EVALUATION OF THE METHODOLOGY FOR DETERMINING THE GLYCAEMIC INDEX OF FOODS WITH SPECIAL REFERENCE TO BLOOD SAMPLING

Y. VAN HEERDEN (B. Dietetics.)

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Supervisor: Prof. C.S. Venter
Co-supervisor: Prof. J.C. Jerling

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To my husband and parents

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ABSTRACT

Background, motivation and objectives

Different types of carbohydrates from different food sources affect blood glucose differently. This physiological effect of carbohydrate containing foods has been quantified and expressed as the glycaemic index (GI) of the food. The GI is defined as the ratio of the incremental area under the blood glucose response curve for a test food containing 50g available carbohydrate to the corresponding area after an equal carbohydrate portion of a standard food is taken by the same subject. The GI of a food can, therefore, be used to guide consumers in choosing a particular food with a predicted known effect on blood glucose levels and homeostasis.

Numerous methodological factors may influence the interpretation of glycaemic response data. One of the major problems regarding labeling foods with GI values is the lack of standardised methodology amongst different researchers in determining the GI. Furthermore, clear directions are needed regarding standardised methodology in accredited laboratories, including clarity on issues such as the reference (standard), total ("available") carbohydrate of the test food, number and characteristics of experimental subjects, capillary versus venous blood samples, analytical method for determination of blood glucose value and the method for calculation of the area under the glucose curve.

A food company commissioned an independent assessment of the GIs of Jungle Oats, Bokomo Oats and Oatso-Easy using methods complying with the most recent internationally accepted methodology and carried out under strictly standardised

conditions. Thus, the area under the curve (AUC) and GI of Jungle Oats, Bokomo Oats and Oatso-Easy was determined using both capillary whole blood and venous plasma. Another objective of the study was to determine if there were significant differences between the GI of the three oats porridges.

Methods

Twenty healthy, non-smoking fasting male students, aged 21-27 years, each consumed 50g available carbohydrate from Jungle Oats, Bokomo Oats, Oatso-Easy and the standard food (glucose) on four different occasions. Finger-prick capillary blood and venous whole blood were collected simultaneously before and at 15 and 30 minute intervals for the first and second hour after ingestion respectively. The capillary whole blood glucose values were determined by using SureStep test strips and SureStep glucometres (Lifescan) and the venous plasma glucose was determined with an enzymatic colorimetric method. The AUC and the GI for the three different oats porridges, taken at four different occasions randomly by the same subjects was calculated using one glucose response as standard.

Results

Statistically significant differences ($p<0.05$) were found between the AUCs of the three different oats porridges for capillary blood and venous plasma. However, no statistically significant differences ($p>0.05$) were found between the mean GIs of the three different oats porridges both for capillary blood and venous plasma (77.1, 67.7 and 78.0 for Oatso-Easy, Jungle Oats and Bokomo Oats, respectively using capillary sampling and 112.4, 112.4 and 113.8 respectively, using venous sampling). The 95%

confidence interval (CI) and standard deviation (SD) of the mean capillary blood glucose were notably smaller than those of the venous plasma.

Conclusions

It can be concluded from the study that the three different oats porridges fell between the intermediate to high categories and that glycaemic responses measured in venous plasma are lower and more variable than those simultaneously obtained in capillary blood.

Recommendations

It is recommended that the methodological guidelines determined by the GI Task Force should be followed. Capillary blood glucose samples are preferred to determine the GI. The last recommendation is that in using the GI to choose carbohydrate foods, patients and consumers should be made aware of the fact that physiological responses to a food may vary between individuals. For example, when advising on the GI, it should be mentioned that the GI of a particular food is usually low, medium or high, but that exceptions can be expected and that these exceptions are normal. Therefore, the label indicating the GI of foods, food products and beverages should be accompanied by clear instructions.

OPSOMMING

Agtergrond, motivering en doelstellings

Verskillende tipes koolhidrate van verskillende voedselbronne beïnvloed bloedglukose response verskillend. Hierdie fisiologiese effek van koolhidraatbevattende voedsels is gekwantifiseer en uitgedruk as die glukemiese indeks (GI) van die voedsel. Die GI word gedefinieer as die verhouding van die inkrementele area onder die bloedglukoseresponskurwe vir 'n toetsvoedsel wat 50g beskikbare koolhidrate bevat tot die ooreenstemmende area nadat dieselfde koolhidraatporsie van die standaard-voedsel ingeneem is deur dieselfde persoon. Die GI van voedsel kan dus gebruik word om verbruikers te lei in die keuse van 'n spesifieke voedsel met 'n voorspelbare effek op bloedglukosevlakke en homeostase.

Verskeie metodologiese faktore mag die interpretasie van die glukemiese respons-data beïnvloed. Een van die belangrikste probleme betreffende die etikettering van voedsel met GI-waardes is die tekort aan gestandaardiseerde metodologie tussen verskillende navorsers in die bepaling van die GI. Verder is duidelike leiding nodig betreffende gestandaardiseerde metodologie in geakkrediteerde laboratoriums, insluitend duidelikheid oor twispunte soos die verwysing (standaard), totale ("beskikbare") koolhidrate van die toetsmaaltyd, getal en eienskappe van die eksperimentele proefpersone, kapillêre teenoor veneuse bloedmonsters, analitiese metode vir die bepaling van die bloedglukosewaarde en die metode vir die berekening van die area onder die glukosekurwe.

'n Voedselmaatskappy het opdrag gegee dat 'n onafhanklike bepaling van die GIs van Jungle Hawermout, Bokomo Hawermout en Oatso-Easy gedoen word, waar metodes gebruik word wat ooreenstem met die mees onlangse internasionale aanvaarbare metodologie en wat uitgevoer word onder streng gestandaardiseerde kondisies. Dus, die area onder die kromme (AUC) en GI van Jungle Hawermout, Bokomo Hawermout en Oatso-Easy is bepaal deur beide kapillêre volbloed en veneuse plasma te gebruik. 'n Ander doelstelling van die studie was om te bepaal of daar enige betekenisvolle verskille tussen die GI van die drie hawermoutpappe was.

Metodes

'n Groep van twintig gesonde, nie-rokende vastende manlike studente, 21-27 jaar oud, het elk 50g beskikbare koolhidrate van Jungle Hawermout, Bokomo Hawermout, Oatso-Easy en die standaardvoedsel (glukose) ingeneem op vier verskillende geleenthede. Vingerprik kapillêre bloed en veneuse volbloed is gelyktydig versamel voor en met 15 en 30 minuutintervalle vir die eerste en tweede uur na inname respektiewelik. Die kapillêre volbloedglukosewaardes is bepaal deur gebruik te maak van SureStep toetsstrokies en SureStep glukosemeters (Lifescan) en die veneuse plasmaglukose is bepaal met 'n ensiematiese kolorimetriese metode. Die AUC en die GI vir die drie verskillende hawermoutpappe wat ewekansig ingeneem is by vier verskillende geleenthede deur dieselfde proefpersone, is bereken deur die glukoserespons as standaard te gebruik.

Resultate

Statisties betekenisvolle verskille ($p<0.05$) is gevind tussen die AUCs van die drie verskillende hawermoutpappe vir kapillêre bloed en veneuse plasma. Geen statisties betekenisvolle verskille ($p>0.05$) is egter gevind tussen die gemiddelde GIs van die drie verskillende hawermoutpappe beide vir kapillêre bloed en veneuse plasma (77.1, 67.7 en 78.0 vir Oatso-Easy, Jungle Hawermout en Bokomo Hawermout, respektiewelik waar kapillêre bloed gebruik is en 112.4, 112.4 en 113.8 respektiewelik, waar veneuse monsters gebruik is). Die 95% vertrouensinterval (CI) en standaardafwyking (SD) van die gemiddelde kapillêre bloedglukose was aansienlik kleiner as die van die veneuse plasma.

Gevolgtrekkings

Die gevolgtrekking kan uit die studie gemaak word dat die GI van die drie verskillende hawermoutpappe tussen die intermediêre tot hoë kategorieë val en dat die glukemiese respons gemeet in veneuse plasma laer is en meer veranderlik is as die wat terselfdertyd in kapillêre bloed verkry is.

Aanbevelings

Dit word aanbeveel dat die metodologiese riglyne opgestel deur die GI Werkgroep gevolg moet word. Kapillêre bloedglukosemonsters word verkies om die GI te bepaal. Die laaste aanbeveling is dat in die gebruik van die GI om koolhidraatvoedsels te kies, pasïente en verbruikers bewus gemaak moet word word van die feit dat fisiologiese response tot 'n voedsel mag varieer tussen individue. Byvoorbeeld,

wanneer advies gegee word oor die GI, moet dit genoem word dat die GI van 'n spesifieke voedsel gewoonlik laag, medium of hoog is, maar dat uitsonderings verwag kan word en dat hierdie uitsonderings normaal is. Daarom moet die etiket wat die GI van 'n voedsel, voedselprodukte en drankies aandui vergesel wees van duidelike instruksies.

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%	Percentage
&	And
β	Beta
<	Less than
>	Greater than
≤	Less and equal than
=	Equal
°C	Degrees Celsius
10-12 hr	Ten to twelve hours
ADA	American Diabetes Association
AUC	Area under the curve
AUC_{min}	Area under the curve (minimum as baseline)
BMI	Body mass index
CB	Capillary blood
CI	Confidence interval
CV	Coefficient of variation
et al	Et alii
DF	Dietary fibre
EDTA	Ethylenediamine tetra acetic acid
FAO/WHO	Food and Agricultural Organization / World Health Organization
g	Gram
GI	Glycaemic index
GIP	Gastric inhibitory polypeptide
GL	Glycaemic load
GTTs	Glucose tolerance tests
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
kg	Kilogram
kg/m^2	Kilogram per square meter
kJ	Kilojoule
LBV	Lower value of the box plot
Ltd	Limited
min	Minute
mL	Millilitre
mmol/L	Millimole per litre
mmol/L/min	Millimole per litre per minute
N	Number
NIDDM	Non-insulin dependent diabetes mellitus
o.c.	Outlier coefficient which is equal to 1.5
PCV	Packed cell volume
PU vir CHO	Potchefstroomse Universiteit vir Christelike Hoër Onderwys
RS	Resistant starch
RST	Reagent strip test
UBV	Upper value of the box plot
SD	Standard deviation
T	Time
UK	United Kingdom
VB	Venous blood
VP	Venous plasma

CHAPTER 1

INTRODUCTION

1.1 Introduction

Different carbohydrate containing foods have different effects on blood glucose responses (Jenkins *et al.,* 1981; Wolever, 1990). Since the early 1980's scientists studied the effects of different carbohydrate foods and their effects on healthy as well as diabetic people. Blood glucose levels were measured at frequent intervals for up to three hours after food was given in a meal. The glycaemic index concept was introduced in 1981 (Jenkins *et al.,* 1981). This index is a ranking of foods which indicate a food's potential to raise blood glucose concentrations, relative to a standard (glucose or white bread) (Jenkins *et al.,* 1981). The glycaemic index (GI) is defined as the incremental area under the curve for the increase in blood glucose after the ingestion of 50g of glycaemic carbohydrate of a test food (unless the total volume exceeds 300ml when 25g of glycaemic carbohydrate from the test food and reference food will be acceptable) in the 2-hour for healthy and 3-hour for diabetic individuals post ingestion period as compared with ingestion of the same amount of glycaemic carbohydrate from glucose taken with 300ml of water spread over a 10 minute period, tested according to a defined procedure by an accredited laboratory in the same individuals under the same conditions using the fasting blood glucose concentrations as a baseline (GI Task Force, 2002).

There is controversy regarding the clinical utility of classifying foods according to their glycaemic responses by using the GI (Bessenen, 2001). Part of the controversy is due to methodological variables that can markedly affect the interpretation of glycaemic responses and the GI values obtained (Wolever, 1990). Variables that

affect the GI value include food-portion size, the method of blood sampling and subject characteristics (Wolever *et al.,* 1991). A task force was appointed in 2002 by the Directorate of Food Control to standardise the procedure for determining the GI in South Africa, as there is currently no international standard besides the method described by the FAO/WHO (1998).

Food portion size has a major effect on the GI value because glycaemic responses are related to the carbohydrate load. Therefore, the 50g carbohydrate portion should not include carbohydrates, such as dietary fibre (DF) and resistant starch (RS) that are not absorbed in the small intestine (GI Task Force, 2002; Wolever, 2003). The FAO/WHO (1998) recommends the use of 50g available carbohydrate for GI testing except when the volume of a low carbohydrate food indicates a smaller load such as a 25g carbohydrate portion.

Glucose as standard food (in determining the GI of foods) has been suggested in South Africa for labeling purposes (GI Task Force, 2002). According to Wolever *et al.* (2003) glucose is a more logical and easily standardised reference food for international use.

The mean area under the curve (AUC) of three trials of the reference food should be used to calculate the GI (WHO/FAO, 1998), because the mean of three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial. Several methods have been used to calculate the area under the glycaemic response curve (FAO/WHO, 1998; Wolever *et al.,* 1991). According to Wolever (2003), the GI is based on the incremental area below the curve and above the fasting level only.

It has recently been confirmed by Wolever *et al.* (2003) that glycaemic responses measured in venous plasma are lower and more variable than capillary blood and that capillary blood allows more accurate determination of the GI as long as capillary blood samples are not contaminated with interstitial fluid due to "milking" of the finger (Wolever, 2003). Current recommendations are, therefore, that capillary blood sampling is preferred for determining the GI (GI Task Force, 2002; Wolever *et al.,* 2003) but venous blood sampling is also acceptable (FAO/WHO, 1998).

Traditionally, researchers included six to eight subjects in studies designed to determine the GI of foods. Based on observations from these relatively small numbers of experimental subjects, Wolever (2003) concluded that GI values are not significantly affected by subject variables such as age (Wolever *et al.*, 1988), ethnicity (Wolever *et al.*, 2003), glucose tolerance status (Jenkins *et al.*, 1983) or presence of type 1 or type 2 diabetes (Wolever *et al.*, 1987) and that variation in GI values in different subjects is, therefore, due to within-subject variation (Wolever, 2003). However, Nell (2001) indicated that if a 10% range for a GI of a food is sought with 80% confidence, between 24 and 90 subjects should be included in a study using venous plasma samples. The GI Task Force (2002) suggested a minimum of 20 subjects for GI testing.

Low GI foods improve overall blood glucose control in people with type 2 diabetes (Brand *et al.,* 1991; Wolever *et al.,* 1992), reduce serum lipids in people with hypertriglyceridaemia (Jenkins *et al.,* 1987) and improve insulin sensitivity (Frost *et al.,* 1998; Riccardi & Rivellesse, 2000). In addition, low GI foods are associated with high concentrations of high-density lipoprotein (HDL) cholesterol (Frost *et al.,* 1999) and reduced risk for the development of type 2 diabetes and cardiovascular disease

(Frost *et al.,* 1998; Salmerón *et al.,* 1997a). When low glycaemic carbohydrates are incorporated into an energy-deficient diet, there is a greater fall in insulin resistance that can be accounted for weight loss alone (Slabber *et al.,* 1994).

These effects prompted the Joint FAO/WHO expert consultation "Carbohydrates in Human Nutrition" (1998) and more recently Riccardi & Rivellese (2000) to endorse the usefulness of the GI in diet planning. However, according to Pi-Sunyer (2002) there are many uncertainties regarding the validity of the GI for determining what foods are "good" and "bad" for one's health. Much more definitive data from controlled clinical trails are needed before any such dietary recommendations are made (Pi-Sunyer, 2002).

Requirements for claims regarding the GI value of carbohydrate-rich foods are included in a new concept regulation regarding food packaging in South Africa (Foodstuffs, Cosmetics and Disinfectants Act, 54/1972). Although it is still a draft regulation, it has been mentioned that according to research in South Africa as well as internationally, the GI concept seems to be acceptable and useful in South Africa (Venter *et al.,* 2003). GI values are generally reproducible from country to country, but in some instances there are variations due to inherent botanical differences. Therefore, our laboratory was commissioned by a food company to determine the GI values of three South African oats porridges.

1.2 Objectives of this study

The objectives of this study were to determine the GI of Jungle Oats, Bokomo Oats and Oatso-Easy using both capillary and venous sampling and to determine whether there were significant differences between the GIs of the products mentioned.

1.3 Structure of the mini-dissertation

The mini-dissertation is divided into five chapters. A short discussion outlines the structure and contents of each chapter.

The first chapter summarizes the methodological issues regarding the determination of the GI and the objectives for the study. The structure of the mini-dissertation is then outlined.

The second chapter of the mini-dissertation consists of a review of the relevant literature. Methodological issues are discussed namely, 1) food portion size, 2) standard (reference) food, 3) repeated testing of the standard food, 4) time of blood sampling, 5) method of area calculation, 6) method of blood sampling, with special reference to capillary blood glucose versus venous plasma glucose and 7) subject characteristics. Finally, the clinical applications of the GI are reviewed and the conclusion is made that the GI concept seems to be acceptable and useful in South Africa.

In Chapter 3 the method of the study is presented according to the most recent laboratory guidelines based on the results of international studies and the recommendations of the South African GI Task Force (2002).

In Chapter 4 the results are presented using both capillary blood glucose and venous plasma glucose to determine the area under the curve (AUC) and the GI of the three different oats porridges.

In Chapter 5 the results of the study are discussed, conclusions are drawn and recommendations for blood sampling and GI food labeling are made.

CHAPTER 2

LITERATURE SURVEY: METHODOLOGY AND CLINICAL UTILITY OF THE GLYCAEMIC INDEX

2.1 INTRODUCTION

Carbohydrates with different physical forms, chemical structures, particle sizes and fibre contents induce distinct plasma and glucose responses (Nell, 2001). The systematic classification of foods according to their glycaemic responses was first undertaken by Otto and Niklas in 1980 (Wolever *et al.,* 1991). One year later, Jenkins and co-workers independently developed the concept known as the GI (Jenkins *et al.,* 1981).

The GI is defined as the ratio of the incremental area under the blood glucose response curve for a test food containing 50g available carbohydrate to the corresponding area after an equicarbohydrate portion of a standard food is taken by the same subject (FAO/WHO, 1998; Wolever, 1990).

It is well known that numerous methodological factors may influence the interpretation of glycaemic response data (Wolever *et al.*, 1991). These issues have been reviewed in detail by Wolever *et al.* (1991). According to Venter *et al.* (2003), one of the major problems regarding labeling foods with GI values is the lack of standardised methodology amongst different researchers in determining the GI. Furthermore, clear directions are needed regarding standardised methodology in accredited laboratories, including clarity on issues such as the reference (standard), total ("available") carbohydrate of the test food, number and characteristics of experimental subjects, capillary versus venous blood samples, analytical method for

determination of blood glucose value and the method for calculation of the area under the glucose curve. A task force was appointed in 2002 by the Directorate of Food Control to standardise the procedure for determining the GI in South Africa, as there is currently no international standard besides the method described by the FAO/WHO (1998). The report of the Task Force will be incorporated in this discussion.

2.2 METHODOLOGICAL ISSUES

Food portion size

Food portion size has a major effect on the GI value because glycaemic responses are related to the carbohydrate load. The GI is an index of the blood glucose raising potential of the absorbable or "glycaemic" or "available" carbohydrate in foods. According to McCance and Lawrence (1929), not all carbohydrates could be utilized and metabolized, therefore, they divided carbohydrates into available (starch and soluble sugars) carbohydrate and unavailable (hemicellulose and cellulose) carbohydrate. However, it is misleading to think of carbohydrate as "unaivalable" because some indigestible carbohydrate is able to provide the body with energy through fermentation. Currently a more appropriate substitute for the terms "available" and "unavailable" would be to describe carbohydrates either as glycaemic (providing carbohydrate for metabolism) or non-glycaemic (FAO/WHO, 1998). Therefore, the 50g carbohydrate portion should not include carbohydrates, such as DF and RS that are not absorbed in the small intestine (GI Task Force, 2002; Wolever, 2003). In practice, however, this is difficult because the analytical method for determination of RS is not widely available and the RS content of most foods is, therefore, unknown. The term RS refers to the sum of starch degradation products that pass into the large intestine, which makes the distinction between starch that is hydrolyzed and the products absorbed in the human small intestine and starch that

reaches the human large intestine either intact or partly hydrolyzed (Englyst *et al.,* 1999). The FAO/WHO (1998) recommends the use of 50g available carbohydrate for GI testing except when the volume of a low carbohydrate food indicates a smaller load such as a 25g carbohydrate portion. Wolever and Bolognesi (1996) fed four different foods at levels 25, 50 and 100g to normal subjects. Although the absolute glycaemic responses differed for the different levels of carbohydrate, the glycaemic responses of foods, relative to that of the reference food containing the same amount of carbohydrate, did not differ significantly. This suggests that the relative glycaemic responses of foods are the same at any level of carbohydrate. However, a larger dose of carbohydrate is preferred for GI testing because the variability of the results obtained increases as the portion size decreases (Wolever & Bolognesi, 1996).

Standard (reference) food

Originally glucose was used as the standard food to determine the GI and was arbitrarily assigned a value of 100 (Wolever *et al.*, 1991). Vorster *et al.* (1990) regarded glucose as the ideal standard. However, subjects may experience the sweetness of glucose as nauseating and the high osmotic load may cause delayed gastric emptying which may effect the results. If glucose is used as standard, it should be purchased in bulk and selected from the same batch. Fifty grams of glucose powder should be weighed in separate portions and dissolved in 200-250mL water (FAO/WHO, 1998).

White bread was later regarded as a more physiologically standard (Wolever, 1990). Almost all starchy food contains some fat and protein (Nell, 2001). Thus, bread stimulates more insulin relative to the blood glucose response than does glucose (Wolever *et al.*, 1991). Fat delays gastric emptying and small intestinal motility. If

bread is used as standard food, each sample should provide 50g available carbohydrate as determined by food composition tables. All bread should come from the same batch and supplier and bread crusts must be removed due to the influence of the Maillard reaction. White bread ingested on different days as standard food should be frozen and thawed according to methods prescribed for test foods to ensure uniformity (Venter *et al.,* 2003).

Wolever *et al.* (1996) proved that results from studies with different standard foods may be compared if adjusted proportionally. Glucose-based values are multiplied by 1.38 to convert them to bread-based values since the glycaemic response of glucose is, on average, 38% greater than that of bread. In order to compare the results of studies where different standards have been used, the standard food should be noted (Nell, 2001). Glucose as standard food (in determining the GI of foods) has been suggested in South Africa for labelling purposes (GI Task Force, 2002). According to Wolever *et al.* (2003), glucose is a more logical and easily standardised reference food for international use.

Repeated testing of the standard food

The mean (AUC) of three trials of the reference food should be used to calculate the GI (WHO/FAO, 1998), because the mean of three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial.

Time of blood sampling

Ideally, measurement is required until the blood glucose response returns to baseline (Wolever *et al.*, 1991). Extending measurement may tend to reduce differences in GI between foods. High GI foods usually result in high peak rises of blood glucose

followed by an undershoot of the baseline. Low GI food, on the other hand, has low peak rises and tends to maintain slightly above the baseline for a prolonged time. These tendencies occur especially in normal subjects. It is, therefore, recommended that for normal subjects, ≤ 2 hours will be sufficient, while 3 hours was chosen for diabetic individuals (Wolever *et al.,* 1991).

Method of area calculation

Several methods have been used to calculate the area under the glycaemic-response curve (FAO/WHO, 1998; Wolever *et al.,* 1991). According to Vorster *et al.* (1990), four different methods have been documented by different research groups to calculate the area under the curve (AUC), namely 1) incremental AUC, 2) net incremental AUC, 3) incremental area with the lowest glucose value as baseline (AUC_{min}) and 4) total AUC. Total AUC includes the area beneath the curve down to a blood glucose of zero and is a measure of the average blood glucose concentration during the period of test. The incremental AUC, on the other hand, is a measure of the change of blood glucose from the fasting condition. According to Wolever (2003), the GI is based on the incremental area below the curve and above the fasting level only as depicted in Figure 2.1a.

The main source of error in determining the GI could be the method of calculating the AUC. According to Jerling *et al.* (2002), there are currently two main schools of approaches namely the Wolever and Potchefstroom approach as summarised in Figure 2.1a and Figure 2.1b. The Potchefstroom approach uses the incremental area with the lowest glucose value as baseline to calculate GIs since hypoglycaemia will not be reflected when the area below fasting level is ignored. Recently, Nell (2001) found that the AUC_{min} method showed less variation than the incremental AUC

method above the fasting level only and suggested that the AUC_{min} method is a more relevant physiological method to use in GI-calculations. However, according to Wolever *et al.* (1991), the GI is based on the area under the blood glucose response curve above the baseline. The overall equation simplifies to: Area = (A + B + C +D/2)t + $D^2t/2(D + \{E\})$, where A, B, C, D and E represent positive blood glucose increments; t is the time interval between blood samples. The AUC_{min} approach uses the incremental area with the lowest glucose value as baseline to calculate the GI since hypoglycaemia will not be reflected when the area below fasting level is ignored (Figure 2.1b)(Vorster *et al.*, 1990). It has to be acknowledged, however, that the Wolever approach has the longest history and is, therefore, used more often in scientific literature.

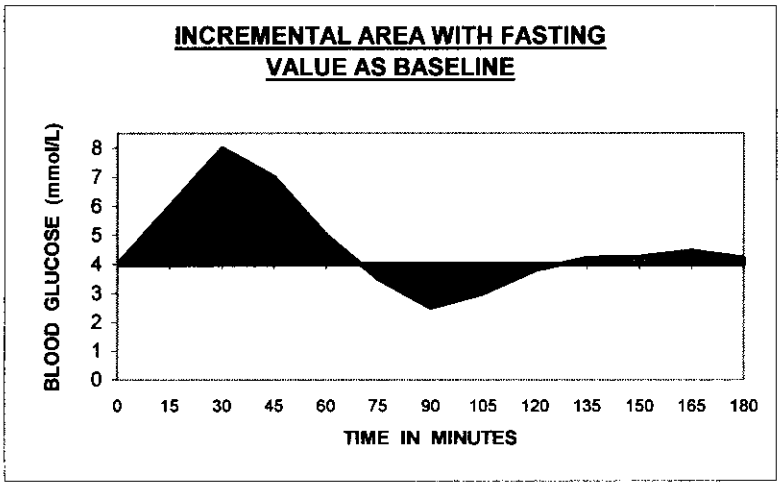

Figure 2.1a. Incremental area with fasting value as baseline

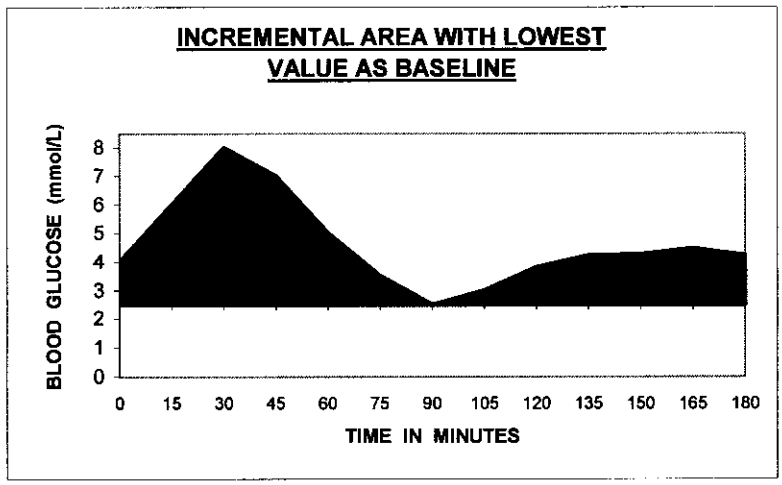

Figure 2.1b. Incremental area with lowest value as baseline

Method of blood sampling

The GI was based on measurement of glucose responses in whole capillary finger-prick blood due to the simplicity and non-invasiveness of the method of blood sampling, allowing for extensive screening of foods (Wolever *et al.,* 1991). Other reasons for the use of capillary blood are the following: it is easier obtain, the rise in the blood glucose concentration is greater than in venous plasma and the results for capillary blood glucose are less variable than those for venous plasma glucose (FAO/WHO, 1998). According to the FAO/WHO (1998), venous plasma glucose can also be used since it yields similar values. An illustration of the difference between simultaneously obtained venous plasma glucose and capillary whole blood is shown in Table 2.1.

Table 2.1. Difference between venous plasma and capillary blood glucose concentrations

	T_0	T_{15}	T_{30}	T_{45}	T_{60}	T_{90}	T_{120}
Venous	5.0	7.1	8.8	8.0	5.6	5.4	4.2
Capillary	4.1	6.3	9.0	8.7	6.7	5.7	3.9

(Adapted from FAO/WHO, 1998).

Glucose uptake at a given insulin concentration increases with increasing blood glucose concentration and different meals produce different glycaemic responses Therefore, the assessment of glycaemic responses to foods may yield different results depending upon whether venous or capillary blood is used (Wolever *et al.,* 1988). Because glycaemic responses in capillary blood are greater than those in venous blood or plasma, smaller differences in glycaemic responses to different foods may be detected. For example, in normal subjects a mixed meal containing spaghetti had a capillary blood glucose response 37% less ($p<0.05$) than one containing bread, but the difference in simultaneously obtained venous whole blood was only 19% and not significant (Wolever *et al.,* 1991). Studies in which GIs were calculated both from analyses of capillary and venous blood have shown no differences in GI values (Granfeldt *et al.,* 1995).

It has recently been confirmed by Wolever *et al.* (2003) that glycaemic responses measured in venous plasma are lower and more variable than capillary blood and that capillary blood allows more accurate determination of the GI as long as capillary blood samples are not contaminated with interstitial fluid due to "milking" of the finger (Wolever, 2003). Current recommendations are, therefore, that capillary blood sampling is preferred in determining the GI (Wolever *et al.,* 2003; GI Task Force, 2002), but venous blood sampling is also acceptable (FAO/WHO, 1998). The issue of blood sampling for GI testing will be discussed further in the following section.

Capillary blood versus venous plasma glucose

The simplest indicator of the adequacy of carbohydrate metabolism of a patient is the blood glucose concentration, however, glucose is rapidly metabolized in the body. Therefore, glucose concentration reflects the immediate status of carbohydrate metabolism and does not allow a retrospective or prospective evaluation of glucose

metabolism. Glucose is measured in different specimens namely, whole blood (capillary or venous), haemolysate, plasma, serum, de-proteinized blood, urine and cerebrospinal fluid (WHO, 2002).

Three major factors influencing glucose values are the laboratory procedure used, the type of sample analyzed (whole blood, plasma or serum) and the source of the blood (venous or arterial) (Eriksson *et al.,* 1983). It is well known that arterial plasma glucose concentrations are greater than those of capillary whole blood because the concentration of glucose in red cells is lower than that in plasma (Wolever & Bolognesi, 1996). Plasma has a higher water content than erythrocytes (93% versus 73%). Therefore, plasma glucose concentrations are about 10-15% higher than those of whole fresh blood (Larson-Cohn, 1976; Teng *et al.,* 1995). After the consumption of a meal, the glucose concentration in arterial blood may differ from 20 to 70% from the concentration in venous blood (Duffy *et al.,* 1973), because as blood flows from the arterial to the venous circulation via the capillaries, peripheral tissues remove some of the glucose. The rise in blood insulin and glucose after eating stimulates glucose removal by tissues, therefore, the difference in glucose concentration between arterial and venous blood is greater postprandially than fasting, leading to a smaller glucose rise in venous blood (Eriksson *et al.,* 1983; Jackson *et al.,* 1973; Wolever *et al.,* 2003). The mean arteriovenous differences are the largest in lean nondiabetic individuals, smallest in diabetic individuals and larger in deep veins than in superficial vessels (Marks, 1996).

Haeckel *et al.* (2002) compared glucose concentrations in venous blood (VB), venous plasma (VP) and capillary blood (CB) in healthy and diabetic subjects during glucose tolerance tests (GTTs). The mean VP/VB ratio from all determinants during the GTTs was 1.148, increasing slightly but statistically not significantly from the healthy to the

diabetic group. The mean VP/CB ratio was 1.048. The VP/CB ratio was related to the nutritional state being 1.084 in the fasted and 0.972 in the postprandial state in healthy nondiabetic subjects ($p<0.001$). In contrast, the VP/CB ratio remained almost constant after a glucose load in diabetic individuals. The VP/CB ratio was higher in diabetic than in nondiabetic individuals ($p<0.001$).

Venous glucose responses may be more variable than capillary responses for several reasons. Blood glucose concentrations oscillate on a minute-by-minute basis, driven, at least in part, by the pulsatile nature of insulin secretion. Presumably, the oscillations of plasma glucose in different tissues in the body are not in phase with each other, because it takes different lengths of time for the pulses of insulin from the pancreas to reach them (Wolever *et al.,* 2003). According to Wolever *et al.* (2003) it is possible that the magnitude of glucose oscillations in forearm venous blood may be greater than those in capillary blood because the vein drains a small volume of tissue with insulin oscillations in phase with each other. However, the glucose oscillations in capillary blood may be damped because arterial blood is derived from all tissues in the body with insulin concentrations oscillating out of phase with each other. There is also a small analytical error associated with measuring glucose and this has a larger proportional effect on the AUC when the rise in glucose is small. For example, a 0.1 mmol/L difference in the fasting glucose concentration results in a 12 mmol/min/L difference in the AUC over 2h, which is 20% of an AUC of 60, but only 6% of an AUC of 200 (Wolever *et al.*, 2003).

When collecting and transporting blood for glucose analysis it is important to inhibit enzymatic degradation of blood glucose (Meinik & Potter, 1982; WHO, 2002). Glycolysis can contribute to the variability in the capillary blood versus venous

plasma glucose relation (Teng *et al.,* 1995; Hussain & Sharief, 2000). In whole blood, glycolysis decreases the glucose concentration by 10-15% per hour at room temperature (Sidebottom *et al.,* 1982; WHO, 2002). Serum glucose once separated from erythrocytes remains stable at room temperature up to 8 hours or for up to 72 hours at 4°C (WHO, 2002). Glycolysis in whole blood is inhibited by sodium fluoride (6 g/L blood) or maleinimide (0.1 g/L blood) and ethylenediamine tetra acetic acid (EDTA) as anticoagulant (1.2-2 g/L blood) (Teng *et al.,* 1995; WHO, 2002). In a study done by Hussain & Sharief (2000), the specimens for glucose analysis were transported to the laboratory and analysed within one hour in order to reduce the impact of glycolysis. Felding *et al.* (2002) reported that glucose concentrations decrease through storage of blood samples stabilized with heparin fluoride and that the decrease seemed unrelated to the glucose concentration. They also confirmed that the ratio between capillary and venous blood glucose is higher and more variable in non-fasting than in fasting persons.

Red blood cells and white blood cells consume glucose. At a packed cell volume (PCV) > 55% Hussain and Sharief (2000) found that the mean difference between venous reagent strip test (RST) and plasma glucose was significantly more than the mean difference between capillary RST and plasma glucose (p = 0.019). This suggests that the higher the haematocrit, the less accurate the venous RST. The American Dietetic Association (ADA) suggests that diagnostic glucose concentrations are measured in venous plasma or serum because the influence of haematocrit is omitted by using plasma or serum (Odum, 1999).

Subject characteristics and number

Many subject characteristics affect the glycaemic response to a given food including health status, type and treatment of diabetes mellitus, basal metabolic index (BMI), age, gender, ethnicity and background knowledge of GI-studies (Jenkins *et al.,* 1984). Wolever *et al.* (1991) also pointed out that subject characteristics, treatment and degree of blood glucose control (diabetics) may have major effects on the absolute glycaemic response obtained. However, if they are standardised they appear to influence the response to all foods similarly and so have only small effects on the resulting GI value. Specific characteristics that have been examined and did not differ significantly include: normal vs. diabetic subjects; non-insulin-dependent diabetes mellitus (NIDDM) subjects on oral agents vs. NIDDM subjects on insulin; children vs. adults; rural Africans vs. healthy Western subjects and NIDDM in good control vs. NIDDM subjects in poor control (reviewed by Wolever *et al.,* 1991). Most GI studies were done with five to 10 subjects (Foster-Powell *et al.*, 2002). However, Nell (2001) indicated that if a 10% range for a GI of a food is sought with 80% confidence, between 24 and 90 subjects should be included in a study using venous plasma samples. The GI Task Force (2002) recommended a minimum of 20 subjects for GI testing. Wolever *et al.* (1991) pointed out that "variability in GI values in different subjects is largely due to within-individual variation", but "when results were expressed as the GI, there was no significance between the subjects".

2.3 CLINICAL UTILITY OF THE GLYCAEMIC INDEX

Consistency of values across space and time

According to Jenkins *et al.* (1988), variability of the "GI values of foods tested in different parts of the world may be due in part to differences in food portion size,

processing, cooking, ripeness, storage, content of antinutrients and nutrient-nutrient interactions". However, it seems that there is a surprisingly broad measure of agreement on the relative glycaemic effect of many foods across space and time, despite the unknowns (Jenkins *et al.,* 1988).

Application in individual subjects

The glycaemic responses in different subjects vary over a wide range. However, when the glycaemic response of a food is expressed relative to that of a standard (reference) food taken by the same subject, the variability between subjects is reduced to the extent that it no longer becomes statistically significant (Wolever, 1990). As pointed out in the previous section, GI values are not significantly affected by subject variables such as age (Wolever *et al.*, 1988), ethnicity (Wolever *et al.*, 2003), glucose tolerance status (Jenkins *et al.*, 1983) or presence of type 1 or type 2 diabetes (Wolever *et al.*, 1987). Variation in GI values in different subjects is, therefore, due to within-subject variation (Wolever, 2003).

Blood glucose responses vary considerably from day-to-day within subjects (FAO/WHO, 1998). For repeated testing of 50g carbohydrate from glucose or bread, the mean coefficient of variation (100 x standard deviation/mean) of the incremental AUC is approximately 15% in subjects with type 2 diabetes, 23 to 25% in nondiabetic subjects and 30% in subjects with type 1 diabetes (Wolever *et al.,* 2003). To obtain representative mean responses to a standard food, it is recommended that the same subject repeat the test at least three times (FAO/WHO, 1998; Wolever *et al.,* 2003). Gosland (as quoted by Venter *et al.,* 2003) states that volunteers from the lay public show an increased within-individual variance as compared to laboratory staff who are more aware of the importance of following a specific protocol.

Application to mixed meals

Coulston *et al.* (1987) claimed that the GI concept lacks clinical utility because the difference in GIs between foods are lost once these foods are consumed in a mixed meal. A mixed meal consists of several carbohydrate sources, therefore, the effect of the lower glycaemic index component is diluted in proportion to the amount of carbohydrate from other foods. There are many foods that do not contain carbohydrates only, but are mixed with other macronutrients, namely protein and fat. Thus, the insulin response to a carbohydrate food varies with the amount of fat, protein or both with which ingested. Wolever *et al.* (1991) stated that the amounts of fat and protein required to have significant effects are large compared with the amounts normally eaten or advised in dietary recommendations. Appropriate calculation of the mixed-meal GI is, therefore, required (Jenkins *et al.,* 2002). The GI of meals containing several carbohydrate foods is expressed as the weighted mean of the GI values of each of the component foods, with the weighting based on the proportion of the total meal carbohydrate provided by each food (Wolever *et al.,* 1991). The amount of a carbohydrate containing food eaten can be expressed as a percentage of the total carbohydrate of the meal multiplied by the GI of that specific food. In this manner the GI acts as a measure of the quality of the carbohydrate intake. In order to calculate the GI of a diet a value of the GI for every food in the diet needs to be assigned (Nell, 2001). A sample calculation is given in Table 2.2. Liu *et al.* (2000) proposed the use of glycaemic load (GL) as a measure of the quantity and quality of the dietary carbohydrates consumed. The GL can be calculated using a food frequency questionnaire. The GL is then calculated by multiplying the carbohydrate content of each food by its GI, then multiplying this value by the frequency of consumption and summing the values from all foods (Liu *et al.,* 2000).

Table 2.2. Sample calculation of mixed-meal GI

	Carbohydrate (g)	% Total carbohydrate	Mean food GI	Meal GI
Jungle Oats (66g)	17.2	38.3	58	22
Milk, fat free (150ml)	7.8	17.4	32	5.6
Sugar (20g)	19.9	44.3	65	28.6
Total	**44.9**	**100**		**56.2**

Therapeutic effects of low-GI diets

There are a number of long-term implications of altering the rate of breakdown and absorption, or, GI of dietary carbohydrate (Venter *et al.,* 2003). Diets with a high GI are associated with greater fluctuations in blood glucose and insulin concentrations (Nell, 2001). Several large-scale, observational studies from Harvard University (Cambridge, MA) indicate a high GL is a significant independent risk factor of developing type 2 diabetes (Salmerón *et al.,* 1997a; 1997b) and cardiovascular disease (Liu *et al.,* 2000). Three intervention studies in adults and in children with type 1 diabetes showed that low GI diets improve glycated haemoglobin concentrations (Frost *et al.,* 1998; Giacco *et al.,* 2000; Gilbertson *et al.,* 2001) and in subjects with cardiovascular disease, low GI diets were shown to be associated with improvements in insulin sensitivity and blood lipid concentrations (Frost *et al.,* 1998; Jenkins & Jenkins, 1987). Two six-year cohort studies, one in men (Salmerón *et al.,* 1997a) and one in women (Salmerón *et al.,* 1997b) have demonstrated diets with high GL and low cereal fibre content are linked with more than twice the risk of type 2 diabetes when compared to diets with low GL or high cereal fibre content. Futhermore, the GI was inversely associated with HDL-cholesterol concentrations in British men and women (Frost *et al.,* 1998). Since low HDL-cholesterol is a feature of the metabolic syndrome, it was suggested that the relationship between GI and HDL be due to the effect of a low GI diet in improving insulin sensitivity (Luscombe *et al.,* 1999). Evidence from both short-term and long-term studies in animals and humans indicate that low GI foods may be useful for weight control (Brand-Miller *et al.,*

2002). The lower energy density and palatability of these foods are important determinants of their greater satiating capacity (Ludwig, 2000). For, athletes, low GI carbohydrate foods were recommended before prolonged exercise to promote carbohydrate availability (Burke *et al.*, 1998). Moderate to high GI foods and drinks are considered appropriate during prolonged exercise and high GI carbohydrates the best choice to enhance glycogen storage after exercise by promoting greater glucose and insulin response (Burke *et al.*, 1998). More recently, evidence was accumulating that a low GI diet might also protect against colon and breast cancer (Foster-Powell *et al.*, 2002).

These effects prompted the Joint FAO/WHO expert consultation "Carbohydrates in Human Nutrition" and, more recently, Riccardi & Rivellese (2000) to endorse the usefulness of the GI in diet planning. However, according to Franz (2000), the usefulness of low GI diets in persons with type 1 diabetes is controversial. According to Pi-Sunyer (2002), there are many uncertainties regarding the validity of the GI for determining what foods are "good" and "bad" for one's health. He believes it would be a mistake to initiate a public health campaign stating that certain widely consumed carbohydrates should be avoided. Much more definitive data from controlled clinical trails are needed before any such dietary recommendations are made (Pi-Sunyer, 2002). Therefore, the American Diabetes Association (ADA) is of the opinion that the evidence of long-term benefit of the use of low GI foods is not sufficient to recommend low GI diets as a primary strategy in meal planning (ADA Position Statement, 2002). However, the European Association for the Study of Diabetes, the Canadian Diabetes Association and the Dietitians Association of Australia all recommend high-fibre, low GI foods for individuals with diabetes as a

means of improving postprandial glycemia and weight control (Foster-Powell *et al.,* 2002).

The GI of foods has important implications for the food industry. Terms such as complex carbohydrates and sugars, which commonly appear on food labels, are now recognized as having little nutritional or physiological significance (Anon., 1994a). The FAO/WHO (1998) recommended that the GI be used to compare foods of similar composition within food groups and that both GI and food composition must be considered when choosing carbohydrate containing foods (Anon., 1994a), therefore, it is important that the GI value is not regarded as the sole determinant of food choice, just as kilojoule value or fat content should not be (Anon., 1994b). Once foods are being labeled for GI or GL they may be classified as being less healthy, when the value surpasses a certain limit, therefore, the sugar and starches industry will have to adapt by defining new strategies concerning the development of low GI carbohydrate sources and the use of carbohydrate combinations that lower GI and the GL. This will open new horizons for the incorporation of sugar replacements (polyols), resistant starches and slow digestible starches in existing types of food matrixes with the goal to maintain a high carbohydrate quantity with a reduced GI or GL. The trend in science and nutrition, to increasingly focus on the glycaemic impact of foods and drinks is now changing into a significant concern. However, it will help to weigh the possible strategic risks related to the promotion of low GI or GL foods for business and marketing managers and will become a major player in the future food markets (Brouns, 2002).

Requirements for claims regarding the GI value of carbohydrate-rich foods are included in a new concept regulation regarding food packaging in South Africa

(Foodstuffs, Cosmetics and Disinfectants Act, 54/1972). Although it is still a draft regulation, it has been mentioned that according to research in South Africa as well as internationally, the GI concept seems to be acceptable and useful in South Africa (Venter *et al.,* 2003). During a Master Class on the GI during the recent 2002 South African Nutrition Congress (2-9 November 2002, Potchefstroom) a group of 36 dietitians and nutritionists critically evaluated the practical application of the GI of foods and reached consensus on the usefulness of the GI concept, stating that there is sufficient experience and exposure amongst South African dietitians to support a labeling initiative for the GI in order to inform the public and promote responsible use of the concept (GI Task Force, 2002). However, they identified a number of areas that need more research for better implementation. Three of these were the best methodology in determining the GI, the GI of traditional and indigenous South African foods/meals and the best way to express the GI on food/drink labels. These issues are given priority in the study reported in this mini-dissertation.

2.4 SUMMARY

Many people have raised concerns about the variation in published GI values for apparently similar foods. This variation may reflect both methodologic factors and true differences in the physical and chemical characteristics of the foods. Another reason for the variation in GI values for apparently similar foods may be that different testing methods are used in different parts of the world. Differences in testing methods include the use of different types of blood samples (capillary or venous), different experimental time periods and different portions of food (Foster-Powell *et al.,* 2002). Recently, seven GI testing laboratories around the world participated in a study to determine the degree of variation in GI values when the

same centrally distributed foods were tested according to the laboratories' normal in-house testing procedures. The results showed that the five laboratories that used finger-prick capillary blood samples to measure changes in postprandial glycaemia obtained similar GI values for the same foods and less intersubject variation (Wolever *et al.*, 2003). Although capillary and venous blood glucose values have been shown to be highly correlated, it appears that the capillary blood samples may be preferable to venous blood samples for reliable GI testing. After the consumption of food, glucose concentrations change to a larger degree in capillary blood samples than in venous blood samples. Therefore, capillary blood may be a more relevant indicator of the physiologic consequences of high GI foods (Wolever *et al.*, 2003). Another important reason GI values for similar foods sometimes vary between laboratories is because of the method used for determining the carbohydrate content of the test foods. GI testing requires that portions of both the reference food and test food contain the same amount of available carbohydrate, typically 25g or 50g. The available carbohydrate portion of test and reference foods should not include resistant starch, but in practice, this can be difficult to ensure because resistant starch is difficult to measure. There is also difficulty in determining the degree of availability of novel carbohydrates, such as sugar alcohols, which are incompletely absorbed at relatively high doses (Foster-Powell *et al.*, 2002). Therefore, Venter *et al.* (2003) considered standardisation of methodology in determining the GI of utmost importance to render the GI universally applicable and acceptable. Furthermore, trained researchers in a well-controlled experimental environment of an accredited laboratory should perform the test with accuracy and precision to warrant reliability and comparability of measurements. Protocols should also comply with research ethical standards (Venter *et al.*, 2003). In the research project described in the following chapters, these recommendations have been implemented.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

The Potchefstroom Institude of Nutrition was commissioned by a food company to carry out a scientific measurement of the GI of Jungle Oats, Bokomo Oats and Oatso-Easy. This study was conducted in accordance with the most recent laboratory guidelines based on the results of international studies (Wolever *et al.,* 2003) and the recommendations of the South African GI Task Force (2002).

3.2 SUBJECTS AND METHODS

Twenty healthy, male students between the ages of 21 and 27, with a mean body mass index (BMI) of 24.55±2.62 kg/m^2 were recruited to take part in the study. Nell (2001) indicated that if a 10% range for a GI of a food is sought with 80% confidence, between 24 and 90 subjects should be included in a study using venous plasma samples. However, the GI Task Force (2002) suggests a minimum of 20 subjects to be recruited based on willingness to comply with the protocol, inclusion and exclusion criteria. The subjects stayed overnight in the Metabolic Unit of the Potchefstroom Institute of Nutrition and were studied after a 10-12 hr fast on four mornings over a four week period. Upon arrival they filled in the necessary informed consent and indemnity forms. All rules and procedures regarding their overnight stay were carefully explained to minimize factors which may influence glucose responses. Subjects should not smoke or exercise 12 hours prior to testing (GI Task Force, 2002). After reception the subjects consumed a standard pre-evening test meal (containing 60% of the total kJ from carbohydrates; 25% from fat; 15% from protein) to optimize carbohydrate metabolic enzyme induction and to standardise

potential "second meal" effects (GI Task Force, 2002; Gresse & Vorster, 1992). The pre-evening test meal is summarized in Table 3.1. The subjects spent a relaxed evening and were instructed to be in bed by 23h00.

Table 3.1. Pre-evening test meal*

Food	Amount	Carbohydrate (g)	Protein (g)	Fat (g)	Energy (kJ)
Milk, fat free	250mL	12.3	8.5	0.5	372.5
Apple (peeled)	80g	11.8	0.2	0.2	212.8
Bread, white (crusts removed)	6X30g	94.3	15.3	3.2	1983.6
Jam	35g	24.5	0.1	0.0	419.3
Cheese, medium fat	60g	1.3	14.9	16.4	855
Margarine, medium fat	10g	0.0	0.0	6.4	240
Total		144.2	39.1	26.7	4083
kJ		2422.56	656.8	1014	4083
% of kJ		59%	16%	%25	100%

* Calculated according to the MRC Food Composition Tables (Langenhoven *et al.*, 1991)

On the test days blood samples were obtained fasting and after subjects randomly consumed a test meal of either 50g glucose powder dissolved in 300ml water or 66g Jungle Oats, 72.8g Bokomo Oats or 105g Oatso-Easy, within 10-15 minutes. In this study the reference food was only measured once. According to the FAO/WHO (1998) and GI Task Force (2002) the reference food requires three measurements (to reduce within-subject variability) and the test food requires one measurement. The nutritional composition of the three different oats porridges is summarized in Table 3.2.

Table 3.2. Macronutrient composition of the three different oats porridges as indicated on the label of the porridges

	Oatso-Easy (40g)	Jungle Oats (40g)	Bokomo Oats (40g)
Energy (kJ)	497	577	672
Protein	4.4	4.9	5.2
Carbohydrate	17.38	22.5	26
Fat	4.18	3.6	2.6
Dietary fibre (60% soluble)	5.5	4.3	4.2

For every given amount of oats porridge, 150ml skimmed milk and 20g sugar was included, except for the Oatso-Easy. The project brief required the foods to be prepared exactly to manufacturers instructions and consumed as eaten by the majority of consumers. The food company provided the research team with data on how oats is normally consumed by consumers. From this information it was clear that the most popular additives were milk and sugar in the case of more than 90% of consumers. The exact amount of ingredients used in the preparation to achieve 50g of available carbohydrate is summarized in Table 3.3.

Table 3.3. Ingredients used in the preparation of the three oats porridges to achieve 50g available carbohydrate

	Oatso-Easy	Jungle Oats	Bokomo Oats
Oats (g)	3x35	66	72.8
Water (mL)	480	450	413
Milk (mL)	-	150	150
Sugar (g)	-	20	20
Total weight of 1 serving (g)	537	515	514

Glycaemic carbohydrate is defined as the carbohydrate available for metabolism and is the summation of the analytical values of mono-, di- and oligosaccharides, starch and glycogen but excludes fructo-oligosaccharides and other non-digestible oligosaccharides and resistant starch (Brand-Miller & Gilbertson, 2001). The Englyst method was used to determine 50g available glycaemic carbohydrate by Englyst

Carbohydrates Research and Services Ltd, Cambridge, United Kingdom. Each meal contained 50g available carbohydrates.

Method of calculation to determine 50g available carbohydrate as eaten

Oatso-Easy

Oatso-Easy(raw):35g=186g cooked (with added water accordingly to manufacturer's instructions)

100g cooked oats=9.6g available glycaemic carbohydrates

186g Oatso-Easy =17.3g available glycaemic carbohydrates

- 35g raw oats=17.3g available glycaemic carbohydrates

Thus: 50g available glycaemic carbohydrate

- 3x35g sachets oats (558g prepared as eaten)=51.9g available glycaemic carbohydrate

Thus, 537.5g = 50g available glycaemic carbohydrate

Bokomo Oats

Bokomo Oats (raw): 36.6g = 201.1g cooked

100g cooked oats=9.6g available glycaemic carbohydrates

201g cooked oats=19.3g available glycaemic carbohydrates

- 36.6g raw oats=19.3g available glycaemic carbohydrates
- +75mL milk =3.5g available glycaemic carbohydrate
- +10g sugar =5g available glycaemic carbohydrate

Total: 27.8g available glycaemic carbohydrate

Thus: 50g available glycaemic carbohydrate

- 73.2g raw oats (402.2g cooked) =38.6g available glycaemic carbohydrate
- +150 mL milk
- +20g sugar

Total: 55.6g available glycaemic carbohydrate

Bokomo Oats prepared with sugar and milk: 572g

572g = 55.6g available glycaemic carbohydrate

Thus, 514g = 50g available glycaemic carbohydrate

Jungle Oats

Jungle Oats (raw): 36.6g = 223g cooked

100g cooked oats=9.6g available glycaemic carbohydrates

223g cooked oats=21.4g available glycaemic carbohydrates

- 36.6g raw oats=21.4g available glycaemic carbohydrates
- +75mL milk =3.5g available glycaemic carbohydrate
- +10g sugar =5g available glycaemic carbohydrate

Total: 29.9g available glycaemic carbohydrate

Thus: 50g available glycaemic carbohydrate

- 73.2g raw oats (446g cooked) =42.8g available glycaemic carbohydrate
- +150 mL milk
- +20g sugar

Total: 59.8g available glycaemic carbohydrate

Jungle Oats prepared with sugar and milk: 616g

616g = 59.8g available glycaemic carbohydrate

Thus, 515g = 50g available glycaemic carbohydrate

Experimental design

A 4x4 factorial design was used in this study. Table 3.4 shows the randomization schedule (Latin square) that was used. Ten subjects participated in the study per day. For example, on day one subject numbers 20, 4 and 2 received Oatso-Easy, subjects 6 and 16 Bokomo Oats, subjects 13, 9 and 12 Jungle Oats and subjects 15 and 14 the glucose standard. The design was a double blinded trial so that neither investigators nor subjects knew who had received what product. The code was only broken after the final statistical analyses had been completed.

Table 3.4. Randomisation to treatment schedule

	Oatso-Easy	Bokomo Oats	Jungle Oats	Standard
Day 1	20,4,2*	6,16	13,9,12	15,14
Day 2	15,14	20,4,2	6,16	13,9,12
Day 3	13,9,12	15,14	20,4,2	6,16
Day 4	6,16	13,9,12	15,14	20,4,2
Day 5	10,8,11	3,19	7,17,1	5,18
Day 6	5,18	10,8,11	3,19	7,17,1
Day 7	7,17,1	5,18	10,8,11	3,19
Day 8	3,19	7,17,1	5,18	10,8,11

* Subject numbers

Capillary whole blood and venous plasma determination

Venous blood samples were obtained by a registered nursing sister using an indwelling catheter placed in a forearm vein and kept open by flushing with 1-2 mL of saline and heparin. The saline and heparin were cleared before each blood sample by withdrawing and discarding 1 mL. Whole capillary blood glucose was measured by three experienced specialized technicians trained and standardised in measuring capillary whole blood glucose using SureStep test strips and SureStep glucometers (Lifescan). This procedure was done in strict compliance with the protocol recommendations of the manufacturer as well as good laboratory practice as described by the GI Task Force. The side of the finger was pricked and the first drop of blood removed. Thereafter, a large drop of blood (without milking the finger) was applied to the strip without touching it (GI task Force, 2002). Venous whole blood and finger-prick capillary blood samples were taken simultaneously before and every 15 minutes for one hour after the test meals, and thereafter every 30 minutes for one hour. Venous blood was collected into sodium-fluoride tubes and centrifuged within 15 minutes to remove the plasma. Thereafter, it was frozen at –84°C until the day of analysis. Plasma glucose was measured in duplicate using the enzymatic colorimetric method (Randox, Cat no GL 2614 for 2 x 500mL reagent, Randox

Laboratories Ltd, Antrim, United Kingdom). Standard laboratory techniques, apparatus and standard reference ranges were used for the analysis of the plasma samples.

Statistical analysis

The statistical analysis was done using the Statistica 6.1 software package. Results were expressed as incremental areas under the glucose curves, ignoring the area beneath the fasting level (AUC) and the GI. For the three different test meals, the mean, 95% confidence interval (CI) and standard deviation (SD) were calculated for the GI taking the capillary whole blood and venous plasma glucose into account. The repeated measures of ANOVA were used with Tukey honest significant difference test in the post hoc analysis.

Ethical considerations

The Ethics Committee of the Potchefstroomse Universiteit vir Christelike Hoër Onderwys approved all procedures and the subjects gave written informed consent.

3.3 LIMITATIONS OF THE STUDY

Due to financial and time constraints the reference food (glucose) was administered only once. However, the mean AUC of three trials of the reference food should be used to calculate the GI (Wolever *et al.,* 2003), because the mean of three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial (Wolever *et al.,* 1991).

3.4 CONCLUSION

There is currently no international standard for the testing of the GI (The GI Task Force, 2002) and it is well known that numerous methodological factors may influence the interpretation of glycaemic response data (Wolever *et al.*, 1991). According to Venter *et al.* (2003) one of the major problems regarding labeling of

foods with GI values is the lack of standardised methodology amongst different researchers in determining the GI. Furthermore, clear directions are needed regarding standardised methodology in accredited laboratories, including clarity on issues such as the reference (standard), total ("available") carbohydrate of the test food, number and characteristics of experimental subjects, capillary versus venous blood samples and the method for calculation of the area under the glucose curve.

This study was conducted in accordance with the most recent laboratory guidelines based on the results of international studies (Wolever *et al.,* 2003) and the recommendations of the South African GI Task Force (2002). Unfortunately, due to financial and time constraints, the reference test was administered only once. This is an important limitation of the study, which could have increased the within-subject variability. Therefore, the results, which are presented in the following chapter, have to be interpreted with caution.

CHAPTER 4

RESULTS

4.1 INTRODUCTION

A food company commissioned an independent assessment of the GIs of Jungle Oats, Bokomo Oats and Oatso-Easy using methods complying with the most recent internationally accepted methodology and carried out under strictly standardised conditions. Thus, the GI and AUC of Jungle Oats, Bokomo Oats and Oatso-Easy was determined using both capillary whole blood and venous plasma. Another aim of the study was to determine if there were significant differences between the GI of the three oats porridges as mentioned. The project brief was to use the oats as consumed by the majority of the target market (for example, with milk and sugar or with water only in the case of Oatso-Easy). Based on the analysis of the glycaemic carbohydrate content of the products (as determined by the Englyst Carbohydrates Research and Services Ltd. in the UK) the amount of cooked product needed to supply 50g glycaemic carbohydrate was calculated.

Statistically significant differences ($p<0.05$) were found between the AUCs of the three different oats porridges for capillary blood and venous plasma. There were no statistical differences ($p>0.05$) found between the GIs (intermediate to high) of Jungle Oats, Bokomo Oats and Oatso-Easy. However, the 95% CI and SD of the capillary blood glucose were smaller than those of the venous plasma.

4.2 SUBJECT CHARACTERISTICS

Table 4.1 summarizes the subject characteristics of the twenty healthy, non-smoking male students that were recruited to take part in the study.

Table 4.1. Subject characteristics

	Mean	SD
Age (years)	24	3
BMI (kg/m^2)	24.55	2.62

SD=standard deviation
BMI=body mass index

4.3 GLUCOSE CONCENTRATIONS IN CAPILLARY BLOOD AND VENOUS PLASMA

The mean capillary blood glucose and venous plasma glucose values (measured at 0, 15, 30, 45, 60, 90 and 120 minutes) for the three different oats porridges and glucose (standard) are listed in Table 4.2 and illustrated in Figure 4.1, 4.2, 4.3 and 4.4. The mean blood glucose concentration was greater in capillary blood than venous plasma at every time point ($p<0.01$) for the three different oats porridges except at T_{15} for Bokomo Oats ($p=0.17$) and glucose standard ($p=0.49$), at T_{45} for Bokomo Oats ($p=0.07$), at T_{90} for glucose standard ($p=0.06$) and at T_{120} for glucose standard ($p=0.08$).

Table 4.2. Mean capillary blood and venous plasma glucose for the three different oats porridges and glucose standard

Time intervals (min)	Oatso-Easy (n=20)		Jungle Oats (n=20)		Bokomo Oats (n=20)		Glucose standard (n=20)	
	Capillary	Venous	Capillary	Venous	Capillary	Venous	Capillary	Venous
	Mean±SD		Mean±SD		Mean±SD		Mean±SD	
T_0	4.8±0.4	4.3±0.5	4.7±0.39	4.4±0.6	4.7±0.3	4.7±0.77	4.8±0.4	4.5±0.9
T_{15}	5.8±0.9	5.1±1.1	6.2±0.8	5.3±1.0	6.4±0.77	6.1±1.1	6.3±0.95	5.9±2.5
T_{30}	7.4±1.0	6.2±1.6	7.4±1.2	6.3±1.2	7.4±0.9	6.8±1.7	8.5±1.2	7.2±2.04
T_{45}	7.0±1.8	5.5±1.5	6.2±1.2	6.2±1.2	6.4±1.2	5.8±1.7	8.06±1.2	7.07±1.7
T_{60}	5.8±0.9	5.1±1.3	5.3±0.9	4.2±1.0	5.7±0.8	5.0±1.4	6.4±1.2	5.3±1.4
T_{90}	5.2±0.4	4.4±1.0	5.2±0.4	4.3±1.0	5.3±0.5	4.5±0.9	5.0±1.1	4.5±1.2
T_{120}	5.2±0.4	4.4±0.7	4.8±0.4	4.2±0.9	4.9±0.4	4.3±0.8	4.2±0.5	3.9±0.9

SD=standard deviation

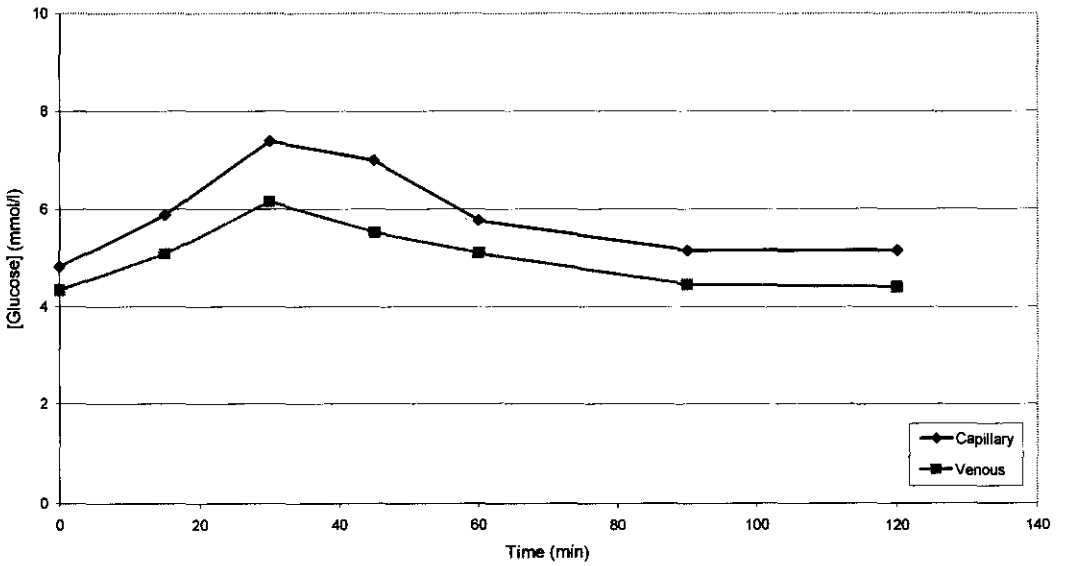

Figure 4.1. Mean capillary blood glucose versus venous plasma glucose after Oatso Easy.

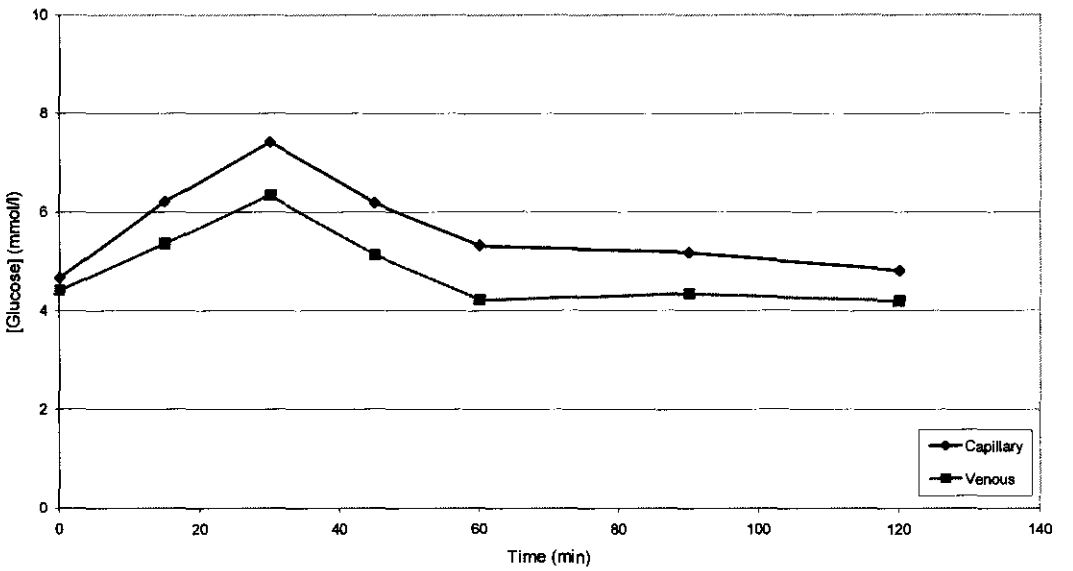

Figure 4.2. Mean capillary blood glucose versus venous plasma glucose after Jungle Oats.

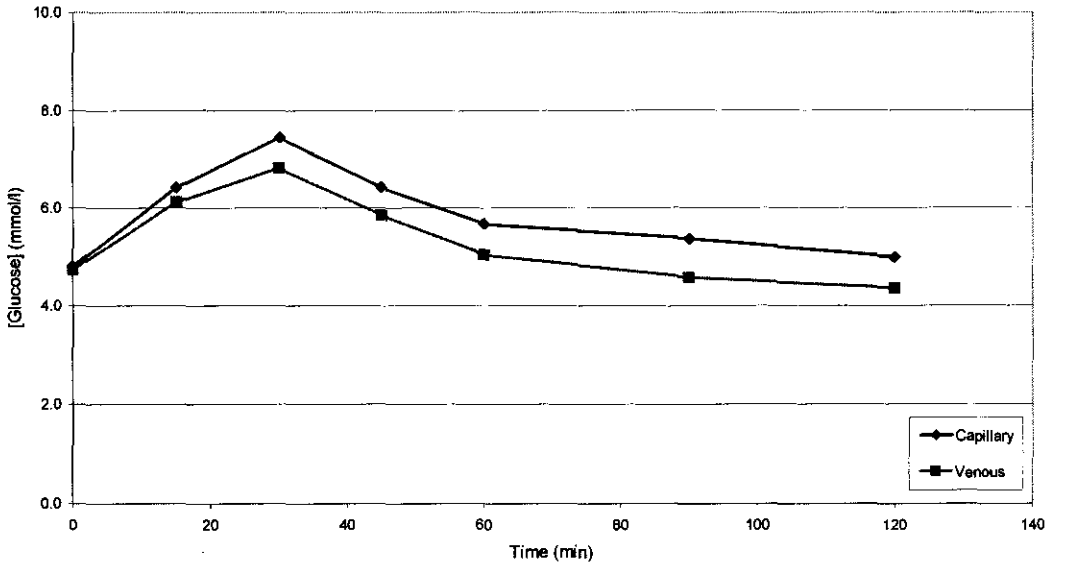

Figure 4.3. Mean capillary blood glucose versus venous plasma after Bokomo Oats.

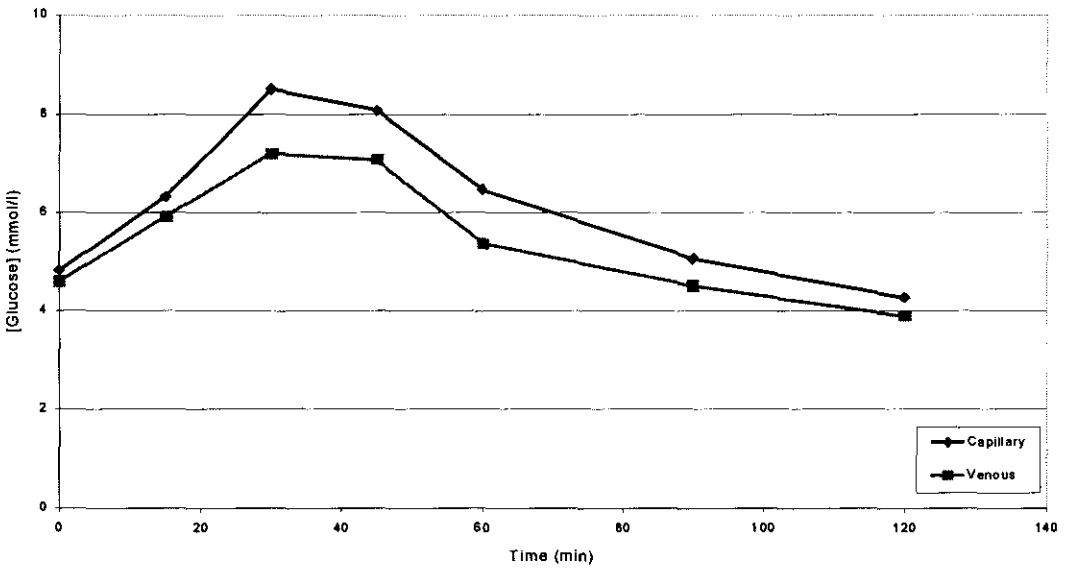

Figure 4.4. Mean capillary blood versus venous plasma after glucose standard.

4.4 THE AUCs FOR THE THREE DIFFERENT OATS PORRIDGES USING CAPILLARY BLOOD AND VENOUS PLASMA

According to Wolever *et al.* (2003), the GI is based on the incremental area below the curve and above the fasting level only. In this study this approach was used to calculate the GI.

Table 4.3 lists the mean AUC, 95% CI and SD (including the extreme values) of the test meals, determined by using capillary blood and venous plasma glucose values. The 95% CI and SD are quite large. These variations (minimum and maximum) also termed extremes, could be due to within-subject variation. To reduce the variability, FAO/WHO (1998) suggested that the standard food should be eaten at least three times with the mean result being used to calculate the GI. In this study, the standard food was tested only once. As the variability of the values from which any ratio is calculated increases, the distribution of the ratio becomes skewed and the mean increases, even if the original values are normally distributed (Wolever *et al.,* 1991). For example, in a study done by Wolever *et al.* (1991) on type 2 diabetics subjects, they stated that the distribution of the resulting GI values was skewed with a mean of 81% and a coefficient of variation (CV) of 22%. The skewness disappeared and the mean and CV were reduced to 80% and 18% respectively when the mean of three standard test meals was used to calculate the GI.

In an attempt to reduce the variation in GI due to differences between subjects, extreme values in AUCs were then excluded in this study (depicted in Table 4.4).

Extreme values were defined as follows:

a. Data point value > UBV + 2*o.c.*(UBV - LBV)

b. or Data point value < LBV - 2*o.c.*(UBV - LBV)

UBV: upper value of the box in the box plot AUC (mean + std.error)
LBV: lower value of the box in the box plot (e.g., the mean - std.error).
o.c.: outlier coefficient which is equal to 1.5

Table 4.3. The mean AUC for the three different oat porridges (including extremes) using capillary blood and venous plasma glucose.

	Capillary blood			Venous plasma		
	Oatso-Easy	**Jungle Oats**	**Bokomo Oats**	**Oatso-Easy**	**Jungle Oats**	**Bokomo Oats**
Valid N	20	20	20	20	20	20
Mean (mmol/L/min)	126.09	122.77	130.08	94.15	76.77	95.45
-95%CI	104.96	89.09	106.09	61.98	51.56	56.45
+95%CI	147.22	156.45	154.06	126.30	101.99	134.45
SD	45.15	71.96	51.24	68.72	53.86	83.33

SD=standard deviation
CI=confidence interval

Table 4.4. The mean AUC for the three different oat porridges (excluding extremes) using capillary blood and venous plasma glucose.

	Capillary blood			Venous plasma		
	Oatso-Easy	**Jungle Oats**	**Bokomo Oats**	**Oatso-Easy**	**Jungle Oats**	**Bokomo Oats**
Valid N	18	18	19	19	19	19
Mean (mmol/L/min)	124.25	105.38	121.41	84.50	67.98	78.98
-95%CI	106.25	80.12	104.82	58.00	49.75	59.68
+95%CI	142.25	130.65	138.01	111.00	86.22	98.29
SD	36.19	50.80	34.43	54.98	37.83	40.04

SD=standard deviation
CI=confidence interval

Figure 4.5 illustrates the mean AUC, SD and 95% CI (excluding the extremes) of the test meals, determined by using capillary blood and venous plasma glucose values. Statistically significant differences ($p<0.05$) were found between the AUCs of the

three different oats porridges for capillary blood and venous plasma, for example Oatso-Easy (p=0.0002), Jungle Oats (p=0.013) and Bokomo Oats (p=0.00002). It can be concluded with 95% confidence that the true mean AUC for Oatso-Easy was between 106.25 mmol/L/min and 142.25 mmol/L/min, for Jungle oats between 80.12 mmol/ L/min and 130.65 mmol/L/min, and Bokomo oats between 104.81 mmol/L/min and 138.02 mmol/L/min, measured in capillary blood. For the plasma venous AUC, the 95% CI level for Oatso-Easy was between 58% and 111%, for Jungle oats was between 49.75 mmol/L/min and 86.22 mmol/L/min and for Bokomo between 59.68 mmol/L/min and 98.29 mmol/L/min.

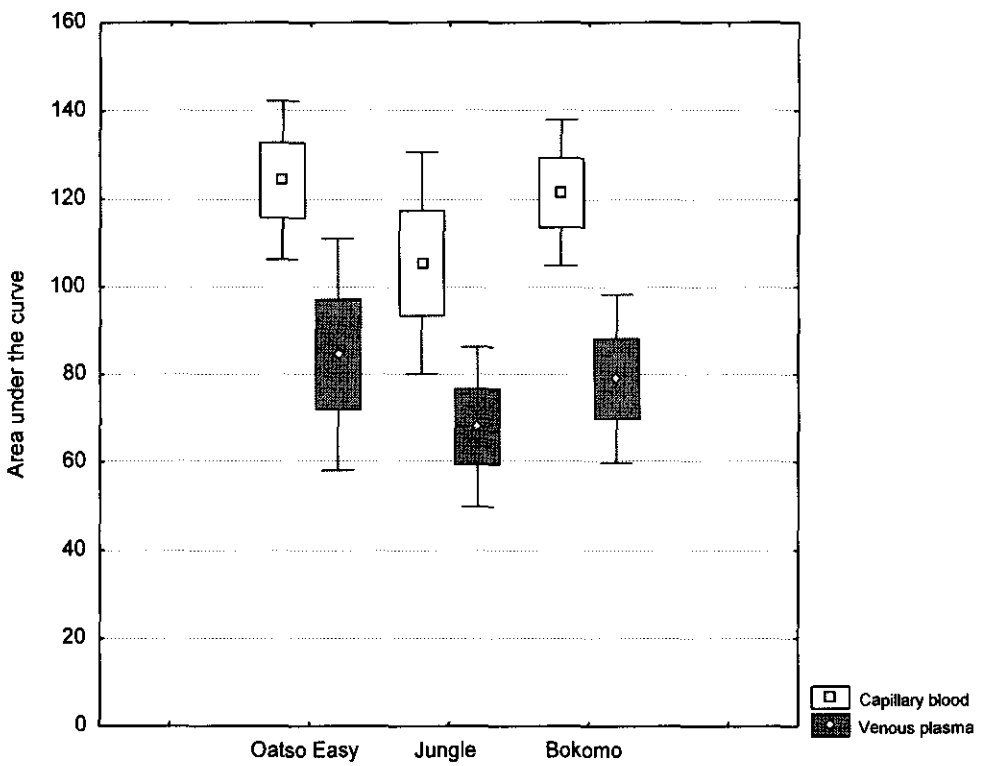

Figure 4.5. The mean AUCs (excluding the extremes) for the three different oats porridges using capillary blood versus venous plasma

4.5 THE GIs OF THE THREE DIFFERENT OATS PORRIDGES USING CAPILLARY BLOOD AND VENOUS PLASMA

The GIs of the three different oats porridges, using capillary blood and venous plasma for each subject, including the extreme values, are listed in Table 4.5. Using capillary blood to determine the GI, most of the values were within normal range. However, a wide range of venous plasma GI values was found. For example, the minimum GI value was 2.78. (venous plasma, Jungle Oats) and the maximum 1663.71 (venous plasma, Bokomo Oats).

Table 4.5. The GIs for individual subjects for the three different oats porridges using capillary blood and venous plasma glucose.

Subject no.	Capillary blood			Venous plasma		
	Oatso-Easy	Jungle Oats	Bokomo Oats	Oatso-Easy	Jungle Oats	Bokomo Oats
1	100.06	69.79	131.90	101.08	255.74	90.51
2	71.10	85.24	68.90	140.89	80.11	121.01
3	85.91	62.33	46.26	92.49	44.89	83.23
4	88.12	59.32	89.01	147.60	75.21	139.67
5	61.85	43.98	151.58	169.01	332.25	27.56
6	52.89	64.04	94.61	15.86	44.84	282.27
7	101.72	80.65	68.86	340.02	376.17	21.79
8	94.44	38.00	68.81	61.75	21.11	127.49
9	64.78	98.25	43.54	50.94	436.26	27.76
10	50.73	54.45	64.81	33.56	17.20	41.70
11	72.51	89.12	52.16	47.57	46.76	52.55
12	47.57	136.98	108.35	17.70	4.27	34.02
13	62.83	35.23	54.74	115.42	2.78	31.02
14	53.25	63.14	43.90	113.14	172.96	1663.71
15	123.89	25.25	236.89	440.22	82.11	32.36
16	63.57	91.48	42.29	60.86	85.71	56.68
17	61.29	33.18	53.00	41.52	65.71	34.07
18	49.80	28.52	51.01	4.83	65.82	105.97
19	67.58	136.07	105.79	245.65	33.49	68.31
20	92.42	91.41	68.25	62.63	41.77	34.02

Table 4.6 lists the mean GIs for the three different porridges excluding the extremes. The mean GIs were 77.01, 67.73 and 77.99 for Oatso-Easy, Jungle Oats and Bokomo Oats respectively, using capillary samples and 112.36, 112.36 and 113.79 respectively using venous samples. No statistically significant differences ($p>0.05$)

were found between the GIs of the three different oats porridges for capillary blood and venous plasma (Oatso-Easy: p=0.09; Jungle Oats: p=0.1 and Bokomo Oats: p=0.2). However, the 95% CI and SD of the capillary blood glucose were smaller than the venous plasma. In a recent inter-laboratory study, Wolever *et al.* (2003) also found that the mean GI was not affected by the type of blood sampling, but for centers using capillary blood sampling, the SD were smaller in all cases than those of the centers using venous blood. Grandfeldt *et al.* (1995) also concluded that there were no significant differences in GI values whether based on capillary blood or venous blood. However, with some products, capillary blood allowed smaller differences to be detected. The three different oats porridges fell between the intermediate and high categories as defined by the draft Regulations Relating to Labelling and Advertising of Foodstuffs in the Foodstuffs, Cosmetics and Disinfectants Act of 1972 (Act no 54 of 1972), which was published for comment on August 8, 2002 (SA, 2002). It can be concluded with 95% confidence that the true mean GI for Oatso-Easy was between 65.58% and 88.45% (intermediate to high), for Jungle Oats between 50.20% and 85.26% (low to high) and Bokomo Oats between 60.57% and 95.39% (intermediate to high) measured in capillary blood. In venous plasma the 95% CI for Oatso-Easy was between 56.33% and 168.39% (intermediate to high), for Jungle Oats between 54.19% and 188.39% (intermediate to high) and for Bokomo Oats between 34.84% and 192.74% (low to high).

Table 4.6. The mean GI for the three different oats porridges (excluding the extremes) using capillary blood and venous plasma glucose

	Capillary blood			Venous plasma		
	Oatso-easy	**Jungle Oats**	**Bokomo Oats**	**Oatso-easy**	**Jungle Oats**	**Bokomo Oats**
Valid N	16	17	16	18	18	18
Mean	77.01	67.73	77.99	112.36	112.36	113.79
-95%CI	65.58	50.20	60.57	56.33	54.19	34.84
+95%CI	88.45	85.26	95.39	168.39	188.39	192.74
SD	21.46	34.10	32.68	112.67	134.99	158.76

SD=standard deviation
CI=confidence interval

4.6 SUMMARY

Current recommendations are that capillary blood sampling is preferred for determining the GI, but that it is acceptable to use venous blood sampling (FAO/WHO, 1998). From this study, the inter-laboratory study done by Wolever *et al.* (2003) and the study done by Wolever & Bolognesi (1996), it can be concluded that glycaemic responses measured in venous plasma are lower and more variable than those from simultaneously obtained capillary blood.

To reduce the variability, FAO/WHO (1998) suggested that the standard food should be taken at least three times with the mean result being used to calculate the GI. Thus, when the mean of three standard food response areas is used in GI calculations, the mean, variability and skewness of the resulting GI distribution are reduced. In this study the standard food was tested only once.

Another concern of researchers is the between-subject variation in the GI of a specific product. Statistically significant differences ($p<0.05$) were found between the AUCs of the three different oats porridges for capillary blood and venous plasma. However, no statistically significant differences ($p>0.05$) were found between the GIs of the three different oats porridges both for capillary blood and venous plasma. The 95% CI and SD of the capillary blood glucose were notably smaller than those of the venous plasma.

The three different oats porridges fell between the intermediate and high categories as defined by the draft Regulations Relating to Labelling and Advertising of Foodstuffs in the Foodstuffs, Cosmetics and Disinfectants Act of 1972 (Act no 54 of 1972) which was published for comment on August 8, 2002 (Jerling *et al.,* 2002).

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The objectives of this study were to determine the GI of Jungle Oats, Bokomo Oats and Oatso-Easy using both capillary and venous sampling and to determine whether there were significant differences between the GIs of the products mentioned. There is controversy regarding the clinical utility of classifying foods according to their glycaemic responses by using the GI (Bessenen, 2001). Part of the controversy is due to methodological variables that can markedly affect the interpretation of glycaemic responses and the GI values obtained (Wolever, 1990). Variables that affect the GI value include food-portion size, the method of blood sampling and subject characteristics (Wolever *et al.,* 1991).

The digestibility of the starch in plant foods is highly variable and is dependent on a number of factors, including the physical structure of both starch and the food matrix (Englyst *et al.,* 1996). For nutritional purposes, starch in foods may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and RS (Englyst *et al.,* 1992). The hypothesis is that the rapidly available glucose (RAG) fraction is rapidly (within 20 minutes) released and absorbed and is a major determinant of the glycaemic response, however, the slowly available glucose (SAG) fraction is released and absorbed slowly and is not expected to contribute to the glycaemic response (Brand-Miller *et al.,* 2001). Englyst *et al.* (1992) reported an *in vitro* method for the measurement of RDS, SDS, RS and three RS fractions in starchy foods, using

controlled enzymatic hydrolysis with pancreatin and amyloglucosidase. The released glucose is measured by colorimetry, using a glucose oxidase kit. In a study done by Englyst *et al.* (1999), they introduced a high performance liquid chromatography (HPLC) endpoint, which allows the use of an internal standard and has the potential advantage of measuring sugars other than glucose. This *in vitro* technique describes RAG, SAG and starch fractions by measuring the amount of glucose released from a test food during timed incubation with digestive enzymes under standardised conditions and could serve as a tool for investigating the importance of the amount, type and form of dietary carbohydrates for health. They found that the correlation between glycaemic response and RAG was highly significant ($p<0.0001$) and a given percentage increase in RAG was associated with the same percentage increase in glycaemic response.

Food portion size has a major effect on the GI value because glycaemic responses are related to the carbohydrate load (Wolever *et al.,* 1991). The GI Task Force (2002) suggests that 50g glycaemic carbohydrate (of the test food) be determined. In this study, the Englyst method described above (Englyst *et al.,* 1999) was used to determine 50g glycaemic carbohydrate by Englyst Carbohydrates Research and Services Ltd; Cambridge, United Kingdom. The test food oats was used with fat free milk and sugar. The standard or reference food must be tested three times (GI Task Force, 2002). Unfortunately, due to financial and time constraints, the reference test was administered only once. This is an important limitation of the study, which could have increased the within-subject variability. The mean AUC of three trials of the reference food should be used to calculate the GI (WHO/FAO, 1998) because the mean of the three trials is more likely to be representative of a subject's true glycaemic response to the reference food than the result of a single trial. As the

variability of the values from which any ratio is calculated increases, the distribution of the ratio becomes skewed and the mean increases, even if the original values are normally distributed (Wolever *et al.,* 1991). Thus, when the mean of three standard food response areas is used in GI calculations, the mean, variability and skewness of the resulting GI distribution are reduced.

Several methods have been used in the past to calculate the area under the glycaemic-response curve (FAO/WHO, 1998). Nell (2001) found highly significant differences ($P<0.01$) between the AUC_{min} and the AUC method ignoring the area below the fasting level. According to Nell (2001), the method of calculation of the AUC is important if the results of other studies are to be compared. The incremental AUC ignoring the area under the fasting glucose level (Wolever approach) was used in this study, because this approach has the longest history and is, therefore, used more often in scientific literature. Statistically significant differences ($p<0.05$) were found between the AUCs for capillary blood glucose and AUCs for venous plasma glucose.

When collecting and transporting blood for glucose analysis it is important to inhibit enzymatic degradation of blood glucose (Meinik & Potter, 1982; WHO, 2002). Glycolysis may contribute to the variability in the capillary blood versus venous plasma glucose relation (Teng *et al.,* 1995; Hussain & Sharief, 2000). In this study, the venous blood samples was collected into sodium-fluoride tubes and centrifuged within 15 minutes to remove the plasma and to reduce the impact of glycolysis.

Current recommendations are that capillary blood sampling is preferred for determining the GI, but that it is acceptable to use venous blood sampling

(FAO/WHO, 1998). The mean blood glucose concentration was greater in capillary blood than venous plasma at every time point ($p<0.01$) except for Bokomo Oats at T_{15} and T_{45} as discussed in Chapter 4. Granfeldt *et al.* (1995) compared capillary and venous blood samples and also found that the venous peak values at 40 minutes were about 0.9 (0.8-1.1) mmol/L lower than the corresponding capillary values ($p<0.05$). It is well known that arterial plasma glucose concentrations are greater than those of capillary whole blood because the concentration of glucose in red cells is lower than that in plasma. After a meal, the glucose concentration in arterial plasma is higher than in venous plasma because of glucose utilization by peripheral tissues (Wolever & Bolognesi, 1996). Blood glucose concentrations oscillate on a minute-by-minute basis (Abdallah *et al.,* 1997), partly as a result of pulses of insulin secretion (Matthews *et al.,* 1983). The oscillations of blood glucose in different tissues in the body are presumably not in phase with each other, because it takes different lengths of time for the insulin pulses from the pancreas to reach them. The magnitude of the oscillations on the forearm venous blood may be greater than those in capillary blood because the vein drains a small volume of tissue with insulin oscillations in phase with each other, whilst arterial blood is derived from all tissues in the body with insulin oscillations out of phase with each other, dampening the glucose oscillations in capillary blood (Wolever *et al.,* 2003). There is also a small analytical error associated with measuring glucose and this has a larger proportional effect on the AUC when the rise in glucose is small. For example, a 0.1 mmol/L difference in the fasting glucose concentration results in a 12 mmol/L.min difference in the AUC over 2h, which is 20% of an AUC of 60, but only 6% of an AUC of 200 (Wolever *et al.,* 2003).

From the results of the study reported here, the inter-laboratory study done by Wolever *et al.* (2003) and the study done by Wolever & Bolognesi (1996) it can be concluded that glycaemic responses measured in venous plasma are lower and more variable than those in simultaneously obtained capillary blood.

The mean GI of oats porridge as determined in eight studies included in the most recent international tables is 58 +- 4 (42 to 75) and 65 to 66 for instant oats porridge (Foster-Powell *et al.,* 2002). The high metabolic responses to oats are surprising, as oats porridges are rich in soluble fibre and it has been shown that soluble, viscous fibers reduce the rate of absorption, flatten the postprandial glycaemia and lower cholesterol levels (Landin *et al.,* 1992; Anderson *et al.,* 1990). The GI of oat bran breakfast cereal may be reduced by enrichment with additional beta-glucan and sweetening with fructose (Jenkins *et al.,* 2002), or by making the porridge from boiled oats kernels (keeping the structure more intact) (Granfeldt *et al.,* 1995). Jenkins *et al.* (2002) confirmed that cooked-extruded oats bran concentrate is effective in lowering postprandial glycaemia and that the enriched products gave a significantly lower glycaemic response than a commercially available cereal naturally high in β-glucan. Furthermore, the reduction in GI per gram of β-glucan in the foods tested showed that the effectiveness of β-glucan fibre was similar to minimally processed oats bran reported in other studies (Foster-Powell & Miller, 1994; Wolever *et al.,* 1994).

A food company provided the research team with data on how oats is normally consumed in South Africa. From this information it was clear that the most popular additives were milk and sugar by more than 90% of consumers. The addition of fat has been shown to delay gastric emptying and enhance gastric inhibitory polypeptide

(GIP) response (Collier & O'Dea, 1983). However, these effects were only seen when the fat content constituted 45-65% of the total calories of the test meal (Wolever, 1993). Jenkins *et al.* (1984) showed that the addition of skimmed-milk, cheese and butter (16g protein and 25g fat) to a 50g carbohydrate portion of bread, either combined or single, had no significant effect on glucose response in type 2 diabetic subjects. Wolever *et al.* (1994) reported a negative relationship between the GI of 102 foods and their protein and fibre content. The relationship between protein and GI is weak with variation in protein accounting for about 17% of the variability in food GI (Wolever *et al.,* 1994). However, according to Vorster *et al.* (1987), 15g sugar added to dried cooked butter beans, a low GI food, increased the GI from 29 to 54. Taking the GI of milk (27 +-4) and sucrose (68 +-5) (Foster-Powell *et al.,* 2002) into account, they might have had an effect on the overall GI of the meals tested in the study reported here. Twenty gram of sugar was added to the Tiger Oats and Bokomo Oats.

To fully exploit the metabolic potential of a low GI, the requirements of a possible GI label should be rigorously examined and recommendations made to international bodies for consideration. Foods that meet specific nutritional criteria and have been tested for their GI by an accredited laboratory, may be eligible to label the GI value and give a short explanation near the nutrition information panel (Venter *et al.,* 2003).

5.2 CONCLUSIONS

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

- Glycaemic responses measured in venous plasma are lower and more variable than those in simultaneously obtained capillary blood

- Statistically significant differences ($p<0.05$) were found between the AUCs of the three different oats porridges for capillary blood and venous plasma
- No statistically significant differences ($p>0.05$) were found between the GIs of the three different oats porridges, both for capillary blood and venous plasma. However, the 95% CI and SD of the capillary blood glucose were smaller than those of the venous plasma
- The three different oats porridges fell between the intermediate to high categories as defined by the draft Regulations Relating to Labeling and Advertising of Foodstuffs in the Foodstuffs, Cosmetics and Disinfectants Act of 1972 (Act no 54 of 1972) which was published for comment on August 8, 2002

5.3 RECOMMENDATIONS

- The first recommendation is that the methodological guidelines determined by the GI Task Force should be followed. Further research to minimize variations in determining the GI of foods should continue.
- The second recommendation is that capillary blood glucose samples are preferred to determine the GI. Venous plasma is associated with greater within-subject variation of both glycaemic responses and GI values and non-normal distribution of GI values.
- The third recommendation is that the reference food must be measured three times to reduce within-subject variability.
- To label the GI on foods, food products and beverages should be accompanied by clear instructions. For example, 1) which foods/beverages/products may be labeled, 2) standardised methodology

in an accredited laboratory, including clarity on issues such as the reference, total ("available") carbohydrate of test food, number and type of subjects, capillary versus venous blood, method of calculation of AUC, 3) way to express the GI on products, using standard deviations and/or 95% CI to reflect variability and 4) claims of benefits of low GI values.

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

BIBLIOGRAPHY

ABDALLAH, L., CHABERT, M. & SYLVESTRE, L. 1997. Cephalic phase responses to sweet taste. *American journal of clinical nutrition,* 65: 737-743.

ADA (American Diabetes Association). 2002. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. (Position statement). *Diabetes care,* 25: 148-198.

ANDERSON, J.W., SPENCER, D.B., HAMILTON, C.C., SMITH, S.F., TIETYEN, J., BRYANT, C.A. & OELTGEN, P. 1990. Oat-bran cereal lowers serum cholesterol and total LDL cholesterol in hypercholesterolemic men. *American journal of clinical nutrition,* 52: 495-499.

ANON. 1994a. The GI Symbol Program.
[web:] http://www.gisymbol.com.auc/pages/index.asp [Date of use: 15 Oct. 2003].

ANON. 1994b. The Glycaemic index.
[web:] http://www.glycemicindex.com/index.html [Date of use: 15 Oct. 2003].

BESSENEN, D.H. 2001. The role of carbohydrates in insulin resistance. *Journal of nutrition,* 131(Suppl.): 2782S-27286S.

BRAND-MILLER, J. & GILBERTSON, H. 2001. Practical aspects of meal planning using the glycaemic index. FAO/Danone Vitapole Workshop. Glycaemic index and health: the quality of the evidence. Bandol, France: Danone Vitapole.

BRAND-MILLER, J.C., HOLT, S.H.A., PAWLAK, D.B. & MCMILLIAN, J. 2002. Glycemic index and obesity. *American journal of clinical nutrition,* 76(Suppl.): 281S-285S.

BRAND-MILLER, J.C., NANTEL, G., SLAMA, G.S. & LANG, V. 2001. Glyvaemic index and health: the quality of the evidence. Danone Vitapole workshop. London : John Libbey.

BROUNS, F. Proposal at the ILSI International Sugars Coordinating Committee meeting, January 2002.

BURKE, L.M., COLLIER, G.R. & HARGREAVES, R. 1998. Glycemic index – a new tool in sport nutrition. *International journal of sport nutrition,* 8: 401-415.

COLLIER, G. & O'DEA, K. 1983. The effect of coingestion of fat on the glucose, insulin and gastric inhibitory polypeptide responses to carbohydrate and protein. *American journal of clinical nutrition,* 37: 941.

COULSTON, A.M., HOLLENBECK, C.D., SWISKOCKI, A.L.M. & REAVEN, G.M. 1987. Effect of source of dietary carbohydrate on plasma glucose and insulin responses to mixed meals in subjects with NIDDM. *Diabetes care,* 10: 395-400.

DUFFY, T., PHILLIPS, N. & PELLEGRIN, F. 1973. Review of glucose tolerance – a problem in methodology. *The American journal of the medical sciences,* 265:117-133.

ENGLYST, H.N., KINGMAN, S.M. & CUMMINGS, J.H. 1992. Classification and measurement of nutritionally important starch fractions. *European journal of clinical nutrition,* 46(Suppl.2): 33S-50S.

ENGLYST, H.N., KINGMAN, S.M., HUDSON, G.J. & CUMMINGS, J.H. 1996. Measurement of resistant starch in vitro and in vivo. *British journal of nutrition,* 75(5):749-755.

ENGLYST, K.N., ENGLYST, H.N., HUDSON, G.J., COLE, T.J. & CUMMINGS, J.H. 1999. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycaemic response. *American journal of clinical nutrition,* 3: 448-454.

ERIKSSON, K.F., FEX. G. & TRELL, E. 1983. Capillary-venous differences in blood glucose values during the oral glucose tolerance test. *Clinical chemistry,* 29: 993.

FAO/WHO Expert Consultation. 1998. Carbohydrates in human nutrition: Report of a Joint FAO/WHO Expert Consultation. Rome, Italy, 14-18 April 1997.

FELDING, P., JENSEN. I. & LINNET, K. 2002. Estimation of *in vivo* capillary or venous blood gucose concentration from analysis on stored venous blood or its plasma and use in quality control of near-patient glucose tests. *Scandinavian journal of clinical and laboratory investigation,* 62: 201-210.

FOSTER-POWELL, K. & MILLER, J.B. 1994. International tables of glycaemic index. *American journal of clinical nutrition,* 59: 66-69.

FOSTER-POWELL, K., HOLT, S.H.A., BRAND-MILLER, J.C. 2002. International table of glycaemic index and glycaemic load values. *American journal of clinical nutrition,* 76: 5-56.

FRANZ, M. Medical nutrition therapy for diabetes mellitus and hypoglycemia of nondiabetic origin. 2000. In: Mahan, LK, Escott-Stump. Krause's food, nutrition, and diet therapy. 10th Ed. Philadelphia: WB Saunders, 2000: p742-780.

FROST, G., LEEDS, A., TREW, G., MARGARA, R. & DORNHORST, A. 1998. Insulin sensitivity in women at risk of coronary heart disease and the effect of a low glycaemic diet. *Metabolism,* 47: 1245-1251.

GIACCO, R., PARILLO, M. & RIVELLESE, A.A. 2000. Long-term dietary treatment with increased amounts of fibre-rich low-glycemic index natural foods improves blood glucose control and reduces the nuber of hypoglycemic events in type 1 diabetic patients. *Diabetes care,* 23: 1461-1466.

GILBERTSON, H.R., BRAND MILLER, J.C., THORNBURN, A.W., EVANS, S., CHONDROS, P. & WERTHER, G.A. 2001. The effect of flexible low glycemic index dietary advice versus measured carbohydrate exchange diets on glycemic control in children with type 1 diabetes. *Diabetes care,* 24(7): 1137-1143.

GI Task Force (see GLYCAEMIC INDEX TASK FORCE)

GLYCAEMIC INDEX TASK FORCE. 2002. Standard operating procedure for the determining of the glycaemic index. (Unpublished report.)

GRANFELDT, Y., HAGANDER, B. & BJöRK, I. 1995. Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. *European journal of clinical nutrition,* 49(3): 189-199.

GRESSE, A. & VORSTER, H.H. 1992. The glycaemic index and second meal effect of a typical African meal in black non-insulin dependent diabetic subjects. *South African journal of food science and nutrition,* 4(3): 64-69.

HAECKEL, R., BRINCK, U., DUBRAVKA, C., JANKA, H-U., PUNTMANN, I., SCHNEIDER, J. & VIEBROCK, C. 2002. Comparability of blood glucose concentrations measured in different sample systems for detecting glucose intolerance. *Clinical chemistry,* 48: 936-939.

HUSSAIN, K. & SHARIEF, N. 1999. The inaccuracy of venous and capillary blood glucose measurement using reagent strips in the newborn period and the effect of haematocrit. *Early human development,* 57: 111-121.

JACKSON, R.A., PETERS, N., PERRY, U.A.G., ROGERS, J., BROUGH, W.H. & PILKINGTON, T.R.E. 1973. Forearm glucose uptake during the oral glucose tolerance test in normal subjects. *Diabetes,* 22(6): 442-458.

JENKINS, D. & JENKINS, A. 1987. The glycemic index, fiber, and the dietary treatment of hypertriglyceridemia and diabetes. *Journal of the American College of Nutrition,* 6:11-17.

JENKINS, A.L., JENKINS, D.J.A., ZDRAVKOVIC, U., WÜRSCH, P. & VUKSAN, V. 2002. Depression of the glycemic index by high levels of β-glucan fiber in two functional foods tested in type 2 diabetes. *Europen journal of clinical nutrition,* 56:622-628.

JENKINS, D.J., WOLEVER, T.M., JENKINS, A.L. 1988. Starchy foods and glycemic index. *Diabetes care,* 11(2): 149-159.

JENKINS, D.J., WOLEVER, T.M., JENKINS, A.L., THORNE, M.J., LEE, R., KALMUSKY, J., REICHERT, R. & WONG, G.S. 1983. The glycemic index of foods tested in diabetic patients; a new basis for carbohydrate exchange favouring the use of legumes. *Diabetologia,* 24: 257-264.

JENKINS, D.J.A., WOLEVER, T.M.S., TAYLOR, R.H., BARKER, H., FIELDEN, H., BALDWIN, J.M., BOWLING, A.C., NEWMAN, H.C., JENKINS, A.L. & GOFF, D.V. 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *American journal of clinical nutrition,* 34: 362-366.

JENKINS, D.J.A., WOLEVER, T.M.S., THORNE, M.J., JENKINS, A.L., WONG, G.S., JOSSE, R.G. & CSIMA, A. 1984. The relationship between glycemic response, digestibility, and factors influencing the dietary habits of diabetics. *American journal of clinical nutrition,* 40: 1175-1191.

JERLING, J.C., PIETERS, M., LESSING, M.C., NELL, T.A. & Y. VAN HEERDEN. 2002. The determination of the glycaemic index of three oats porridges. (Report to for Tiger Brand food company in November.) Potchefstroom. 12p. (Unpublished.)

LANDIN, K., HOLM, G., TENGBORN, L. & SMITH, U. 1992. Guar gum improves insulin sensitivity, blood lipids, blood pressure, and fibrinolysis in healthy men. *American journal of clinical nutrition,* 56: 1061-1065.

LANGENHOVEN, M.L., KRUGER, M., GOUWS, E. & FABER M. 1991. MRC food composition tables. 3rd ed. Tygerberg : Medical Research Council. 245 p.

LARSON-COHN. U. 1976. Differences between capillary and venous blood glucose during oral glucose tolerance test. *Scandinavian journal of clinical and laboratory investigation,* 36: 805-808.

LIU, S., WILLET, W.C., STAMPFER, M.J., HU, F.B., FRANZ, M., SAMPSON, L., HENNEKENS, C.H. & MANSON, J.E. 2000. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. *American journal of clinical nutrition,* 71(6): 1455-1461.

LUDWIG, D.S. 2000. Dietary glycemic index and obesity. *Journal of nutrition,* 130(Suppl.): 280S-283S.

LUSCOMBE, N.D., NOAKES, M. & CLIFTON, P.M. 1999. Diets high and low in glycemic index versus high monounsaturated fat diets: effects on glucose and lipid metabolism in NIDDM. *European journal of clinical nutrition,* 53(6): 473-478.

MARKS, V. 1996. Blood glucose: its measurement and clinical importance. *International journal of clinical chemistry,* 251:3-17.

MATTHEWS, D.R., LANG, D.A., BURNETT, M. & TURNER, R.C. 1983. Control of pulsatile insulin secretion in man. *Diabetologia,* 24: 231-237.

McCANCE, R.A. & LAWRENCE, R.D. 1929. The carbohydrate content of foods. *Medical Research Council special report series.* London: Her Majesty's Stationary Office.

MEINIK, J. & POTTER, J.L. 1982. Variance in capillary and venous glucose during oral glucose tolerance test. *American journal of medical technology,* 6: 543-545.

NELL, T.A. 2001. The variation and application of the glycaemic index of foods. Potchefstroom: PU vir CHO. (Thesis – Ph.D.) 145p.

ODUM, L. 1999. Measurement of glucose and diagnosis of diabetes mellitus. *Ugeskrift for laeger,* 161(7): 928-931.

PI-SUNYER, F.X. 2002. Glycemic index and disease. *American journal of clinical nutrition,* 76(Suppl.1): 290S-298S.

RICCARDI, G. & RIVELLESE, A.A. 2000. Dietary treatment of the metabolic syndrome - the optimal diet. *British journal of nutrition,* 83 (Suppl. 1): S143-155.

SA see SOUTH AFRICA

SALMERON, J., ASCHERIO, A., RIMM, E.B., COLDITZ, G.A., SPEIGELMAN, D., JENKINS, D.J., STAMPFER, M.J., WING, A.L. & WILLET, W.C. 1997a. Dietary fibre, glycemic load, and risk of NIDDM in men. *Diabetes care,* 20(4): 545-550.

SALMERON, J., MANSON, J.E., STAMPFER, M.J., COLDITZ, G.A., WING, A.L., WILLET, W.C. 1997b. Dietary fibre, glycemic l;oad, and risk of non-insulin-dependent diabetes mellitus in women. *Journal of the American Medical Association,* 277(6): 472-477.

SIDEBOTTOM, R.A., WILLIAMS, P.R. & KANAREK, K.S. 1982. Glucose determinations in plasma and serum: Potential error related to increased hematocrit. *Clinical chemistry,* 28(1): 190-192.

SLABBER, M., BARNARD, H.C., KUYL, J.M., DANNHAUSER, A. & SHALL, R. 1994. Effects of a low-insulin-response, energy-restricted diet on weight loss and plasma insulin concentrations in hyperinsulinaemic obese females. *American journal of clinical nutrition,* 60: 48-53.

SOUTH AFRICA. DEPARTMENT OF HEALTH. 2002. Government Notice. Regulations relating to labeling and advertising of foodstuffs. (R. 1055). Government Gazette: 23714, 8 August. 87p.

TENG, F.Y., PUNLA, O. & KOOS, B.J. 1995. Predictive value of hemocue capillary whole blood glucose measurements in pregnancy. *Journal of the Society for Gynecologic Investigation,* 2: 618-622.

VENTER, C.S., SLABBER, M. & VORSTER, H.H. 2003. Labelling of foods for glycaemic index: Advantages and problems. South African journal of clinical nutrition (in press).

VORSTER, H.H., VENTER, C.S. & SILVIS, N. 1990. The glycaemic index of foods; a critical evaluation. *South African journal of food science and nutrition,* 2(1): 13-17.

VORSTER, H.H., VAN TONDER, E., KOTZE, J.P. & WALKER, A.R.P. 1987. Effects of graded sucrose additions on taste preference, acceptability, glycemic index, and insulin response to butter beans. *American journal of clinical nutrition,* 1987,40: 575-579.

WOLEVER, T.M.S. 1990. The glycaemic index. *World review of nutrition and dietetics,* 62: 120-185.

WOLEVER, T.M.S. 1993. Effect of dietary fiber on carbohydrate, lipid and protein metabolism. CRC handbook of dietary fiber in human nutrition. 2nd ed. Boca Raton : CRC Press. 360p.

WOLEVER, T.M.S. 2003. Carbohydrate and the regulation of blood glucose and metabolism. *Nutrition reviews,* 61(5): S40-S48.

WOLEVER, T.M.S. & BOLOGNESI, C. 1996. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *Journal of nutriton,* 126: 2798-2805.

WOLEVER, T.M.S., JENKINS, D.J.A., COLLIER, G.R., LEE, R., WONG, G.S. & JOSSE, R.B. 1988. Metabolic response to test meals containing different carbohydrate foods: Relationship between rate of digestion and plasma insulin response. *Nutrition research,* 8: 573-581.

WOLEVER, T.M.S., JENKINS, D.J., JENKINS, A.L. & JOSSE, R.G. 1991. The glycemic index: methodology and clinical implications. *American journal of clinical nutrition,* 54(5): 846-854.

WOLEVER, T.M.S., JENKINS, D.J.A., JOSSE, R.G., WONG, G.S. & LEE, R. 1987. The glycemic index: similarity of values derived in insulin-dependent and non-insulin-dependent diabetic patients. *Journal of the American College of Nutrition,* 6: 295-305.

WOLEVER, T.M.S., JENKINS, D.J., VUKSAN, V., JENKINS, A.L., WONG, G.S. & JOSSE, R.G. 1992. Beneficial effect of low-glycemic index diet in overweight NIDDM subjects. *Diabetes care,* 15: 562-564.

WOLEVER, T.M.S., KATZMAN-RELLE, L., JENKINS, A.L., VUKSAN, V., JOSSE, R.B. & JENKINS, D.J. 1994. Glycaemic index of 102 complex carbohydrate foods in patients with diabetes, *Nutrition research,* 14(5): 651-669.

WOLEVER, T.M.S., VORSTER, H.H., BJöRK, I., BRAND-MILLER, J., BRIGHENTI, F., MANN, I.J., RAMDATH, D.D., GRANDFELDT, Y., HOLT, S., PERRY, T.L., VENTER, C. & XIAOMEI, W. 2003. Determination of the glycaemic index of foods: Interlaboratory study. *Europan journal of clinical nutrition,* 57: 475-482.

WOLEVER, T.M.S., VUKSAN, V. & PALMASON, C. 1996. Less variation of postprandial blood glucose after starchy test meals than oral glucose. *Nutrition research,* 16(6): 899-905.

WORLD HEALTH ORGANIZATION (WHO). 2002. Laboratory diagnosis and monitoring of diabetes mellitus. [Web:] http://www.diabetes.org.uk/infocentre/carerec/newdiagnostic.htm. [Date of access: 4 Jun. 2003].