.4	50.7-132.9

Regression analysis was used to identify outliers, by observing studentized residuals.[112] The AUC values of subject A2 were identified as an outlier.

When the AUC values of Muesli were compared after removal of the outlier (Figure 9), the mean AUC $\pm$ SE as measured by MPQIDG (122$\pm$20.2) was higher than that measured by YSI (95$\pm$17.9), but the difference was not significant (p=0.32926). Pearson's correlation coefficient was r=0.9 (p<0.0001), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient was r_c=0.9, which reflects a high reproducibility/agreement. The removal of the outlier had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient.
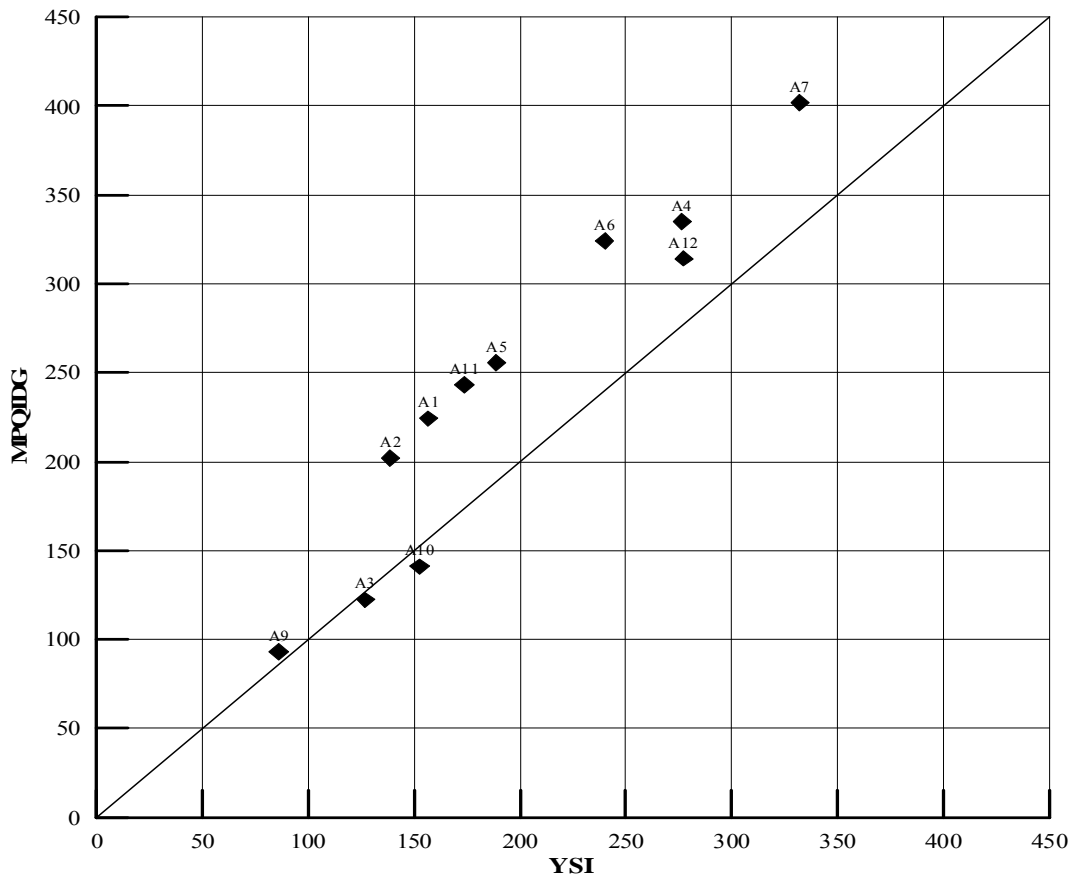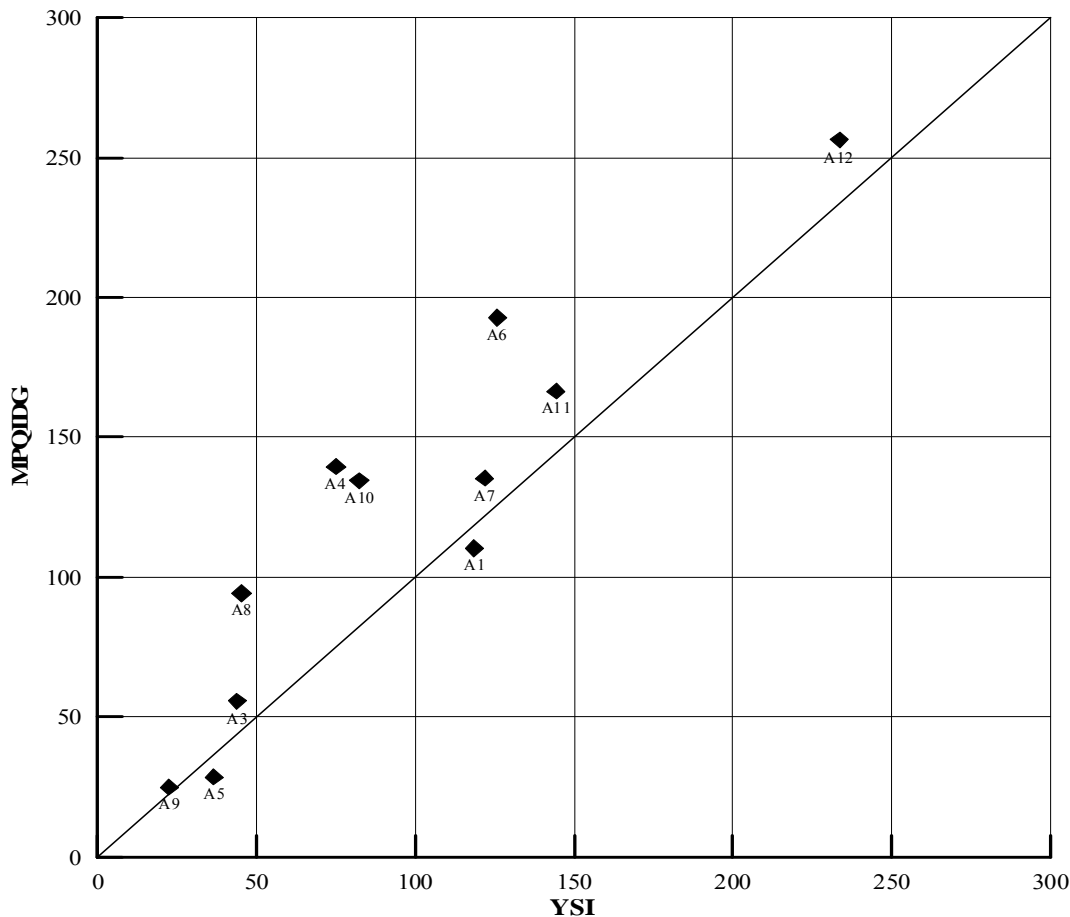
Figure 9: AUC (above fasting baseline) values of Muesli as determined by the MPQIDG vs. YSI, after removal of the outlier

The very good and significant correlation (r=0.9; p=0.0002) between the AUC values of Muesli and the very good agreement (r_c=0.9), using the two instruments could probably be due to the fact that the subjects were more efficient at obtaining the larger samples of blood for the YSI by the time they tested Muesli, in comparison to Glucose 1, although not as efficient as for Glucose 3. The correlation between the AUC of Muesli was very good, in spite of the fact that the correlation of the absolute blood glucose readings at 0min for Muesli was poor (r=0.3; p=0.4519), probably due to the reasons mentioned before, and at 90min for Muesli was only acceptable (r=0.7; p=0.1078)(Table 18). The reason for the poor correlation at 90min for Muesli could probably be attributed to faulty operation by the technician on duty on that particular day. The correlation of the absolute blood glucose readings at 15min for Muesli was also much better than for Glucose 1 and 2 and very good and significant (r=0.9; p=0.0008). The tendency for the mean AUC_{MPQIDG} (Glucose 3) to be higher than the mean AUC_{YSI} could possibly be due to the fact that plasma values are usually 10-15% higher than whole blood glucose values [84] and this was not taken into consideration when the AUC readings were compared.

- **Apple juice**

When the AUC values of Apple juice, as determined IL in the group of healthy subjects (Group 1), using the YSI and MPQIDG, were compared (Table 30), the mean AUC $\pm$ SE as measured by MPQIDG (98 ± 14.7) was higher than that measured by YSI (88 ± 11.8), but the difference was not significant ($p=0.62204$). Pearson's correlation coefficient was $r=0.9$ ($p<0.0001$), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient was $r_c=0.9$, indicating a high reproducibility or agreement (Table 31).

Table 30: AUC (above fasting baseline) values of Apple juice as determined in a group of healthy subjects (Group 1), using the MPQIDG and YSI

Subject ID	AUC (above fasting baseline) of Apple juice using MPQIDG, IL (Group 1)	AUC (above fasting baseline) (Group 1)
A1	80.1	67.4
A2	125.4	119.2
A3	67.5	50.2
A4	114.3	100.1
A5	53.8	48.5
A6	94.2	61.4
A7	131.5	149.4
A8	121.9	78.3
A9	27.4	42.3
A10	72.4	76.5
A11	66.5	85.7
A12	225.0	174.5
Mean	98	88
SE	14.7	11.8
Subejcts	12	12
95% CI	63.5-133.2	59.6-116

Outliers were then identified using regression analysis, by observing studentized residuals.[112] The AUC values of subject A7 were identified as an outlier.

When the AUC values of Apple juice were compared after removal of the outlier (Figure 10), the mean AUC $\pm$ SE as measured by MPQIDG (95 ± 15.0) was higher than that measured by YSI (88 ± 11.0), but the difference was not significant ($p=0.49199$). Pearson's correlation coefficient was $r=0.9$ ($p<0.0001$), indicating a very good and highly significant correlation and Lin's concordance correlation coefficient was $r_c=0.9$, which reflects a high reproducibility/agreement. The removal of the outlier had no effect on either the Pearson correlation coefficient or Lin's concordance correlation coefficient.
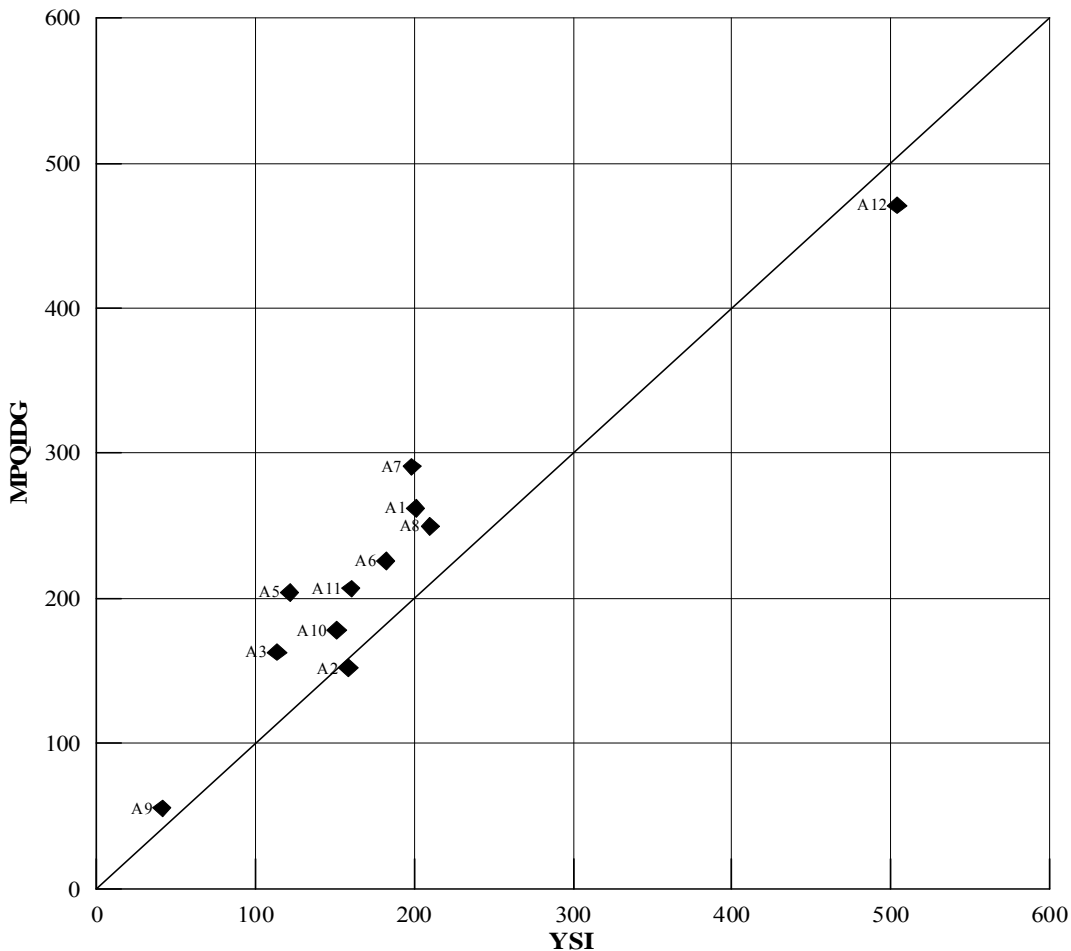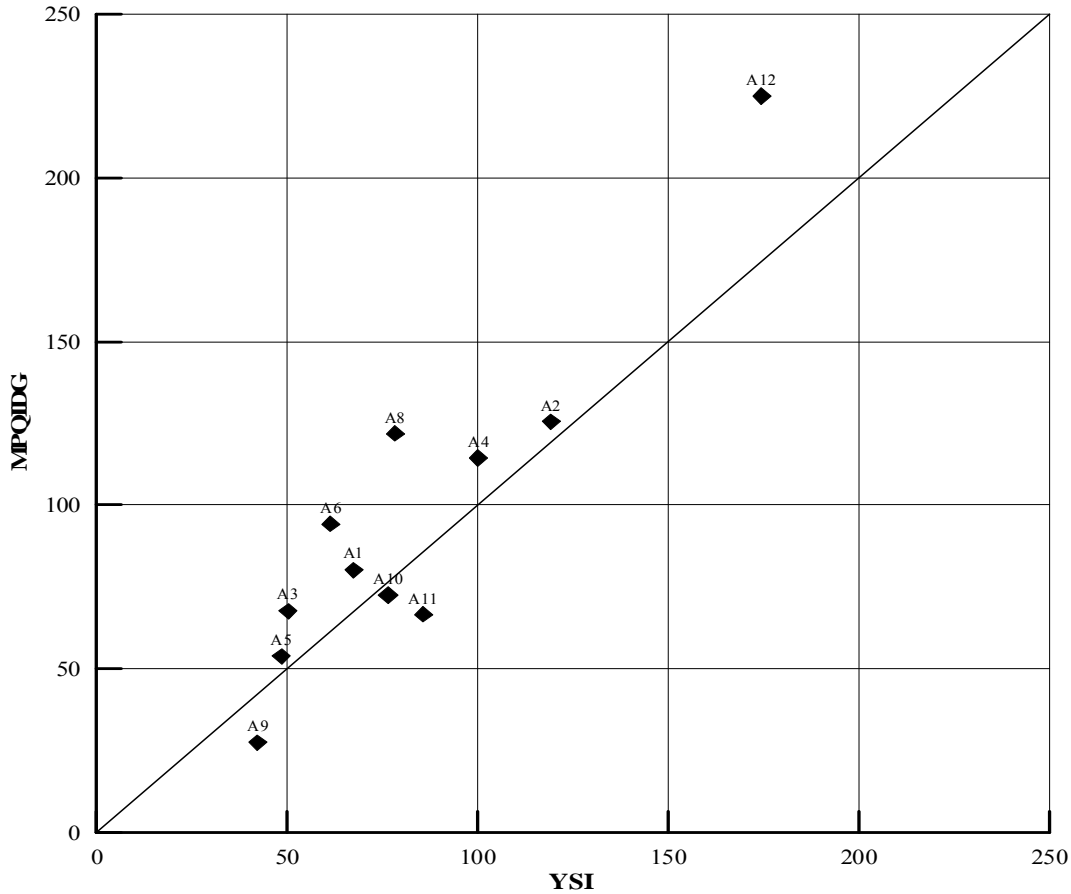
Figure 10: AUC (above fasting baseline) of Apple juice as determined by the MPQIDG vs. YSI, after removal of the outlier

The very good and highly significant correlation (r=0.9; p<0.0001) between the AUC values of Apple juice and the very good agreement (r_c=0.9), using the two instruments (Figure 9), could probably be due to the fact that the subjects were even more efficient at obtaining the larger samples of blood for the YSI by the time they tested Apple juice, in comparison to Glucose 1 and 2. The correlation between the AUC values of Apple juice was very good and highly significant (r=0.9; p<0.0001) in spite of the fact that the correlation of the absolute blood glucose readings at 0min, 90min and 105min of Apple juice was poor (r=0.02; p=0.9515; r=0.4; p=0.2454 and r=0.3; p=0.7072, respectively)(Table 18). The poor correlation at 90min and 120min for Apple juice could probably be due to the reasons mentioned before.

Table 31 is a summary of Pearson's correlation coefficient (r)(p-value), as well as Lin's concordance correlation coefficient (r_c), which shows reproducibility/agreement, between the two instruments for the AUC values of Glucose 1, 2 and 3, as well as the Muesli and Apple juice, after removal of outliers. The correlations were acceptable and significant for Glucose 1 and very good and highly significant for Glucose 2 and 3, Muesli and Apple juice. The agreement was acceptable for Glucose 1, good for Glucose 2 and very good for Glucose 3, Muesli and Apple juice, respectively.

Table 31: Pearson's correlation coefficient (r), p-values and Lin's concordance correlation coefficient (r_c), between the MPQIDG and YSI for the AUC values of the Muesli and Apple juice, after removal of outliers

Food	r	p-value	r_c
Glucose 1	0.7	0.0081	0.7
Glucose 2	0.9	< 0.0001	0.8
Glucose 3	0.9	< 0.0001	0.9
Muesli	0.9	< 0.0001	0.9
Apple juice	0.9	< 0.0001	0.9

The mean within-subject CV of the AUC values of repeated tests of the reference food for the 12 subjects, that were observed by Velangi et al (2003), was greater for AUC_{OTU} (32.1$\pm$4.4%) than AUC_{YSI} (28.1$\pm$3.2%), implying greater within-subject variability (CV) of blood glucose responses for the OTU, although this was not statistically significant (p=0.3).[34] Contradictorily, the mean CV of the repeated tests of the reference food for the 12 healthy subjects of Group 1 (IL) in Part 1 of this study was greater for AUC_{YSI} (28.4$\pm$16.3%) than AUC_{MPQIDG} (24.0$\pm$11.6%)(not shown in a table), indicating less variability (CV) of blood glucose responses for the MPQIDG, although this was not statistically significant (p=0.4319). However, both values compared well with the CV of repeated tests of the reference food mentioned in the literature, i.e. 25$\pm$12 [56] and there was no statistically significant difference between the CVs of the AUC_{YSI} (p=0.5546) and AUC_{MPQIDG} (p=0.8433) respectively, when compared to the CV of repeated tests of the reference food (25$\pm$12) mentioned in the literature [56], using the analysis of variance (two-tailed t-test).

The mean within-subject CV of the AUC values of repeated tests of the reference food for the mixed group of 11 subjects of Group 3 (EL; Muesli) in Part 1 of this study was also greater for AUC_{YSI} (28.4$\pm$16.3%) than AUC_{MPQIDG} (25.6$\pm$18.9)(not shown in a table), indicating less within-subjects variability (CV) of blood glucose responses for EL testing (i.e. MPQIDG in a mixed group of subjects, EL), although this was not statistically significant (p=0.693). The mean within-subject CV of the AUC values of repeated tests of the reference food for the mixed group of eight subjects of Group 4 (EL; Apple juice) in Part 1 of this study was also greater for AUC_{YSI} (28.4$\pm$16.3%) than AUC_{MPQIDG} (25.9$\pm$17.4)(not shown in a table, indicating less variability (CV) of blood glucose responses for EL testing (i.e. MPQIDG in a mixed group of subjects, EL), although this was also not statistically significant (p=0.7674). The CV of repeated tests of the reference foods for the EL tests of both Muesli (25.6$\pm$18.9) and Apple juice (25.9$\pm$17.4) also compared well with the CV of repeated tests of the reference food mentioned in the literature (25$\pm$12) [56] and there was no statistically significant difference between the CV of the AUC for Muesli (EL) (p=0.9213) and AUC Apple juice (EL) (p=0.9215) respectively, when compared to the CV of repeated tests of the reference food (25$\pm$12) mentioned in the literature [56], using the analysis of variance (two-tailed t-test).

In conclusion

There was an acceptable and significant correlation (p$\leq$0.05) and an acceptable agreement between the AUC values of Glucose 1, using the two instruments. However, there was good agreement between the AUC values of Glucose 2 and a very good and highly significant correlation (p<0.0001) between the

AUC values for Glucose 2 and 3, Muesli and Apple juice and very good agreement between the AUC values of Glucose 3, M and A, respectively.

When the mean AUC values were considered, the mean AUC readings as measured by MPQIDG were always higher than that measured by YSI, though not statistically significant for any of the food products ($p > 0.2$). The EL testing (MPQIDG in a mixed group of subjects, EL) also always consistently showed less within-subject variability (CV) of blood glucose responses for both Muesli and Apple juice, when compared to IL testing (YSI in a group of healthy subjects), although not statistically significant. As far as outliers are concerned for the AUC values of the three glucose determinations, Muesli and Apple juice, not one subject was identified as an outlier on more than one occasion.

The poor correlation of absolute blood glucose readings at 0min (fasting) for all the foods tested (except for Glucose 3, after removal of outliers), 15min for Glucose 1 and 2, 90min for Muesli and Apple juice, and 105min for Apple juice seemed not to have influenced the correlation and agreement between the AUC values for Glucose 3, Muesli and Apple juice, or the GI value of Muesli. However, it seemed to have had an effect on the AUC values for Glucose 1 and the GI value of Apple juice and to a lesser extent the AUC value of Glucose 2. This is reflected in the following (refer to Tables 24 and 31):

The **correlation (r)** was acceptable and significant for the AUC values of Glucose 1 ($r=0.7$; $p=0.0081$), good and significant for the GI value of Apple juice ($r=0.8$; $p=0.0043$) and very good and highly significant for the AUC values of Glucose 2 ($r=0.9$; $p<0.0001$), Glucose 3 ($r=0.9$; $p<0.0001$), Muesli ($r=0.9$; $p<0.0001$) and Apple juice ($r=0.9$; $p<0.0001$) and the GI value of Muesli ($r=0.9$; $p=0.0003$) respectively, after removal of outliers.

The **agreement (r_c)** was acceptable for the AUC value of Glucose 1 ($r_c=0.7$) and the GI value of Apple juice ($r_c=0.7$), good for the AUC value of Glucose 2 ($r_c=0.8$) and very good for the AUC-value of Glucose 3 ($r_c=0.9$), Muesli ($r_c=0.9$) and Apple juice ($r_c=0.9$) and the GI-value of Muesli ($r_c=0.9$), respectively, after removal of outliers. This could probably be due to the fact that, out of the 45 time intervals, more than 50% (27) showed a very good, significant correlation ($r=0.9$; $p\leq0.02$) between the absolute blood glucose readings obtained on the two instruments and of these, 13 were highly significant ($p<0.0001$). 15.6% (seven) of the 45 time intervals showed a good, significant correlation ($r=0.8$; $p\leq0.04$), 4.4% (two) showed an acceptable correlation ($r=0.7$; $p\leq0.1$), 2.2% (one) showed a fair, significant correlation ($r=0.6$; $p\leq0.05$) and 17.7 (nine) of the 45 time intervals showed a poor correlation ($r=0.5$ or less; $p>0.1061$ or more. The correlation (r) between all of the determinations at time intervals 30, 45, 60, 75 and 120min was good to very good ($r=0.8$–0.9) and significant ($p\leq0.05$)(Table 18).

4.1.3 Sub-problem

How did the GI values of the two foods that were determined in the mentioned four ways, compare when using the three methods of calculating the AUC (IAUC, AUC_{min} and AUC_0).

4.1.3.1 AUCminimum (AUC_{min})

Tables 32 and 33 show the mean GI values of Muesli and Apple juice, which were determined in four ways, using the AUCmin method of calculating GI values.

Table 32: GI values of Muesli, which were determined in four ways, using the AUC$_{min}$ method of calculating AUC

Subject ID	MPQIDG; EL (Group 3)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
A1		46	34	52
A2		71	55	40
A3	87		75	57
A4		38	32	35
A5		38	13	24
A6		39	55	42
A7		61	35	35
A8		61	67	39
A9	54		40	40
A10		47	47	30
A11		62	38	47
A12	71	51	91	62
A13	71			
A14 (Type 2)	49			
A15	36			
A16 (Type 1)	101			
A17 (Type 1)	104			
A18 (Type 2)	64			
A19 (Type 1)	77			
A20 (Type 2)	52			
MEAN (GI)	**70**	**51**	**49**	**42**
SE	6.3	4	6	3.2
SUBJECTS	11	9	12	12
95% CI	55.9-84.1	41.8-60.2	35.7-62.3	35.0-49.0

Type 1: Type 1 diabetic subject. Type 2: Type 2 diabetic subject; the rest of the subjects were healthy individuals.

Table 33: GI values of Apple juice, which were determined in four ways, using the AUC_{min} method of calculating AUC

Subject ID	MPQIDG; EL (Group 4)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
1			40	58
2		74	71	66
3		62	69	49
4		86	42	48
5		34	22	47
6		69	47	63
7		32	54	61
8		35	60	53
9	28		84	104
10		62	89	68
11		52	39	45
12		35	79	56
13	28			
14 (Type 2)	29			
15				
16 (Type 1)				
17 (Type 1)	47			
18 (Type 2)	56			
19 (Type 1)	66			
20 (Type 2)	40			
MEAN (GI)	**47**	**54**	**58**	**60**
SE	7	6	6	4.6
SUBJECTS	8	10	12	12
95% CI	30.3-63.7	40.4-67.6	44.7-71.3	49.8-70.2

Type 1: Type 1 diabetic subject.
Type 2: Type 2 diabetic subject
The rest of the subjects were healthy individuals

4.1.3.2 Total AUC (AUC_0)

Tables 34 and 35 show the mean the GI values of Muesli and Apple juice, which were determined in four ways, using the AUC_0 method of calculating GI values, respectively.

Table 34: GI values of Muesli, which were determined in four ways, using the AUC_0 method of calculating AUC

Subject ID	MPQIDG; EL (Group 3)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
A1		86	86	76
A2		96	92	86
A3	88		78	79
A4		84	84	88
A5		80	84	81
A6		86	86	78
A7		92	86	84
A8		106	90	78
A9	74		97	87
A10		85	101	81
A11		92	91	87
A12	98	79	88	80
A13	74			
A14 (Type 2)	100			
A15	86			
A16 (Type 1)	72			
A17 (Type 1)	132			
A18 (Type 2)	90			
A19 (Type 1)	101			
A20 (Type 2)	77			
MEAN (GI)	**90**	**89**	**89**	**82**
SE	5.1	2	1.7	1.2
SUBJECTS	11	9	12	12
95% CI	78.6-101.4	84.4-93.6	85.12-92.8	79.5-84.5

Type 1: Type 1 diabetic subject
Type 2: Type 2 diabetic subject
The rest of the subjects were healthy individuals

Table 35: GI values of Apple juice, which was determined in four ways, using the AUC_0 method of calculating AUC

Subject ID	MPQIDG; EL (Group 4)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
A1	89		84	80
A2		88	98	94
A3		100	85	89
A4		90	77	91
A5		77	85	81
A6		81	80	80
A7		91	89	76
A8		83	76	108
A9	55		96	88
A10		67	80	85
A11		73	84	83
A12		68	78	77
A13	92			
A14 (Type 2)	99			
A15				
A16 (Type 1)				
A17 (Type 1)	56			
A18 (Type 2)	91			
A19 Type 1)	99			
A20 (Type 1)	72			
MEAN (GI)	**82**	**82**	**84**	**86**
SE	6.4	3.5	2	2.6
SUBJECTS	8	10	12	12
95% CI	66.9-97.1	74.1-89.9	79.6-88.4	80.3-91.7

Type 1: Type 1 diabetic subject
Type 2: Type 2 diabetic subject
The rest of the subjects are healthy individuals

The GI values obtained using the AUC_{min} method of calculation were significantly different to that of the IAUC method, for one of the four GI determinations of Muesli (Table 36) and two of the four GI determinations of Apple juice (Table 37).

Though Venter et al (2003) were of the opinion that this is the best method of calculating the AUC, as it takes into consideration hypoglycemia as well as hyperglycemia [23], and has also been found to show the least variation [81], the AUC_{min} method of calculating GI values has its limitations:

- Like the AUC_0, this method of calculation causes an unrealistically high AUC, especially when there is an undershot of the baseline, as the whole area from the minimum reading to the fasting reading is added to the AUC. [21]
- It does not yield similar GI values to the IAUC method if diabetic subjects are used for GI tests, as professed [23] and a low GI food/drink, such as Muesli and Apple juice used in this study, could yield a false higher GI value, due to this method of calculation.
- Vorster et al seem to be the only researchers worldwide that prefer this method of calculation. [23]

The exclusion of this method of calculation as a suitable method to calculate the AUC for the purposes of GI tests is confirmed by Brouns et al (2005), based on the data set of five foods tested in healthy subjects, who formed part of the inter-laboratory study.[39] It was found that GI values based on AUC_{min} correlated significantly with the subjects' glycemic response to the reference food (in this case glucose), which suggests that this is not a good method to calculate GI determinations, as the GI values obtained depend to a large extent on the glucose tolerance status of the subject.[21] The GI values of foods should be independent of the glucose tolerance of the subject.

It was found in this study that very high and probably false GI values were yielded when the AUC_0 method was used, as the GI values of the two foods that were tested in Part 1 of this study are actually low (42–54 for Muesli and 39–44 for Apple juice), whereas this method yields GI values of 82–90 for Muesli and 82–86 for Apple juice (about twice the GI values found by the investigator)(Table 35). The GI values of foods that truly have a high GI value, will then probably be >100 GI, which is unrealistic. The reason for these unrealistically high GI values could probably be due to the fact that the whole area under the curve from 0 to the fasting value is added in the calculation of the AUC, yielding a false high AUC. This method has also been criticized as insensitive for detecting differences between the postprandial glycemic responses of different meals.[61] This study confirmed that, as the GI values of Muesli and Apple juice were very similar (82–90 for Muesli and 82–86 for Apple juice), when calculated using the AUC_0 method.

The GI values obtained using this method of calculation were significantly different to that of the IAUC method, for all four GI determinations of Muesli (Table 36), as well as Apple juice (refer to Table 37). Different methods were used in the past to calculate the AUC. However, the Expert Consultation Group on carbohydrates in Human Nutrition decided to standardize on the AUC (above the fasting baseline), as most published GI data was calculated using this method, which ignores the area beneath the fasting concentration.[3]

Table 36 is a summary of the analysis of variance (two-tailed t-test)(p-values), which was done to compare the mean GI values of Muesli, which were determined in the mentioned four ways, when AUC_{min} and AUC_0 were compared to IAUC. As can be seen from this table, there was a significant difference between the mean GI values of Muesli, when calculated using the AUC_{min} method as opposed to the IAUC method, in the case of the original EL-test (Group 3). There was, however a highly significant difference between the mean GI values of Muesli, when calculated using the AUC_0 method as opposed to the IAUC, in the case of all four tests that were performed.

Table 36: The analysis of variance (two-tailed t-test) for Muesli, when comparing AUC_{min} and AUC_0 to IAUC (p-values)

	MPQIDG; EL (Group 3)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
AUC_{min}	**0.0474**	0.8438	0.8430	1.0000
AUC_0	**<0.0000**	**<0.0000**	**<0.0000**	**<0.0000**

The values in bold print show a significant difference

Table 37 is a summary of the analysis of variance (two-tailed t-test)(p-values), which was done to compare the mean GI values of Apple juice, which were determined in the mentioned four ways, when AUC_{min} and AUC_0 were compared to IAUC. As can be seen from this table, there was a significant difference between the mean GI values of Apple juice, when calculated using the AUC_{min} method as opposed to the IAUC method, in the case of the IL tests, using the MPQIDG and YSI. There was, however a highly significant difference between the mean GI values of Apple juice, when calculated using the AUC_0 method as opposed to the IAUC method, in the case of all four tests that were performed.

Table 37: The analysis of variance (two-tailed t-test) for Apple juice, when comparing AUC_{min} and AUC_0 to IAUC (p-values)

	MPQIDG; EL (Group 4)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
AUC_{min}	0.3815	0.1253	**0.0377**	**0.0125**
AUC_0	**< 0.0000**	**< 0.0000**	**< 0.0000**	**< 0.0000**

The values in bold print show a significant difference

Wolever et al (1985) reported a large difference in the GI values of the same food, determined by different investigators, whereas if the same method was used to calculate the GI, the GI values are very similar, suggesting that the different GI values (same food) could be attributed to different methods of data analysis rather than differences in responses to foods.[56] This is confirmed by Venter et al (2003), who were of the opinion that the main source of error in determining the GI could be the method of calculating the AUC.[23]

In conclusion

The AUC_{min} and AUC_0 methods of calculating the AUC seem inappropriate for calculating the AUC for GI tests. Using the IAUC to calculate the AUC for GI tests has been confirmed by Brouns et al (2005).[21] The results of this study are supportive of this decision.

4.1.4 Research problem

What was the degree of variance and was there a significant difference between the mean GI values of the same two foods, which were determined in four ways, i.e. Group 1 (healthy subjects) using YSI, IL; Group 1 (healthy subjects) using MPQIDG, IL; the same group of healthy subjects using MPQIDG, EL (Group 2) and two mixed groups of subjects (i.e. healthy, type 1 and type 2 diabetic subjects) who had already used MPQIDG, EL to determine the GI values of Muesli (Group 3) and Apple juice (Group 4) ?

As mentioned before, the overall aim of the study (Part 1 and 2) was to compare the mean GI values of foods (as determined EL, using a mixed group of subjects and the MPQIDG) to the mean GI values of the same foods (as determined IL, using a healthy group of subjects and laboratory equipment), in order to determine whether:

- EL testing is an acceptable alternative for IL testing, and
- there is a significant difference between the mean GI values obtained, using the two methods.

In par 4.1.4.1-4.1.4.2 the over arching research problem (Part 1) will be answered; based on the findings presented in par 4.1.1-4.1.3.

4.1.4.1 Muesli

Table 38 summarizes the GI values of Muesli, which were determined in the mentioned four ways.

Table 38: GI values of Muesli, determined in four ways

Subject ID	MPQIDG; EL (Group 3)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
A1		58	40	55
A2		62	76	33
A3	51		39	27
A4		52	51	43
A5		39	14	15
A6		58	68	63
A7		69	43	46
A8		38	48	22
A9	52		25	23
A10		32	76	45
A11		59	71	76
A12	78	51	67	54
A13	37			
A14 (Type 2)	33			
A15	61			
A16 (Type 1)	48			
A17 (Type 1)	61			
A18 (Type 2)	49			
A19 (Type 1)	65			
A20 (Type 2)	65			
MEAN (GI)	**54.4**	**51.8**	**51.5**	**41.8**
SE	3.9	3.8	5.9	5.3
SUBJECTS	11	10	12	12
95% CI	45.5-62.5	43.5-60.5	38.3-63.7	30.6-53.4

Type 1: Type 1 diabetic subject.
Type 2: Type 2 diabetic subject
The rest of the subjects were healthy individuals

The ANOVA test for variance (the SAS System; the GLM procedure, Release 8.2) was used (Figure 11).
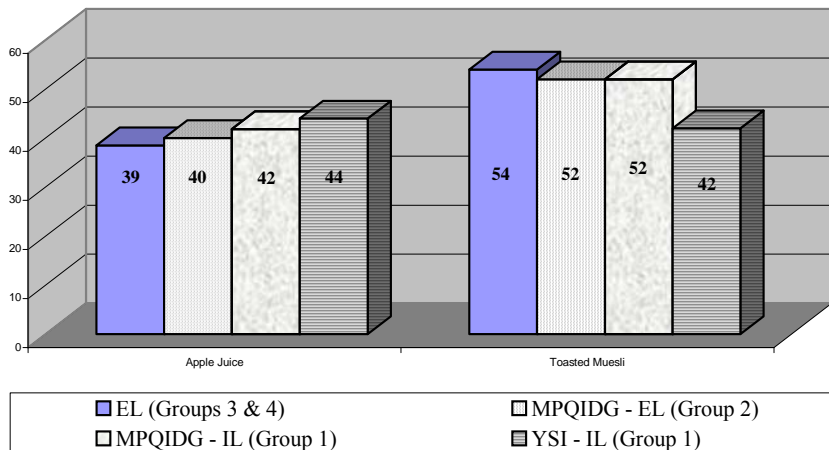
Figure 11: Comparison of the mean GI values of Apple juice and Muesli, determined in four ways

The mean GI values of Muesli, as determined by Group 1 (IL), using the MPQIDG (52±5.9), by Group 2 (EL), using the MPQIDG (52±3.8) and by Group 3 (EL), using the MPQIDG (54±3.9), all had higher GI values than the GI value of Muesli, as determined by Group 1 (IL), using the YSI (42±5.3) (38 and Figure 11). However, in spite of the fact that the GI value of Muesli, as determined by Group 1 (IL), using the YSI (42±5.3), was 12 points lower than the GI value of Muesli, as determined by Group 3 (EL), using the MPQIDG (54±3.9), there was no significant difference (p=0.2897) (not shown in a table) between any of the mean GI values of Muesli, which was determined in four ways (ANOVA test for variance). This is in line with the results of the inter-laboratory study, which showed that the difference between two GI values is true and not a chance finding, if the GI values of the "same" food differ by more than 18, as the average SD of laboratory mean GI values for five foods was 9.0.[39]

The SE values of the GI tests conducted IL on Muesli by Group 1 were 5.9 for the MPQIDG and 5.3 for the YSI respectively, whereas the SE values of the GI tests conducted EL were 3.8 and 3.9 for Groups 2 and 3 respectively, showing that IL tests do not necessarily yield lower SE values than EL tests (Table 38).

The 95%CI limits of all the GI tests conducted on Muesli was <30. In fact, the CI limits of the EL tests conducted by Groups 2 and 3 was <20, whereas the CI limits of the two IL tests conducted by Group 1, using both instruments, was >20 but <30. This compares well with the results of the capillary laboratories in the inter-laboratory study [39], which all had a 95% CI <30 , whereas the venous laboratories had a 95% CI >50.[21]

4.1.4.2 Apple juice

Table 39 summarizes the GI values of Apple juice, which was determined in the mentioned four ways.

Table 39: GI values of Apple juice, determined in four ways

Subject ID	MPQIDG; EL (Group 4)	MPQIDG; EL (Group 2)	MPQIDG; IL (Group 1)	YSI; IL (Group 1)
A1	58		29	32
A2		65	66	76
A3		64	47	41
A4		38	42	57
A5		14	27	30
A6		59	33	31
A7		24	41	57
A8		63	62	37
A9	21		28	43
A10		17	41	42
A11		26	28	45
A12		27	58	41
A13	33			
A14 (Type 2)	35			
A15				
A16 (Type 1)				
A17 (Type 1)	24			
A18 (Type 2)	44			
A19 (Type 1)	51			
A20 (Type 2)	43			
MEAN (GI)	**38.6**	**39.7**	**41.8**	**44.3**
SE	4.6	6.5	4	3.8
SUBJECTS	8	10	12	12
95% CI	27.8-49.2	24.7-54.7	32.9-50.7	35.7-52.3

Type 1: Type 1 diabetic subject.
Type 2: Type 2 diabetic subject.
The rest of the subjects were healthy individuals.

The ANOVA test for variance (the SAS System; the GLM procedure, Release 8.2) was used. Although the mean GI values of the three tests determined by MPQIDG were all lower than the mean GI value as obtained by YSI (Figure 11), there was no significant difference between the mean GI values of A, when it was determined in the mentioned four ways (p=0.85). Whereas all the mean GI values of Muesli, using the MPQIDG (IL and EL) were non-significantly higher than the mean GI value of Muesli, using the YSI (IL), this was not the case with Apple juice (Table 39 and Figure 11). The mean GI values of Apple juice, as determined by Group 1 (IL), using the MPQIDG (42$\pm$4.0), by Group 2 (EL), using the MPQIDG (40$\pm$6.5) and by Group 4 (EL), using the MPQIDG (39$\pm$4.6) were all lower than the GI value of Apple juice, as determined by Group 1 (IL), using the YSI (44$\pm$3.8). However, in spite of the fact that the GI value of Apple juice, as determined by Group 1 (IL) using the YSI (44$\pm$3.8) was 5 points lower than the GI value of Apple juice, as determined by Group 4 (EL) using the MPQIDG (38.6$\pm$4.6), there was no significant difference (p=0.8454) (not shown) between any of the mean GI values of Apple juice, which was determined in four ways (ANOVA test for variance). This is in line with the results of the inter-laboratory study which showed that the difference between two GI values is true and not a chance finding,

if the GI values of the "same" food differ by more than 18 points (95% CI limits), as the average SD of laboratory mean GI values for five foods was 9.0.[39]

The SE of the GI tests conducted on Apple juice by Group 1 (IL) was 4.0 for the MPQIDG and 3.8 for the YSI, respectively, whereas the values of the GI tests conducted by Groups 2 and 4 (EL) were 6.5 and 4.6, respectively (Table 39). For Apple juice, the SE values of the GI tests that were conducted EL were higher than those obtained with the GI tests conducted IL, which was opposite to that found with the GI results for Muesli. This shows that GI tests that are conducted EL seem not to consistently yield either higher or lower SE values.

The 95% CI limits of all the GI tests conducted on Apple juice was ≤30. In the case of Apple juice, the 95% CI limits of the EL tests conducted by Groups 4 and 2 were >20, but ≤30, whereas the 95% CI limits of the two IL tests conducted by Group 1, using both instruments, were <20. These results compare well with the results of the capillary laboratories in the inter-laboratory study [39], which all had 95% CI limits <30 (Table 43), whereas the venous laboratories had 95% CI limits >50.[21]

Traditionally, either a group of healthy or a group of diabetic subjects is used to conduct GI tests.[22,30] GI tests are also usually conducted IL, using one of the instruments described under 2.4.1.1. However, although studies on different population samples result in slightly different GI values for the same food, the rank order of GI for different foods has been found to be essentially the same between healthy and diabetic subjects (r=0.756; p<0.01) [1,32], and type 1 and type 2 diabetic subjects (r=0.96, p<0.01 and r=0.928, p<0.0001) (Jenkins et al, 1986 and Wolever et al, 1987, as reported by Wolever et al, 1991[2]). Part 1 of this study confirmed this, as there was no significant difference between the mean GI values obtained IL, using a group of healthy subjects and the YSI, when compared to the mean GI values obtained EL on the same two foods (i.e. Muesli and Apple juice), using a mixed group of subjects (healthy, type 1 and type 2 diabetic subjects) and the MPQIDG (p=0.2897 for Muesli and p=0.8454 for Apple juice)(not shown)

In conclusion
There was thus no significant difference between the mean GI values of two foods (Muesli and Apple juice), when determined in the mentioned four ways. However, this finding should be interpreted with caution and cannot be generalized from the findings of Part 1 of the study, as only two foods were tested. This could be regarded as a limitation of Part 1.

4.2 Part 2

Eleven subjects, six healthy and five diabetic (type 2), five males and six females, were recruited and tested EL, under well-controlled conditions.[3,39] One healthy subject was unable to complete all the tests and therefore withdrew from the study. The age of the subjects who partook in the study varied from to 30-62 (45$\pm$12y) and their BMI from 20-35 kg/m^2 (27$\pm$5)(Table 40).

Table 40: Characteristics of subjects (N=10) who partook in Part 2 of the study

Subject	Type of Subject	Age	BMI
B1	Healthy	42	20
B2	Healthy	43	24
B3	Healthy	43	28
B4	Healthy	31	23
B5	Healthy	30	26
B6	Diabetic (Type 2)	62	29
B7	Diabetic (Type 2)	59	25
B8	Diabetic (Type 2)	55	30
B9	Diabetic (Type 2)	57	32
B10	Diabetic (Type 2)	30	35
Mean		**45**	**27**
SD		12	5

4.2.1 Sub-problem

What was the degree of variance between the mean GI values of each of these five foods obtained in each of the five capillary laboratories in the inter-laboratory study, using healthy subjects and laboratory equipment [39], when compared to the weighted mean GI values of the rest of the capillary laboratories, including the extra-laboratory (EL) results on a group of mixed subjects using the MPQIDG, in order to simulate participation in the inter-laboratory study.

The GI values, SD and 95% CI of the five foods, as determined by the mixed group of ten subjects who took part in Part 2 of the study, are displayed in Table 41. The weighted mean GI values, SD and 95% CI of the five foods, as determined by a comparative group of 47 healthy subjects from five capillary laboratories (laboratories A–E), who took part in the inter-laboratory study [39], are displayed in Table 42.

Table 41: GI values (SD) and 95% CI of the five foods, as determined EL

Subject	Potato	Bread	Rice	Spaghetti	Barley
B1	74	76	52	40	45
B2	86	85	42	67	52
B3	79	83	39	38	36
B4	80	37	27	65	41
B5	56	64	46	33	38
B6 (Type 2)	113	80	61	19	24
B7 (Type 2)	101	86	63	57	21
B8 (Type 2)	55	73	56	40	47
B9 (Type 2)	89	62	45	47	36
B10 (Type 2)	74	91	85	20	67
MEAN (GI)	**81**	**74**	**51**	**43**	**41**
SD	18	16	16	17	13
SUBJECTS	10	10	10	10	10
95% C I	69.6-92.1	64.0-83.7	41.6-61.4	32.2-53.1	32.2-49.0

Type 2: Type 2 diabetic subject
The rest of the subjects were healthy individuals

Table 42: Weighted mean GI values (SD) and 95% CI of the five foods, as determined by the five capillary laboratories that took part in the inter-laboratory study [39]

Laboratory	Potato	Bread	Rice	Spaghetti	Barley
A	86	79	55	39	36
B	93	64	63	44	31
C	90	65	85	70	46
D	99	69	63	44	25
E	88	79	77	42	39
Weighted MEAN (GI)	**91**	**71**	**69**	**48**	**35**
Weighted Mean (SD)	25	21	23	18	16
Number of subjects	47	47	47	47	47
Weighted 95% CI	84.3-98.7	65.0-76.9	62.3-75.2	42.5-52.8	30.8-39.7

The 95% CI and mean GI value of each food as determined by each capillary laboratory [laboratories A-E that took part in the inter-laboratory study [39]], as well as EL, were compared to the weighted mean 95% CI and GI values of a comparative group of subjects from the rest of the laboratories. The significance of differences was calculated using the analysis of variance (two-tailed t-test). Table 43 shows the mean GI values, SD and 95% CI of the five foods, as determined by each laboratory and the weighted mean GI values, SD and 95% CI of a comparative group from the rest of the laboratories.

Table 43: Mean GI value (SD) and 95% CI of the individual laboratories, as well as the weighted mean GI value (SD) and 95% CI of the comparative group

		Individual laboratories				Weighted means (comparative group)			
Lab	Food	Mean GI value	SD	N	95% CI	Mean GI value	SD	N	95% CI
EL	Potato	80.9	18.2	10	69.6-92.1	91.5	25.1	47	84.3-98.7
EL	Bread	73.8	15.9	10	64.0-83.7	71.0	20.7	47	65.0-76.9
EL	Rice	51.5	16.0	10	**41.6-61.4**	68.7	22.6	47	62.3-75.2
EL	Spaghetti	42.6	16.9	10	32.2-53.1	47.6	18.1	47	42.5-52.8
EL	Barley	40.6	13.5	10	32.2-49.0	35.2	15.7	47	30.8-39.7
A	Potato	86.1	29.7	8	65.5-106.7	90.6	23.6	49	84.0-97.0
A	Bread	78.7	28.4	8	59.1-98.3	70.2	18.2	49	65.1-75.3
A	Rice	54.9	24.1	8	38.2-71.5	67.0	22.5	49	60.7-73.3
A	Spaghetti	38.7	13.2	8	29.6-47.9	48.1	18.3	49	43.0-53.2
A	Barley	36.0	15.4	8	25.3-46.7	36.2	15.4	49	31.8-40.5
B	Potato	93.3	32.5	10	73.1-113.4	89.3	22.5	47	82.8=95.7
B	Bread	64.2	15.4	10	54.6-73.7	72.9	20.5	47	67.1-78.8
B	Rice	62.6	25.0	10	47.1-78.0	65.9	22.6	47	59.4-72.4
B	Spaghetti	44.1	19.8	10	31.8-56.3	47.3	17.6	47	42.3-52.4
B	Barley	31.4	18.7	10	19.8-43.0	37.1	14.5	47	33.0-41.3
C	Potato	89.9	23.9	9	74.3-105.5	90.0	24.6	48	83.0-97.0
C	Bread	64.6	21.6	9	50.5-78.7	72.6	19.5	48	67.1-78.2
C	Rice	85.0	28.6	9	66.3-103.7	61.6	19.9	48	56.0-67.2
C	Spaghetti	69.9	18.8	9	**57.6-82.2**	42.4	14	48	38.5-46.4
C	Barley	46.2	15.4	9	36.2-56.3	34.3	14.7	48	30.1-38.4
D	Potato	98.5	20.6	10	85.8-111.3	88.2	24.8	47	81.1-95.3
D	Bread	69.4	3.6	10	67.2-71.7	71.8	21.8	47	65.6-78.0
D	Rice	63.3	8.1	10	58.3-68.3	65.7	25.0	47	58.6-72.9
D	Spaghetti	43.8	9.2	10	38.0-49.5	47.4	19.2	47	41.9-52.9
D	Barley	24.5	7.3	10	**20.2-29.1**	38.6	15.5	47	34.2-43.0

Table 43/..,.

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Table 43 (continued)

		Individual laboratories				**Weighted means (comparative group)**			
E	Potato	88.3	21.3	10	75.2-101.5	90.3	25.1	47	83.2-97.2
E	Bread	78.9	26.1	10	63.4-94.3	69.8	18.2	47	64.6-75.0
E	Rice	76.9	12.9	10	68.9-84.9	62.8	23.9	47	56.0-69.7
E	Spaghetti	42.1	10.8	10	35.5-48.8	47.7	18.9	47	42.3-53.2
E	Barley	39.3	13.0	10	31.2-47.4	35.5	15.8	47	31.0-40.0

95% CI limits of individual laboratories in bold print fall outside the 95% CI limits of the comparative group of the rest of the laboratories

When the 95% CI limits of each food, as determined by each laboratory, was compared to the weighted mean 95% CI limits of the comparative group from the rest of the laboratories, the laboratories whose 95% CI limits fell outside the 95% CI limits of the comparative group mean were identified (Table 43).

Table 44 shows the number of test subjects each laboratory used for the GI tests, as well as the total number of test subjects in the comparative group, with which the results of each laboratory were compared. It also shows the calculated t- and p-values. Table 45 shows the same information, but the values are listed according to food type.

Table 44: Analysis of variance (two-tailed t-test) for the five foods as determined in the inter-laboratory study [39], as well as the EL values (calculated t- and p-values)

Laboratory	Food	N (lab)	Calculated t-value	N (comparative group)	p-value
EL	Barley	10	1.1	47	0.276
	Bread	10	0.5	47	0.619
	Potato	10	-1.5	47	0.14
	Rice	10	-2.8	47	**0.007**
	Spaghetti	10	-0.8	47	0.427
A	Barley	8	0	49	1.0
	Bread	8	0.8	49	0.427
	Potato	8	-0.4	49	0.691
	Rice	8	-1.3	49	0.199
	Spaghetti	8	-1.8	49	0.078
B	Barley	10	-0.9	47	0.372
	Bread	10	-1.5	47	0.14
	Potato	10	0.4	47	0.691
	Rice	10	-0.4	47	0.691
	Spaghetti	10	-0.5	47	0.619
C	Barley	9	2.1	48	**0.041**
	Bread	9	-1.0	48	0.3
	Potato	9	0	48	1.0
	Rice	9	2.4	48	**0.019**
	Spaghetti	9	4.2	48	**<0.0001**
D	Barley	10	-4.4	47	**<0.0001**
	Bread	10	-0.7	47	0.487
	Potato	10	1.4	47	0.167
	Rice	10	-0.5	47	0.619
	Spaghetti	10	-0.9	47	0.372
E	Barley	10	0.8	47	0.427
	Bread	10	1.0	47	0.322
	Potato	10	-0.3	47	0.765
	Rice	10	2.6	47	**0.012**
	Spaghetti	10	-1.3	47	0.199

The values in bold print show a significant difference from the weighted mean GI values ($p \leq 0.05$)

Table 45: Analysis of variance (two-tailed t-test) for the five foods as determined in the inter-laboratory study [39], as well as the EL values (calculated t- and p-values)

Food	Laboratory	N (lab)	Calculated t-value	N (comparative group)	p-value
Barley	EL	10	1.1	47	0.276
	A	8	0	49	1.0
	B	10	-0.9	47	0.372
	C	9	2.1	48	**0.041**
	D	10	-4.4	47	**<0.0001**
	E	10	0.8	47	0.427
Bread	EL	10	0.5	47	0.619
	A	8	0.8	49	0.427
	B	10	-1.5	47	0.14
	C	9	-1.0	48	0.3
	D	10	-0.7	47	0.487
	E	10	1.0	47	0.322
Potato	EL	10	-1.5	47	0.14
	A	8	-0.4	49	0.691
	B	10	0.4	47	0.691
	C	9	0	48	1.0
	D	10	1.4	47	0.167
	E	10	-0.3	47	0.765
Rice	EL	10	-2.8	47	**0.007**
	A	8	-1.3	49	0.199
	B	10	-0.4	47	0.691
	C	9	2.4	48	**0.019**
	D	10	-0.5	47	0.619
	E	10	2.6	47	**0.012**
Spaghetti	EL	10	-0.8	47	0.427
	A	8	-1.8	49	0.078
	B	10	-0.5	47	0.619
	C	9	4.2	48	**<0.0001**
	D	10	-0.9	47	0.372
	E	10	-1.3	47	0.199

The values in bold print show a significant difference from the weighted mean GI values ($p \leq 0.05$)

The results of the analysis of variance (two-tailed t-test) can be summarized as follows (Tables 44 and 45). The 95% CI (Table 43) revealed similar **trends** with regards to agreement:
Laboratories A and B had no food of which the mean GI value showed a significant difference ($p \leq 0.05$) from the weighted mean GI value of the rest of the laboratories. (The 95% CI for all the foods of laboratories A and B also overlapped with the weighted mean 95% CI of the comparative group from the rest of the laboratories.)
Laboratories D, EL and E each had one food of which the mean GI value showed a significant difference ($p \leq 0.05$) from the weighted mean GI values of the rest of the laboratories. (The 95% CI limits for all but

one food of laboratories D and EL, and all of the foods of laboratory E, overlapped with the weighted mean 95% CI limits of the comparative group from the rest of the laboratories).

Laboratory C had three foods (Rice, Barley and Spaghetti) of which the mean GI values showed a significant difference ($p \leq 0.05$) from the weighted mean GI values of the rest of the laboratories. (However, the 95% CI limits for all but one food overlapped with the weighted mean 95% CI limits of the comparative group from the rest of the laboratories).

Foods for which the mean GI value of no laboratory showed a significant difference from the weighted means of the rest of the laboratories: Bread and potato.

Food for which the mean GI value of one laboratory (i.e. laboratory C) showed a significant difference from the weighted means of the rest of the laboratories: Spaghetti.

Food for which the mean GI value of two laboratories (i.e. laboratory C and D) showed a significant difference from the weighted means of the rest of the laboratories: Barley.

Food for which the mean GI value of three laboratories (i.e. laboratories C, E and EL) showed a significant difference from the weighted means of the rest of the laboratories: Rice.

In the presentation of results of this kind, it should be kept in mind that the mean GI value of a specific laboratory is influenced by the GI values of the same food, as determined by the other laboratories. To illustrate this point, the reason why the mean GI value of Rice of laboratories E and EL showed a significant difference from the weighted mean GI value of the rest of the laboratories could possibly be due to the fact that Rice was also one of the three foods for which laboratory C showed a significant difference form the rest of the laboratories. These differences could possibly have been caused by sources of error in the inter-laboratory study, e.g. analytical variation, blood sample handling and storage, between-subject variation, within-subject variation and even differences in food preparation.[34] It could also have been attributed to lifestyle-confounding factors.

When the GI values of the five foods, as obtained by laboratories A-E and EL, were compared using ANOVA, the results showed similar **trends** with regards to agreement:

Bread: There was no significant difference between the mean GI values obtained ($p=0.4$).

Potato: There was no significant difference between the mean GI values obtained ($p=0.6991$).

Barley: There was a significant difference between the mean GI values obtained ($p=0.0276$). This was probably mainly due to the fact that the mean GI value obtained by laboratory D differed significantly from three laboratories [i.e. laboratories C ($p=0.0017$), E ($p=0.0239$) and EL ($p=0.0141$)]. The only laboratories from which the mean GI value, as was obtained by laboratory D did not differ significantly, were laboratories A ($p=0.0918$) and B ($p=0.2848$). Though the mean GI value, as was obtained by laboratory A, did not differ significantly from any other laboratory, the mean GI value, as was obtained by laboratory C differed significantly from laboratoriy B as well ($p=0.0276$). The mean GI values obtained by laboratories E and EL did not differ significantly from any other laboratory, except for differing significantly from laboratory D ($p=0.0239$ and $p=0.0141$, respectively).

Rice: There was a significant difference between the mean GI values obtained ($p=0.0051$). This was probably mainly due to the fact that the mean GI value obtained by laboratory C differed significantly from four laboratories [i.e. laboratories A ($p=0.0033$), B ($p=0.0187$), D ($p=0.0225$) and EL ($p=0.0006$)]. The only laboratory from which the mean GI value, as was obtained by laboratory C, did not differ significantly, was laboratory E ($p=0.3818$). The mean GI value obtained by laboratory E also differed significantly from laboratories A ($p=0.0253$) and EL ($p=0.0066$).

Spaghetti: There was a significant difference between the mean GI values obtained ($p=0.0008$). This was probably mainly due to the fact that the mean GI value, obtained by laboratory C differed significantly from all five the laboratories [i.e. laboratories A ($p=0.0001$), B ($p=0.0006$), D ($p=0.0005$) and EL ($p=0.0003$)]. The mean GI values as were obtained by all the other laboratories did not differ significantly from any other laboratory.

In conclusion
There was a significant difference between the mean GI value of one food (i.e. Barley for laboratory D, Rice for EL and laboratory E) and the weighted mean GI values of the rest of the laboratories for these foods. However, there was a significant difference between the mean GI values of three foods (Rice, Barley and Spaghetti) for laboratory C and the weighted mean GI values of the rest of the laboratories for these foods (Table 44). EL therefore performed similarly to laboratories D and E and better than laboratory C. Laboratories A and B performed the best in this regard.

There was no significant difference between the mean GI values, as obtained by any of the laboratories for bread (p=0.4) and potato (p=0.6991), using ANOVA. However, there was a significant difference between the mean GI values, as obtained by some of the laboratories for barley (p=0.0276), rice (p=0.0051) and spaghetti (p=0.0008). In the case of barley, laboratory D differed significantly from three other laboratories; in the case of rice, laboratory C differed significantly from four other laboratories and in the case of spaghetti, laboratory C differed significantly from all the other laboratories.

4.2.2 Sub-problem

How did the standard error (SE), 95% CI limits and deviation from group mean GI value of each of the five foods [as determined by each of the five capillary laboratories in an inter-laboratory study, using healthy subjects and laboratory equipment [39], as well as by the investigator in the current extra-laboratory (EL study), using a mixed group of subjects (i.e. healthy, type 1- and type 2 diabetic subjects) and the MPQIDG], compare to the weighted means of the rest of the laboratories for these parameters?

Table 46 shows the SE, 95% CI limits and deviation from weighted group mean GI value for each of the five foods of each individual laboratory in comparison to the weighted means of the rest of the laboratories, for these parameters.

Table 46: SE, 95% CI and deviation from weighted mean GI value of each of the five foods of each laboratory, as well as the weighted means of the rest of the laboratories

Food	Lab	Individual laboratory					Weighted means of the rest of the laboratories				
		Mean GI value	SE	N	95% CI	Deviation from group mean GI value	Weighted mean GI value	Weighted mean SE (per food)	N	Weighted 95% CI (per food)	Weighted deviation from group mean GI value (per food)
Potato	A	86.1	**10.5**	8.0	**41.2**	4.5	90.6	7.4	49.0	29.1	5.5
Potato	B	93.3	**10.3**	10.0	**40.3**	4.0	89.3	7.4	47.0	28.8	5.7
Potato	C	89.9	**8.0**	9.0	**31.2**	0.1	90.0	7.9	48.0	30.7	6.3
Potato	D	98.5	6.5	10.0	25.5	**10.3**	88.2	8.2	47.0	31.9	4.3
Potato	E	88.3	6.7	10.0	26.3	2.0	90.3	8.1	47.0	31.8	6.1
Potato	EL	80.9	5.8	10.0	22.5	**10.6**	91.5	8.3	47.0	32.6	4.3
Bread	A	78.7	**10.0**	8.0	**39.2**	**8.5**	70.2	5.3	49.0	20.3	6.2
Bread	B	64.2	4.9	10.0	19.1	**8.7**	72.9	6.2	47.0	23.8	6.0
Bread	C	64.6	**7.2**	9.0	**28.2**	**8.0**	72.6	5.7	48.0	22.0	6.2
Bread	D	69.4	1.1	10.0	4.5	2.4	71.8	6.9	47.0	26.9	7.4
Bread	E	78.9	**8.3**	10.0	**30.9**	**9.1**	69.8	5.4	47.0	21.3	5.9
Bread	EL	73.8	5.0	10.0	19.7	2.8	71.0	6.1	47.0	23.7	7.3
Rice	A	54.9	**8.5**	8.0	**33.3**	**12.1**	67.0	5.8	49.0	20.7	11.8
Rice	B	62.6	**7.9**	10.0	**30.9**	3.3	65.9	5.8	47.0	20.7	13.7
Rice	C	85.0	**9.5**	9.0	**37.4**	**23.4**	61.6	5.5	48.0	19.7	9.7
Rice	D	63.3	2.6	10.0	10.0	2.4	65.7	6.9	47.0	25.1	13.9
Rice	E	76.9	4.1	10.0	16.0	**14.1**	62.8	6.6	47.0	23.8	11.4
Rice	EL	51.5	5.1	10.0	10.8	**17.2**	68.7	6.4	47.0	24.9	10.8
Spaghetti	A	38.7	4.7	8.0	18.3	**9.4**	48.1	4.8	49.0	18.8	8.6
Spaghetti	B	44.1	**6.3**	10.0	**24.5**	3.2	47.3	4.5	47.0	17.5	9.9
Spaghetti	C	69.9	**6.3**	9.0	**24.6**	**27.5**	42.4	4.5	48.0	17.7	5.2
Spaghetti	D	43.8	2.9	10.0	11.5	3.6	47.4	5.2	47.0	20.3	9.8
Spaghetti	E	42.1	3.4	10.0	13.3	5.6	47.7	5.1	47.0	19.9	9.4
Spaghetti	EL	42.6	**5.3**	10.0	**20.9**	5.0	47.6	4.7	47.0	18.3	9.5

Table 46/…

Table 46 (continued)

Food	Lab	Individual laboratory					Weighted means of the rest of the laboratories				
		Mean GI value	SE	N	95% CI	Deviation from group mean GI value	Weighted mean GI value	Weighted mean SE (per food)	N	Weighted 95% CI (per food)	Weighted deviation from group mean GI value (per food
Barley	A	36.0	**5.4**	8.0	**21.4**	0.2	36.2	4.3	49.0	17.0	8.1
Barley	B	31.4	**5.9**	10.0	**23.2**	5.7	37.1	4.2	47	16.4	7.3
Barley	C	46.2	**5.1**	9.0	**20.1**	**11.9**	34.3	4.4	48.0	17.2	6.1
Barley	D	24.5	2.3	10.0	9.1	**14.1**	38.6	5.0	47.0	19.4	5.5
Barley	E	39.3	4.1	10.0	16.2	3.8	35.5	4.6	47.0	17.9	7.7
Barley	EL	40.6	4.3	10.0	16.8	5.4	35.2	4.5	47.0	17.8	7.3

SE (Individual laboratory): Values in bold print are higher than the weighted group mean for each food
95% CI limits: Values in bold print are higher than the weighted group mean for each food
Deviation from group mean GI value: Values in bold print are higher than the weighted group mean for each food

When comparing the SE, 95% CI and deviation from group mean GI values of each of the five foods for each laboratory with the weighted means of the rest of the laboratories for these parameters, the results showed the following:

Standard error (SE)
Laboratories of which the SE of the GI values of all five foods was lower than the weighted mean SE of the rest of the laboratories: Laboratory D.
Laboratory of which the SE of the GI values of four of the five foods was lower than the weighted mean SE of the rest of the laboratories: Laboratory E and EL.
Laboratories of which the SE of the GI values of only one of the five foods was lower than the weighted mean SE of the rest of the laboratories: Laboratories A and B.
Laboratory of which the SE of the GI values of none of the five foods was lower than the weighted mean SE of the rest of the laboratories: Laboratory C.

Based on the number of foods of which the SE was lower than the weighted mean SE of the rest of the laboratories, laboratory D performed the best. Laboratory E, together with EL, performed second best and laboratories A and B third best. Laboratory C performed worst.

95% CI
Laboratory of which the 95% CI limits of all five foods were smaller than the weighted mean CI limits of the rest of the laboratories: Laboratory D.
Laboratories of which the 95% CI limits of four of the five foods were smaller than the weighted mean CI width of the rest of the laboratories: Laboratories E and EL.
Laboratories of which the 95% CI limits of only one of the five foods was smaller than the weighted mean CI widths of the rest of the laboratories: Laboratories A and B.
Laboratory of which the 95% CI limits of only one of the five foods was smaller than the weighted mean CI widths of the rest of the laboratories: Laboratory C.

Number of foods of which the 95% CI limits was smaller than the weighted means of the rest of the laboratories: One laboratory (i.e. laboratory D) performed better than EL, and laboratories E and EL performed second best. Laboratory C performed worst.
Number of foods of which the 95% CI limits <30: Laboratories D and EL performed the best.

Deviation from group mean GI values
Laboratory of which the deviation from group mean GI values of all five foods was smaller than the weighted mean deviation from group mean GI values of the rest of the laboratories: None.
Laboratory of which the deviation from group mean GI values of four of the five foods was smaller than the weighted mean deviation from group mean GI values of the rest of the laboratories: Laboratory B.
Laboratories of which the deviation from group mean GI values of three of the five foods was smaller than the weighted mean deviation from group mean GI values of the rest of the laboratories: Laboratories D, E and EL.
Laboratory of which the deviation from group mean GI values of two of the five foods was smaller than the weighted mean deviation from group mean GI values of the rest of the laboratories: Laboratory A.
Laboratory of which the deviation from group mean GI values of only one of the five foods was smaller than the weighted mean deviation from group mean GI values of the rest of the laboratories: Laboratory C.
Laboratory of which the deviation from group mean GI value of all five foods was lower than 18 [39]: Laboratories A, B, D, E and EL.
Laboratory of which the deviation from group mean GI value of two of the five foods was >18 [39]: Laboratory C.

Number of foods of which the deviation from group mean GI value was smaller than the weighted means of the rest of the laboratories: One laboratory (i.e. laboratory A) performed better than EL and laboratories D, E and EL performed second best. Laboratory C performed worst.
Number of foods of which the deviation from group mean GI value was <18 [39]: All the laboratories except for laboratory C, which performed worst.

Extra-laboratory (EL) performed as follows:
SE: The SE of one of the five foods was higher than the weighted mean SE of the rest of the laboratories.
95% CI limits: The 95% CI limits of only one of the five foods was higher than the weighted mean CI limits of the rest of the laboratories for that food. No 95% CI limits of EL was >30.
Deviation from group mean GI value: The deviation from group mean GI value of two of the five foods was higher than the weighted means of the rest of the laboratories. The deviation from the group mean GI value was >18 for none of the five foods.
The analysis of variance (two-tailed t-test): Number of foods of which the mean GI values were significantly different from the weighted mean GI values of the rest of laboratories for that food: Only one laboratory (i.e. Laboratory D) performed better than EL.

Coefficient of variance (CV)
For the EL GI tests, the CV of repeated tests of the reference food was 20±11 for the mixed group of diabetic and healthy subjects (not shown). This compares well and is lower than the mean CV (i.e. 23.4±2.1) of repeated tests of the reference food in the inter-laboratory study [39], although this was not statistically significant (p=0.3). The CV for the mixed group of subjects (20±11) was also lower than, but not significantly different from the CV of repeated tests of the reference food mentioned in the literature, i.e. 25±12 for healthy persons (p=0.6952) and higher, but not significantly different from the CV of repeated tests of the reference food mentioned in the literature, i.e. 16±7 for type 2 diabetic subjects (not on insulin) [56] (p=0.6957), using the analysis of variance (two-tailed t-test).

In conclusion

When considering the SE, only laboratory D showed less variability than EL; when considering the 95% CI limits, only laboratory D showed less variability than EL; when considering the deviation from weighted group mean GI value, only laboratory B showed less variability than EL and when considering the mean GI values, only laboratories A and B showed less variability than EL. The CV (of repeated tests of the reference food of the mixed group of subjects of EL) was lower than the CV of repeated tests of the reference food of the group of healthy subjects who partook in the inter-laboratory study (23.4 ± 2.1) [39] and lower than the CV of repeated tests of the reference food mentioned in the literature (25 ± 12), although neither of these were significant (p=0.03 and p=0.6952, respectively).

4.2.3   Research Problem

What was the degree of variance between the mean GI values obtained EL (mixed group of subjects and MPQIDG) and the weighted mean GI values obtained IL (by a group of healthy subjects from five experienced capillary GI testing centers in the inter-laboratory study, using laboratory equipment) on three glucose (reference food) and five food (i.e. Rice, Barley, Spaghetti, Instant potato and White bread) determinations?[39]

The GI values, SD and 95% CI of the five foods, as determined by the mixed group of ten subjects who took part in Part 2 of the study, are displayed in Table 41. The mean GI values, as well as the weighted mean GI values, SD and 95% CI of each of the five foods, as determined by a group of 47 healthy subjects of the capillary laboratories, who took part in the inter-laboratory study, are displayed in Table 42. The mean GI values (SD) of laboratories A–E, including EL are shown in Figure 12.

The mean GI values for each of the five foods, as determined EL, were compared to the weighted mean GI values of the five experienced capillary laboratories (Laboratories A–E) that partook in the inter-laboratory study [39], and the significance of differences was calculated using the analysis of variance (two-tailed t-test). The mean GI value of one of the five foods, i.e. Rice, that was tested EL showed a significant difference (p=0.007) from the weighted mean GI value of the five capillary laboratories (refer to Tables 41and 42).

The mean GI values for each of the five foods, as determined EL, were also compared to the mean GI values, as determined IL by laboratories A-E (Table 48), using ANOVA.
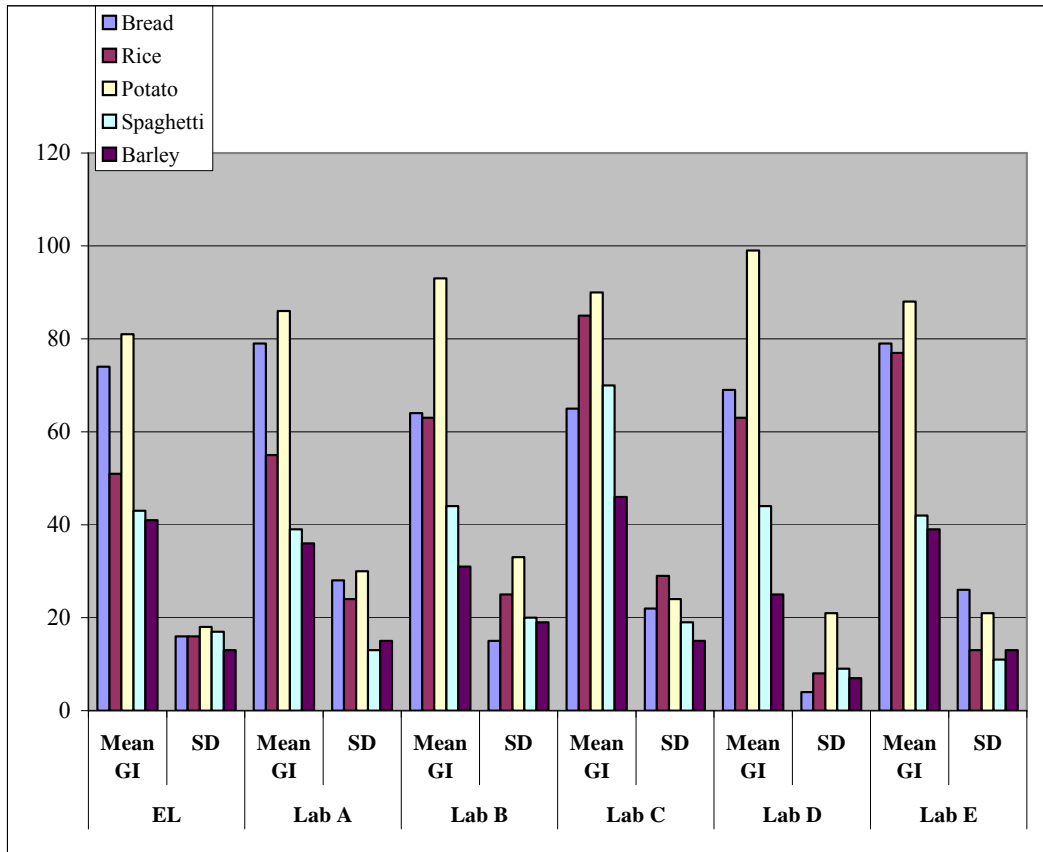
Figure 12: Comparison of the mean GI (SD) values [EL and 5 capillary laboratories[39]]

Table 44 shows the mean GI values of the five foods, as well as the average SD between laboratories (as determined in the inter-laboratory study [39], together with the 95% CI. Table 45 shows the same information, but with the results of EL added.

Table 47: Mean GI values (average SD) of the five foods as determined in the inter-laboratory study [39]

Laboratory	Potato	Bread	Rice	Spaghetti	Barley	Interlab SD
A	86.0	78.7	54.8	38.7	36.0	
B	93.3	64.2	62.6	44.1	31.4	
C	89.9	64.6	85.0	69.6	46.2	
D	98.5	69.4	63.3	43.8	24.5	
E	88.3	78.9	76.9	42.1	39.3	
Mean (GI)	**91**	**71**	**69**	**48**	**35**	
Average SD	5	7	12	12	8	9.0
Laboratories	5	5	5	5	5	
95% CI	86.9-95.5	64.8-77.5	57.9-79.2	36.7-58.6	28.3-42.6	54.9-70.7

Table 48: Mean GI values (average SD) of the five foods as determined in the inter-laboratory study [39]**, as well as the EL values**

Laboratory	Potato	Bread	Rice	Spaghetti	Barley	Interlab SD
EL	81.0	74.0	51.0	43.0	41.0	
A	86.0	78.7	54.8	38.7	36.0	
B	93.3	64.2	62.6	44.1	31.4	
C	89.9	64.6	85.0	69.6	44.2	
D	98.5	69.4	63.3	43.8	24.5	
E	88.3	78.9	76.9	42.1	39.3	
Mean GI	**90**	**72**	**66**	**47**	**36**	
Average SD	6	7	13	11	8	8.9
Laboratories	6	6	6	6	6	
95% CI	84.7-94.3	66.3-76.9	55.2-76.0	37.8-55.9	30.3-42.5	54.9-69.1

The mean GI value (51±16) of one of the five foods, i.e. Rice, as determined EL, was significantly different (p=0.007) (Table 44) from the weighted mean GI value (69±23) (Table 47) of the rest of the laboratories (i.e. laboratories A–E). However, the GI value of one of the five foods, as determined by experienced capillary GI testing laboratories D and E, also showed a significant difference (p≤0.05) from the weighted mean GI values of the rest of the laboratories. The GI values of three of the five foods, as determined by experienced capillary GI testing laboratory C, showed a significant difference (p≤0.05) from the weighted mean GI values of the rest of the laboratories (Table 44).

Using ANOVA, the mean GI value of rice, as determined EL, differed significantly from that of laboratories C (p=0.0006) and E (p=0.0057); the mean GI value of barley differed significantly from that of laboratory D (p=0.0141) and the mean GI value of spaghetti differed significantly from that of laboratory C (p=0.0003). However, when rice is concerned, the mean GI value, as obtained by laboratory C differed significantly from four laboratories [i.e. laboratories A (p=0.0033), B (p=0.0187), D (p=0.0225) and EL (p=0.0006)]. The only laboratory from which the mean GI value, as was obtained by laboratory C did not differ significantly, was laboratory E (p=0.3818). The mean GI value, as obtained by laboratory E also differed significantly from laboratories A (p=0.0253) and EL (p=0.0066). Where barley is concerned, the mean GI value, as obtained by laboratory D differed significantly from three laboratories [i.e. laboratories C (p=0.0017), E (p=0.0239) and EL (p=0.0141)]. The only laboratories from which the mean GI value, as was obtained by laboratory D did not differ significantly, was laboratories A (p=0.0918) and B (p=0.2848). However, the mean GI value, as was obtained by laboratory A did not differ significantly from any other laboratory, whereas the mean GI value, as was obtained by laboratory C differed significantly from laboratoriy B as well (p=0.0276). The mean GI values, as were obtained by laboratories E and EL did not differ significantly from any other laboratory, except for differing significantly from laboratory D. Where spaghetti is concerned, the mean GI value, as was obtained by laboratory C differed significantly from all five the laboratories [i.e. laboratories A (p=0.0001), B (p=0.0006), D (p=0.0005) and EL (p=0.0003)]. The mean GI values, as were obtained by all the other laboratories did not differ significantly from any other laboratory.

The finding that the mean GI value of Rice, as determined EL, was significantly different from the weighted mean GI value of the Rice from the rest of the laboratories, could possibly be due to the fact that the mean GI value (85±28.6) of the Rice, as determined by laboratory C (Table 47), was significantly different (p=0.019) (Table 44) from the weighted mean GI value of the rest of the laboratories, as well as the finding that the GI value (76.9±12.9) of the Rice, as determined by laboratory E (Table 47), was also significantly different (p=0.012) (Table 44) from the weighted mean GI value of the rest of the laboratories. A few sources of error in the inter-laboratory study, e.g. analytical variation, blood sample handling and storage, between-subject variation, within-subject variation and even differences in food preparation [34], could have affected the finding. Even lifestyle-confounding factors (refer to Addendum 7) could also have had an effect.

It is also worthwhile noting that, of all five foods that were tested in the inter-laboratory study, as well as by EL, Rice showed the most variation (Table 45). The mean GI value of three laboratories showed a significant difference from the weighted mean GI value of the rest of the laboratories. The SE and 95% CI limits of laboratories A, B and C for Rice were also higher than the weighted means of the rest of the laboratories for these parameters, and the deviation from the group mean GI value of laboratories A, C, E and EL for Rice was higher than the weighted mean of the rest of the laboratories.

It would seem that, even in some of the most experienced capillary GI testing laboratories in the world, about one in every five GI tests could be erroneous, as six of the 30 foods that were tested (i.e. five foods tested by each of the five international capillary laboratories, as well as EL) had GI values that differed significantly from the weighted mean GI values of the foods of the rest of the laboratories. The finding

that the GI value of one of the investigator's foods was significantly different from the mean GI value of the rest of the laboratories is therefore not extra-ordinary. In fact, two of the five laboratories showed similar results and one laboratory had three foods of which the GI values were significantly different from the weighted mean GI values of the rest of the laboratories.

If the mean GI value for the seven foods, which was determined by YSI and OTU, are treated as duplicate estimates, the average SD of the duplicates will be 9.2 (Velangi et al, 2005), which is similar to the average between laboratory SD of the five capillary laboratories that partook in the inter-laboratory study.[39] The only source of error in their study was the difference in glucose analysis, whereas sources of error other than analytical differences, such as blood sample handling and storage, between-subject variation, within-subject variation and possible difference in food preparation accounted for the majority of between-laboratory variance. They concluded that, if glucometres were used to determine GI values, the differences between laboratories might be considerably greater than in the inter-laboratory study conducted by Wolever et al (2003).[34]

However, this was not the case in this present study. In the inter-laboratory study [39], the major finding was that the average inter-laboratory SD of laboratory mean GI values was 9.0. (Table 47). Once EL GI values had been added (Table 48), the inter-laboratory SD was lowered slightly to 8.9, showing that the GI results, as determined EL, seem not to affect this finding negatively. Contrary to this, the addition of the two venous laboratories, who originally took part in the inter-laboratory study, affected the average inter-laboratory SD and caused it to be raised to 10.6.[39] This could possibly be due to the fact that EL testing seems to cause less within-subject variability, as a result of controlling lifestyle-confounding factors in a non-stressful manner, resulting in reducing the day-to-day variation of glycemic responses within subjects, which was the main cause of between laboratory variation in the inter-laboratory study.[39] This is reflected in the SE of EL which, together with laboratory D was lower than the weighted mean SE of the rest of the laboratories, for each food. EL also fared well when compared to the rest of capillary laboratories for 95% CI, 95% CI limits, and deviation from weighted mean GI values and CV.

As the literature search revealed, the following lifestyle-confounding factors influence blood glucose response and could therefore influence the GI: food [21,23,59], alcohol [21,42,43] and caffeine [40,41] consumption, activity [44,45,81], smoking [21], medication [21,23], sleeping habits [46], length of overnight fast [21], stress [38], illness [5,48] and menstrual cycle.[47] It would therefore seem that strict control of diet, activity and other factors during the day before a GI test is conducted would improve the reproducibility of GI results.

In the present investigation, the following methodology was followed:
- standardized techniques, i.e. the subjects were trained in using the MPQIDG, lancet and test strips (Addendum 3).
- measures to reduce the effect of day-to-day within-subject variation in glycemic responses, i.e. control had to be taken over lifestyle-confounding factors, regarding their consumption of caffeine [40,41], alcohol [42,43] and medication.[23] They also had to standardize their exercise [23], sleeping [46] and smoking habits [21]. Female subjects were advised to not conduct a GI test during their menstrual period [47], and all subjects were advised to not conduct a GI test if they have an infection in their bodies.[5,48]
- involvement of subjects, by having them choose a standard meal [21,23], which had to be consumed before 20h00 on the night before testing, although they were also allowed to have a small snack and drink, e.g. a fruit or a biscuit, if preferred and coffee or tea before 22h00. All subjects, however, had to decide beforehand whether he/she wanted to take only coffee or tea, or the small snack as well, and had to follow the same procedure for every test.

All the above factors, especially reduced stress due to EL testing, could have helped to reduce within-subject variability (CV) of glycemic responses. **Further and specific reseach is needed to confirm this.**

The EL tests (using a mixed group of subjects and the MPQIDG, EL) of Part 1 showed less variation than the IL testing (healthy subjects and YSI). There was also no significant difference between the mean GI values of Muesli and Apple, as determined by a group of healthy subjects, using the YSI (IL); the same group of healthy subjects, using the MPQODG (IL); the same group of healthy subjects, using the MPQIDG (EL) and two mixed groups of subjects, using the MPQIDG (EL), respectively (refer to 4.1.4).

The EL testing of Part 2 of this study showed less variation than IL testing (healthy subjects and laboratory equipment). EL testing (using a mixed group of subjects and MPQIDG) consistently showed less variation than one experienced GI testing laboratory (i.e. laboratory C) and similar variation to two experienced laboratories (laboratories D and E) (refer to 4.2.3).

From the recent findings it seems as though:
- there is a reduction in the effect of stress on GI testing by having the subjects conduct the GI tests in their natural (home) environment, i.e. EL (or it could be due to using a group of mixed subjects.Well-controlled diabetics can decrease variability through the effect of the treatment);
- using type 2 diabetic subjects as part of a mixed team of test subjects could help to reduce the variability in GI tests, as type 2 diabetic subjects (with or without insulin) have the lowest CV (reference food), i.e. 16 ± 7 for type 2 diabetic persons not on insulin and 15 ± 4 for type 2 diabetic persons on insulin, in contrast to 29 ± 19 for type 1 diabetic persons and 25 ± 12 for healthy individuals [56] The fundamental question remains whether they should form part of a test group as their glucose metabolism is under control of hypoglycemic agents;
- consistency in controlling for lifestyle-confounding factors (in a way that does not increase stress levels in the subjects) could help to reduce within-subject variability/variability in GI tests. The GI tests have to fit into the subject's lifestyle, rather than the subject's lifestyle has to be adjusted to the GI tests. This ensures better compliance and does not cause unnecessary stress in the subject, which is known to cause more variability.[38] The investigator illustrated the point by an example (refer to Addendum 7);
- including regular, trained test subjects in the GI testing team, who are interested in conducting GI tests for a prolonged period of time, instead of using a new group of test subjects for every group of foods that needs to be tested for GI, could also help to reduce variability in GI tests. Choosing test subjects for GI testing who are routine persons (i.e. a person who would e.g. get up at a specific time every morning, go for a walk soon after rising, always eat immediately after the walk, then take a shower and get dressed for the day, have supper at the same time every day and go to bed at the same time every night) could also help significantly, as any changes in lifestyle factors can affect GI tests.

4.3 Reflection on research hypotheses

The following research hypotheses were posed:

4.3.1 There would be a significant correlation ($p \leq 0.05$) between the actual blood glucose readings as obtained IL, when a group of healthy subjects (Group 1), and the YSI and MPQIDG were used.

- There was a good to excellent correlation between most (36 out of the 45 readings) of the blood glucose readings ($p \leq 0.05$), except for all the FBG readings, as well as the 15min readings of Glucose 1 and 2, the 90min reading of Muesli and 90 and 105min readings of Apple juice. The correlation between the fasting values was poor for Glucose 1 and 2, as well as for Muesli and Apple juice and fair for Glucose 3. The correlation between the values taken at 15min was poor for Glucose 1 and 2 and also at 90 and 105min for Apple juice. However, out of the 45 time intervals, 27 showed a very good correlation ($r = 0.9$; $p \leq 0.02$) between the absolute blood glucose readings obtained on the two instruments and of these, 13 were highly significant ($p < 0.0001$). Seven out of the 45 time intervals showed a good and significant correlation ($r = 0.8$; $p \leq 0.04$), two showed an acceptable correlation ($r = 0.7$; $p \leq 0.1$), one showed a fair, significant correlation ($r = 0.6$; $p \leq 0.05$) and eight out of the 45 time intervals showed a poor correlation ($r = 0.5$ or less; $p > 0.1$). The correlation (r) between all of the determinations at time intervals 30, 45, 60, 75 and 120min was significant ($p \leq 0.05$).

  It can be concluded that there was a good and significant ($r = 0.6$-0.98; $p \leq 0.05$) correlation between the absolute blood glucose readings, as obtained on the YSI and MPQIDG, after removal of outliers, at all the time points, except for at 0min (all but one product), at 15min (two of the five products), at 90min (Muesli and Apple juice) and at 105min (Apple juice).

4.3.2 There would be a significant correlation ($p \leq 0.05$) and agreement between the GI and AUC values as obtained IL, when a group of healthy subjects (Group 1), and the YSI and MPQIDG were used.

- The **correlation** (r) was acceptable and significant for the AUC values of Glucose 1 ($r = 0.7$; $p = 0.0081$), good and significant for the GI value of Apple juice ($r = 0.8$; $p = 0.0043$) and very good and highly significant for the AUC values of Glucose 2 ($r = 0.9$; $p < 0.0001$), Glucose 3 ($r = 0.9$; $p < 0.0001$), Muesli ($r = 0.9$; $p < 0.0001$) and A ($r = 0.9$; $p < 0.0001$) and the GI value of Muesli ($r = 0.9$; $p = 0.0003$), respectively.
  The **agreement** (r_c) was acceptable for the AUC value of Glucose 1 ($r_c = 0.7$) and the GI value of Apple juice ($r_c = 0.7$), good for the AUC value of Glucose 2 ($r_c = 0.8$) and very good for the AUC value of Glucose 3 ($r_c = 0.9$), Muesli ($r_c = 0.9$) and Apple juice ($r_c = 0.9$) and the GI value of Muesli ($r_c = 0.9$), respectively.

4.3.3 There could possibly be a significant difference ($p \leq 0.05$) between the mean GI values of the two foods that were determined in the mentioned four ways, using the IAUC calculation, when compared to the mean GI values of the two foods, using the other two most commonly used methods of calculating the AUC, i.e. AUC_{min} and AUC_0.

- There was a significant difference between the mean GI values of Muesli, when calculated using the **AUC_{min} method** as opposed to the IAUC method, in the case of the original EL test (Group 3, using the MPQIDG)($p = 0.0474$). There was also a significant difference between the mean GI values of Apple juice, when calculated using the AUC_{min} method as opposed to the

IAUC method in the case of both the IL tests (Group 1, using both the YSI and MPQIDG)(p=0.0125 and p=0.0377, respectively). There was, however a highly significant difference between the mean GI values, when calculated using the **AUC$_0$ method** as opposed to the IAUC method, in the case of all four tests that were performed for both Muesli and Apple juice (p=0.0000 in all cases).

From these finding it can be recommended that the AUC$_{min}$ and AUC$_0$ methods of calculating the AUC should not be used to calculate the AUC for GI tests.

4.3.4. **There would be no significant difference between the mean GI values of the five foods obtained when a mixed group of subjects (i.e. healthy and type 2 diabetic subjects) did three glucose (reference food) and five foods (i.e. Rice, Barley, Spaghetti, Instant potato and White bread) determinations using the MPQIDG, EL, and the weighted mean GI values obtained when a group of healthy subjects from five experienced capillary GI testing centres in the inter-laboratory study used laboratory equipment to test the same foods intra-laboratory.[39]**

- When the mean GI-values of all five foods of each laboratory were compared to the weighted means of the rest of the laboratories, including EL, there was no significant difference for Laboratories A and B (p>0.05). There was a significant difference for one of the foods for Laboratories D (p<0.0001), E (p=0.012) and EL (p=0.007), respectively. However, three of the foods for Laboratory C showed a significant difference from the weighted means of the rest of the laboratories (p=0.041, p=0.019 and p<0.0001).

  It can be concluded that, when comparing the mean GI values of all five foods of each laboratory to the weighted means of the rest of the laboratories, EL showed similar variability to Laboratories D and E and less variability than Laboratory C.

- When the mean GI values were compared using ANOVA, the laboratories that differed significantly from more than one laboratory for a specific food was laboratory C (three foods) and laboratories A, D, E and EL (one food each). Laboratory B did not differ significantly from more than one laboratory for any food.

  It can be concluded that, when comparing the mean GI values of all five foods of each laboratory using ANOVA, EL showed similar variability to Laboratories A, D and E and less variability than Laboratory C, which confirms the findings mentioned above.

4.3.5. **The standard error (SE), 95% CI limits and deviation from group mean GI value of each of the five foods [as determined by each of the five capillary laboratories in an inter-laboratory study using healthy subjects and laboratory equipment, as well as by the investigator in the current EL study, using a mixed group of subjects (i.e. healthy, type 1 and type 2 diabetic subjects) and the MPQIDG], would be less or equal to the weighted means of the rest of the laboratories, for these parameters.**

Comparing the SE, 95% CI limits and mean GI-values of each laboratory with the weighted means of the rest of the laboratories, for these parameters, the results showed:
- only Laboratory D showed smaller SE values than the weighted means of the rest of the laboratories for more foods than EL;
- only laboratory D had 95% CI limits that was smaller than the weighted means of the rest of the laboratories for more foods than EL;

- laboratories A, B, D and E showed similar deviations from the weighted mean GI values of the rest of the laboratories to EL.

It can therefore be concluded that, when considering the SE, EL showed the least variability, together with laboratory D; when considering the 95% CI limits, only laboratory D showed less variability than EL; when considering the deviation from the weighted group mean GI value, only laboratory A showed less variability than EL and when considering mean GI values, only laboratories A and B showed less variability than EL.

4.4 Limitations of the study

4.4.1   The YSI can analyze whole blood or plasma.[84] However, in this study whole blood was used for the YSI, which yields results that are 10–15% lower than that of plasma.[83,84] The investigator only discovered from the literature, after Part 1 of the Study had been completed, that the MPQIDG, like many other glucose metres, yield plasma results, even although whole blood is used.[37] The one, however, can be calculated from the other, if the packed cell volume is known.[21] Although this should not have affected the correlation between the actual blood glucose readings obtained on the two instruments, or the GI, as the GI is an index, it might have affected the AUC values. Velangi et al (2005) found that the mean AUC obtained on the glucose meter (OTU) was about 10% greater than the mean AUC of the YSI due to the fact that the plasma readings obtained using glucometres that determine plasma values are usually about 10–15% lower than the whole blood glucose readings.[34] If the investigator had been aware of this fact, the packed cell volume could have been determined, so that results from plasma obtained from the MPQIDG could have been compared with the results of plasma, as calculated from the whole blood samples used for the YSI. This should preferably be done in all future comparisons between whole blood or plasma readings, obtained from different instruments that are used for glucose analysis.

4.4.2   The results of Part 1 showed that there was no significant difference between the mean GI values of two foods (Muesli and Apple juice), when determined in the mentioned four ways. However, this finding should be interpreted with caution and cannot be generalized from the findings of Part 1 of the study, as only two foods were tested. This could be regarded as a limitation of Part 1.

5. CONCLUSION AND RECOMMENDATIONS

GI tests are usually conducted IL, using a conventional group of subjects, i.e. either healthy or type 1 or type 2 diabetic subjects and laboratory equipment, such as the YSI. In some GI testing centres, subjects have to eat a standard dinner in the laboratory and sleep in beds provided in the laboratory. In other centres subjects can eat a standard dinner at home and sleep in their own beds the night before a GI test is conducted. They then go to the laboratory for the GI test on the morning of the test. It was proposed in this study that in EL testing, the subjects eat a standard dinner at home and sleep in their own beds the night before a GI test is conducted and then conduct the GI test in their natural (home) environment, using the MPQIDG.

The results of Part 1 of the study showed that:

- there was a good and significant correlation (p≤0.05) between the absolute blood glucose readings, as obtained on the YSI and MPQIDG (IL), at all time intervals (after removal of outliers), except for at 0min (all but one product), at 15min (two of the five products), at 90min (Muesli and Apple juice) and at 105min (Apple juice). There was a very good *(r=0.9; p<0.0001)* correlation and good agreement (r_c=0.8) between all the absolute blood glucose readings at all time points, as obtained on the YSI and MPQIDG, after removal of outliers. The means of all these values differed significantly (p=0.0000), where the absolute blood glucose readings, obtained using the YSI were consistently lower than the readings obtained using the MPQIDG. This could possibly be due to the fact that the new test strips of the MPQIDG were calibrated to yield plasma results as determined by the YSI [36,37], whereas whole capillary blood was used on the YSI in this study. It is well known that capillary plasma, as measured by YSI, yields blood glucose readings that are 10-15% higher than readings obtained using whole capillaryblood.[84] However, the packed cell volume must be known in order to calculate the one from the other.[21] However, the GI values should not have been affected by the fact that the YSI yields lower blood glucose readings than the MPQIDG, as the GI is a calculated index value (refer to 3.5.7);
- apart from an acceptable and significant correlation (p≤0.05) and an acceptable agreement between the AUC values of Glucose 1, using the two instruments and a good agreement between the AUC values of Glucose 2, there was a very good and highly significant correlation (r=0.9; p<0.0001) between the AUC values for Glucose 2 and 3, M and A and very good agreement (r_c=0.9) between the AUC values of Glucose 3, Muesli and Apple juice, after removal of outliers. When the mean AUC values were considered, the mean AUC readings as measured by MPQIDG were always higher than that measured by YSI, before and after removal of outliers, though not statistically significant for any of the food products (p>0.2);
- the AUC_{min} and AUC_0 methods of calculating the AUC seem inappropriate to calculate the AUC for GI tests, as it mostly differs significantly (p≤0.05) from the GI values obtained when using the IAUC method for calculating GI values, as recommended by the FAO/WHO [3];
- GI values obtained EL (using a mixed group of subjects and the MPQIDG) seemed not to yield GI values that were significantly different to the GI values obtained IL (using a conventional group of subjects and the YSI). Results obtained EL showed less variability than those obtained IL in Part 1 of the study, regarding one of the foods that was tested, i.e. Muesli (SE was lower). EL testing (MPQIDG in a mixed group of subjects) consistently showed less within-subject variability (CV of repeated tests of the reference foods) of blood glucose responses for both Muesli and Apple juice, when compared to IL testing (YSI in a group of healthy subjects), although not statistically significant.

Although the results of Part 1 showed that there was no significant difference between the mean GI values of two foods (Muesli and Apple juice), when determined in the mentioned four ways, this finding should be interpreted with caution and cannot be generalized from the findings of Part 1 of the study, as only two

foods were tested. However, the findings of Part 1 of the study could be valued in the context of the findings of Part 2 of the study.

The results of Part 2 of the study showed that:

- when the mean GI values of all five foods of each laboratory were compared to the weighted means of the rest of the laboratories, EL showed similar variability to laboratories D and E and less variability than laboratory C. This was confirmed when the mean GI values of all five foods of each laboratory were compared using ANOVA;
- when considering the SE, only laboratory D showed less variability than EL; when considering 95%CI limits, only laboratory D showed less variability than EL; when considering deviation from group mean GI values, laboratories D and E showed similar deviations from the weighted mean GI values to EL and when considering mean GI values, only laboratory A and B showed less variability than EL;
- EL consistently showed less variability than one of the experienced, capillary GI testing laboratories that took part in an inter-laboratory study [39], regarding mean GI values, SE, 95% CI limits and deviation from the group weighted mean GI values. EL testing showed less within-subject variability (CV of repeated tests of the reference food) of glycemic responses than the rest of the laboratories (who also partook in the inter-laboratory study).[39]

The reason results obtained EL showed less variability in GI testing can probably be attributed to the following:

- a reduction in the effect of stress on GI testing by having the subjects conducting the GI tests in their natural (home) environment. **Further and specific reseach is needed to confirm this**;
- consistency in controlling for lifestyle-confounding factors; in a way that did not increase stress levels in the subjects. **Further and specific reseach is needed to confirm this**;
- using a group of mixed subjects.Well-controlled diabetics can decrease variability through the effect of the treatment;
- using trained, experienced test subjects.
- Using a mixed group of subjects and the MPQIDG to conduct GI-tests EL therefore seems to be an acceptable alternative to using a conventional group of subjects, IL. However, well-controlled diabetics can decrease variability through the effect of the treatment. The power of this influence ought to be detemined. It was not possible to determine it in this study due to the small number of diabetics used in the test group (n=5).

The fundamental question remains whether diabetics should form part of a test group as their glucose metabolism is under control of hypoglycemic agents. Type 2 diabetic subjects (with or without insulin) have the lowest CV of the reference food of all test subjects.[56] **More and specific research in this regard is warranted.**

The investigator wishes to recommend the following:

- that for all GI calculations the IAUC method ought to be used, as is also suggested by the Expert consultation on carbohydrates [3] and the international committee for the standardization of GI testing methodology.[21] Other methods of calculating AUC seem to yield significantly different GI values, especially AUC_0;
- that all the recommendations made by the international committee for the standardization of GI testing methodology [21] be heeded, except for the following considerations:
  instrument used for glucose analysis: the results of this study show that the MPQIDG seems to be an acceptable alternative to the YSI for GI testing. However, the OTU did not show acceptable agreement regarding AUC and GI values [34], and therefore the performance of glucose metres should be evaluated specifically, before being used for GI tests;

type of subjects used for GI tests: The results from this study showed that using a mixed group of subjects (healthy and type 2 diabetic subjects)(EL) seemed to be an acceptable alternative to using a conventional group of subjects (IL). Type 2 diabetics (with or without insulin) have the lowest CV of the reference food of all test subjects. However the fundamental question remains whether diabetics should form part of a test group as their glucose metabolism is under control of hypoglycemic agents;

- subjects should be trained in using the MPQIDG (as outlined in 2.4.1.2);
- subjects should be informed about lifestyle-confounding factors and the control thereof, in a way that will not increase their stress levels (as outlined in 4.2.3);
- EL testing for GI tests should be explored further, as it is a more cost-effective way of doing GI tests and it is the way food is usually consumed in real life.

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Addendum 1

Informed Consent

Title of the Study: A Comparison of the Glycemic Index (GI) results obtained from two techniques, using a group of healthy and a group of mixed subjects.

Patient Consent Form (Part 1)

Research Study

I, _____ willingly agree to participate in this study which has been explained to me by **Elizabeth Delport.** The Department of Human Nutrition, University of Pretoria, is conducting this research study.

Purpose of the study

It has been explained to you that you have the privilege of being a healthy human being, which qualifies you to be invited to participate in this research study. This study involves the consumption of a glucose solution on three separate occasions, a fruit juice on one and a cereal on another separate occasion in a laboratory, in order to investigate the effect these products have on blood glucose levels and calculated Glycemic Index (GI) of a group of 12 healthy subjects, when analyzed with laboratory equipment, i.e. the YSI Analyzer (YSI) and the Medisense Precision QID glucometre (MPQIDG). As you are probably aware, the effect of these foods on the blood glucose and calculated GI of a mixed group of subjects, when tested with the MPQIDG, has already been determined EL. Those of you, who did not partake in the original EL tests, will be asked to test these two foods EL as well. This study wishes to investigate, whether the mean GI values of two foods, when determined IL by using YSI and MPQIDG, will be similar to those obtained using the MPQIDG, EL.

Description of Procedures

You should understand that this study involves research. Eligibility criteria are standards used to assure that patients who enter this are medically appropriate candidates for this therapy and that they have the carefully defined medical condition as outlined by the study protocol. For your own well being, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions be made to these criteria for admission to the study.

This study involves oral treatment over a period of 15 minutes with 50g glucose powder dissolved in water, on three separate occasions, $\pm$ 500mL fruit juice on one occasion and a cereal mixed with 250ml water on another occasion in a laboratory. The consumption of these five products will be spread out over a period of 5 weeks to two months, depending on all the subjects' schedules and will usually be done on Tuesdays or Saturdays. Before consumption of the products, you will obtain a FBG value for the YSI, as well as a second one, as a control, using a lancet to prick your finger. Another fasting value will be obtained by you, using the MPQIDG, a well as a second one, as a control. Another two blood glucose values will be obtained (one for the YSI and another using MPQIDG) every 15 min., until the last two values fall on or below the fasting values, as obtained by the MPQIDG. No test will continue for longer than 2h.

For the nights before these mornings on which the GI tests are going to be conducted, you will be given three standard recipes, of which you are expected to prepare one meal and have the prescribed portion for your supper. This meal should be consumed before 20h00 on the night before a test. You may take a small snack, such as one fruit or one biscuit and 1 cup of tea or coffee, with milk and a standard amount of sugar, if desired. However, this is optional. Please decide beforehand what you would like to do and keep to your decision throughout the duration of the study. Nothing should be consumed after 22h00, except water. The laboratory GI tests will start at 7h55 am sharp on the mornings agreed upon beforehand. Please try to be at the laboratory at 7h30 am. Please try to start all EL GI tests at approximately the same time on the mornings that the GI tests will be conducted. Do not consume anything on the morning of the test, except for water.

Risks and Discomforts

The consumption of the glucose solution used in this program may cause all, some or none of the side effects listed. (The fruit juice and cereal should not cause any side effects). In addition there is always the risk of very uncommon or previously unknown side effects occurring. **Side effects and/or complications of therapy:** dizziness, trembling, shakiness, weakness, nervousness, hunger, perspiring freely, rapid heartbeat, fatigue or nausea and/or vomiting (in extreme cases).

The medical personnel will be checking you closely to see if any of these side effects are occurring. You will be given a tub of yogurt at the end of the test to counteract any of these side effects.

Cost of GI Tests

Novo Nordisk will supply the glucometres and test strips, the glucose will be supplied by the investigator, the fruit juice possibly by Ceres Fruit Juices, the cereal by Tradecor and the yoghurt by the investigator. The blood samples will be taken by you yourself and analyzed by laboratory personnel, using YSI. The investigator will supply the solutions required for the operation of YSI. All this will be done free of charge.

Benefits

The study does not have any specific, immediate benefit for you.
However, if the results of this study confirms the hypotheses, it will have the following benefits:
1.   GI Tests will be more affordable to food companies.
2.   More GI tests will be able to be done more quickly.
3.   Mixed groups of subjects will be able to be used for GI tests.
4.   Patients will obtain better blood glucose control, since the GI of more foods will be known.
5.   Possibly a prevention of Diabetes and lifestyle diseases, if the general public follows a lower GI lower fat diet.

Alternatives

The only alternative to blood glucose determinations for GI tests, using laboratory equipment (e.g. YSI), is blood glucose determinations using a glucometre (e.g. MPQIDG). Blood glucose determinations using laboratory equipment can, however be done on either venous or capillary blood, although for international standardization of GI Tests, it has been recommended that whole capillary blood be used, since this method of doing GI tests shows fluctuations in blood glucose due to food consumption better than venous blood and is fairly non-invasive, especially when the GI's of many foods have to be determined (Wolever

et al, 1991). There are of course many glucometres available on the market, but the MPQIDG has been chosen, since it showed the smallest difference from the reference method across the entire range of mean glucose levels in a study done by Engel et al (1998). The MPQIDG with the new microflo test strip is also uniquely designed to minimize user errors (Velazquez, date unknown). Furthermore, clinically acceptable user proficiency in capillary blood glucose testing can be maintained in most subjects with recurrent intensive education (Kabadi et al, 1994).

Voluntary participation

Participation in this study is voluntary. Each participant will receive a compensation of R100.00, upon completion of the clinical part of part 1 of this study. You are free to withdraw your consent to participate in this treatment program at any time without prejudice to your subsequent care. Refusing to participate will involve no penalty or loss of benefits, except for the compensation of R100.00, which will only be given to those who complete the project. You are free to seek care from a physician of your choice at any time. If you do not take part in or withdraw from the study, you will continue to receive care. In the event that you withdraw from the study, a replacement will be sought to fill your place.

Confidentiality

A record of the forms containing your blood glucose results for the different foods that will be tested for this study, will be kept in a file in a confidential form at the rooms of the investigator and also in a computer file at said office. During their required reviews, representatives of the University or laboratory may have access to medical records, which contain your identity. However, no information by which you can be identified will be released or published.

I have read all the above, had time to ask questions, received answers concerning areas I did not understand and I willingly give my consent to participate in this program. Upon signing this form, I will receive a copy.

Subject's Signature:_____ (Date)_____

Witnesses' Signatures: **1.** _____**2.**_____(Date)___--_____

Investigator's Signature:_____ (Date)_____

Informed consent

Title of the Study: **A Comparison of the Glycemic Index (GI) results obtained from two techniques, using a group of healthy and a group of mixed subjects.**

Patient Consent Form (Part 2)

Research Study

I, _____ willingly agree to participate in this study which has been explained to me by Elizabeth Delport. The Department of Human Nutrition, University of Pretoria, is conducting this research study.

Purpose of the study

It has been explained to you that you have the privilege of being a healthy or type 2 diabetic individual, which qualifies you to be invited to participate in this research study. This study involves the consumption of a glucose solution on three separate occasions and five foods on five different occasions extra-laboratory (EL), in order to investigate the effect these products have on the blood glucose and calculated Glycemic Index (GI) of a group of 5 healthy and 5 type 2 diabetic subjects, using the Medisense Precision QID glucometre (MPQIDG). As you are probably aware, the effect of these foods on the blood glucose and MPQIDG, has already been determined IL, by groups of 8 – 12 healthy subjects of 5 international, experienced capillary laboratories, using laboratory equipment, such as the YSI Analyzer (YSI). This study wishes to investigate how the GI values of the above mentioned five foods, when calculated by using laboratory equipment to analyze capillary blood glucose values of healthy subjects, as obtained intra-laboratory (IL) by 5 experienced, international laboratories, compare with blood glucose values obtained EL by using MPQIDG and the same five foods.

Description of Procedures

You should understand that this study involves research. Eligibility criteria are standards used to assure that patients who enter this are medically appropriate candidates for this therapy and that they have the carefully defined medical condition as outlined by the study protocol. For your own well being, as well as to ensure that the results of this study can be useful for making treatment decisions regarding other patients with similar diseases, it is important that no exceptions be made to these criteria for admission to the study.

This study involves oral treatment over a period of 15 minutes with 50g glucose powder dissolved in water, on three separate occasions, and five foods on five different occasions, EL. The consumption of these eight products will be spread out over a period of 8 weeks to three months, depending on all the subjects' schedules. Before consumption of the products, a FBG value will be obtained by yourself, using the MPQIDG, as well as a second one, as a control, using as lancet to prick your finger. Another blood glucose value will be obtained using MPQIDG every 15 min., until the last two values fall on or below the fasting values, but not longer than 2h (healthy subjects) and not longer than 3h (diabetic subjects).

For the nights before the mornings on which the GI tests are going to be conducted, you will be given three standard recipes, of which you are expected to prepare one meal and have the prescribed portion for your supper. This meal should be consumed before 20h00 on the night before the tests are conducted. You may take a small snack, such as one fruit or one biscuit and 1 cup of tea or coffee, with milk and a standard amount of sugar, if desired. However, this is optional. Please decide beforehand what you would like to do and keep to your decision throughout the duration of the study. Nothing should be consumed after 22h00, except water. Please try to start all GI tests at approximately the same time on the morning of the tests. Do not consume anything on the morning of the test, except for water.

Risks and Discomforts

The consumption of the glucose solution used in this program may cause all, some or none of the side effects listed. (The five foods should not cause any side effects). In addition there is always the risk of very uncommon or previously unknown side effects occurring. Side effects and/or complications of therapy: dizziness, trembling, shakiness, weakness, nervousness, hunger, perspiring freely, rapid heartbeat, fatigue or nausea and/or vomiting (in extreme cases).

Cost of GI Tests

Novo Nordisk will supply the glucometres and test strips and the glucose will be supplied by the investigator. The test foods will be supplied to you by the investigator, upon receiving it one of the principal investigators of the inter-laboratory study (Wolever et al, 2003). The blood samples will be taken by yourself for analysis by the MPQIDG. All this will be done free of charge.

Benefits

The study does not have any specific, immediate benefit for you.
However, if the results of this study confirms the hypotheses, it will have the following benefits:
1.   GI Tests will be more affordable to food companies.
2.   More GI tests will be able to be done more quickly.
3.   Mixed groups of subjects will be able to be used for GI tests.
4.   Patients will obtain better blood glucose control, since the GI of more foods will be known.
5.   The prevention of Diabetes and lifestyle diseases, if the general public follows a lower GI lower fat diet.

Alternatives

The only alternative to blood glucose determinations for GI tests, using laboratory equipment (e.g. YSI), is blood glucose determinations using a glucometre (e.g. MPQIDG). Blood glucose determinations using laboratory equipment can, however be done on either venous or capillary blood, although for international standardization of GI Tests, it has been recommended that whole capillary blood be used, since this method of doing GI tests shows fluctuations in blood glucose due to food consumption better than venous blood and is fairly non-invasive, especially when the GI's of many foods have to be determined (Wolever et al, 1991). There are of course many glucometres available on the market, but the MPQIDG has been chosen, since it showed the smallest difference from the reference method across the entire range of mean glucose levels in a study doen by Engel et al (1998). "The Precision QID glucometre with the new microflo test strip is also uniquely designed to minimize user errors" (Velazquez, date unknown). Furthermore, "clinically acceptable user proficiency in capillary blood glucose testing can be maintained in most subjects with recurrent intensive education" (Kabadi et al, 1994).

Voluntary participation

Participation in this study is voluntary. Each participant will receive a compensation of a GI Guide e-book and booklet, upon completion of the clinical part of part 2 of this study. You are free to withdraw your consent to participate in this treatment program at any time without prejudice to your subsequent care. Refusing to participate will involve no penalty or loss of benefits. You are free to seek care from a physician of your choice at any time. If you do not take part in or withdraw from the study, you will continue to receive care. In the event that you withdraw from the study, a replacement will be sought to fill your place.

Confidentiality

A record of the forms containing your blood glucose results for the different foods that will be tested for this study, will be kept in a file in a confidential form at the rooms of the investigator and also in a computer file at said office. During their required reviews, representatives of the University or laboratory may have access to medical records, which contain your identity. However, no information by which you can be identified will be released or published.

I have read all the above, had time to ask questions, received answers concerning areas I did not understand and I willingly give my consent to participate in this program. Upon signing this form, I will receive a copy.

Subject's Signature:_____(Date)_____

Witnesses' Signatures: **1.**_____**2.**_____ _(Date)___-_____

Investigator's Signature: _____(Date)_____

Skills assessment checklist

Testing procedure:

Calibrate glucometre with calibrator of new box of strips (if applicable) and check that test strips have not yet expired (Medisense User's Guide, 2000)

Open foil package by tearing at the notches in the foil and remove one end of foil packet to expose contact bars at the end of the electrode (Medisense user's Guide, 2000).

Insert electrode into Sensor by pushing it in gently until it stops, with contact bars facing upwards, and removing foil packet from other end of electrode packet (Kabadi et al, 1994; Medisense User's Guide, 2000).

Fingerprick sampling:

Site selection (Kabadi et al, 1994).

Site cleaning (Kabadi et al, 1994) or wash hands with soap and water and dry thorougly. (Medisense User's Guide, 2000)

Obtain a blood sample by using spring-loaded device with a chisel or blade type lancet and enough force to make a good puncture or incision in the skin of the finger (Warnick et al, 1994), as well as appropriate technique (Medisense User's Guide, 2000).

Obtain adequate drop (Medisense User's Guide, 2000; Kabadi et al, 1994).

Softly touch blood to the target area while the display reads "rdy" (Medisense User's Guide, 2000)

Do not smear sample (Kabadi et al, 1994), although this seems to cause no significant difference in readings obtained, in some of the newer metres, like the MPQIDG and its new test strips (Abbott White Paper, 1997).

Blood drop covered area (Kabadi et al, 1994).

FOOD INTAKE RECORD

Name:_____   **Date of Supper:**_____

Time	Type of Food/Drink	Quantity

Addendum 5

YSI GI TEST FORM

DATE:_____   NAME:_____

STARTING TIME: _____   PRODUCT:_____

BLOOD VALUES

TWO INITIAL READINGS	( )
SECOND READING (after 15 min)	
THIRD READING (after 30 min)	
FOURTH READING (after 45 min)	
FIFTH READING (after 60 min)	
SIXTH READING (after 1h15 min)	
SEVENTH (after 1h30min)	
EIGHTH READING (after 1h45min)	
NINTH READING (after 2 hours)	
TENTH READING (after 2h15min)	
ELEVENTH (after 2h30min)	
TWELFTH (after 2h45min)	
THIRTEENTH (after 3 hours)	

MPQIDG GI TEST FORM

DATE:_____   NAME:_____

STARTING TIME: _____   PRODUCT:_____

BLOOD VALUES

TWO INITIAL READINGS	(_____)
SECOND READING (after 15 min)	
THIRD READING (after 30 min)	
FOURTH READING (after 45 min)	
FIFTH READING (after 60 min)	
SIXTH READING (after 1h15 min)	
SEVENTH (after 1h30min)	
EIGHTH READING (after 1h45min)	
NINTH READING (after 2 hours)	
TENTH READING (after 2h15min)	
ELEVENTH (after 2h30min)	
TWELFTH (after 2h45min)	
THIRTEENTH (after 3 hours)	

Control Of Lifestyle-confounding Factors: Case Scenario

Mrs A (35y old), mother of three children, is interested in becoming a test subject. She usually trains for 1h in the mornings in the gymnasium on Mondays, Wednesdays and Fridays, drinks 2 cups of regular coffee per day (one at breakfast and the other at lunch), is on 0.5 mg thyroid medication, always has curry and Rice with banana and shambals on Friday evenings, as well as a glass of wine and 2 blocks of chocolate and smokes five cigarettes per day at more or less regular times. It would therefore make sense for her to conduct a GI test every Saturday morning, as she has the same meal every Friday evening. The fact that she exercises regularly, smokes, is on medication, consumes caffeine on a regular basis and has a glass of wine on the night before a test will not affect the GI tests she conducts each Saturday morning, unless she changes her routine, e.g. does not exercise on the Friday before a GI test or decides to run a marathon on a Friday [21,38,81] and/or smokes more or less than five cigarettes on the day before a GI test and/or does not have coffee or has five cups of regular coffee on the Friday before her test and/or drinks a whole bottle of wine instead of one glass of wine on the night before a test. If any of these should happen, she should be advised to not test that Saturday or to test on another day, provided her routine is essentially the same as on Fridays. If a permanent drastic change occurs in her lifestyle, e.g. if her doctor increases her thyroid medication and/or if she decides to switch from regular to decaffeinated coffee and/or if she decides to stop smoking and/or if she cannot exercise for a while or decides to start training for the comrades and/or decides to stop drinking alcohol, she should inform the investigator thus and repeat at least one, but preferably two or three tests of the reference food. [21]

However, she should also, before she does any GI tests, be advised of the following:
- To refrain from doing GI tests during periods of hormonal imbalance, such as during menstruation or when pregnant or when she starts to go through menopause [47], unless she is on HRT, which will help to stabilize her hormones and therefore blood glucose levels.
- To refrain from doing her initial reference food tests when she is on vacation, if all her other food tests are going to be done when she works, due to the possible change in stress levels [38,40] and to refrain from doing a GI test when she has abnormal stress.
- To refrain from doing a GI test if she went to be very late or did not sleep well the night before a GI test [46].
- To decide on a standard, favourite meal and to preferably consume this meal [21], or at least one that has a similar macronutrient distribution and content, on the evening before GI tests are conducted, preferably before 8h00 pm.
- To only have a small snack e.g. 1 biscuit (if desired) with a standard number of cups of regular tea/rooibos tea or regular/decaffeinated coffee (if desired) every evening before a GI test is conducted and to have this before 22h00, after which she should try to consume only water. However, she should try to keep to the same protocol re. evening tea and snack for her reference tests and all the food tests that are done thereafter.
- To conduct all her GI tests at more or less the same time on Saturday mornings, e.g. 7h00 and not 5h00 on one occasion and 9h00 on another occasion, since there is a significant positive correlation (p=0.02) between time of day and AUC [38]. This will also automatically control the fasting time [21], without causing unncessary stress in the subject.
- To not skip breakfast or lunch on Fridays, to have more or less the same type of breakfast and lunch every Friday, or at least a meal of the same macronutrient distribution and content and to preferably have breakfast, lunch and supper on most days, due to the effect on GI tests of CHO restriction during the days before a GI test [23].
- To not conduct GI tests when ill, e.g. influenza, a cold, toothache or another infection [5,48].

- To not conduct GI tests when she is consuming medication or supplements temporarily [23]. However, medication (like diabetic medication, thyroid medication and the like or vitamin supplements), which are consumed regularly, should be consumed at the same time, i.e. before or after GI tests for all tests of the reference food and test foods [2,23,38].
- To refrain from conducting a GI tests within three days of having obtained a vitamin injection, as the latter also seems to have an effect on FBG readings, until such time as this has been specifically researched and found to not have an effect on day-to-day variation in blood glucose readings.